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EFFECT OF CYTOCHROME C SUPPLEMENTATION ON AEROBIC RUNNING PERFORMANCE

IRVIN E. FARIA¹, ERIK W. FARIA², DARYL L. PARKER¹

¹Department of Kinesiology and Health Science, California State University, Sacramento, CA; ²Exercise Physiology Laboratories, University of New Mexico, Albuquerque

ABSTRACT

EFFECT OF CYTOCHROME C SUPPLEMENTATION ON AEROBIC RUNNING PERFORMANCE. Irvin E. Faria, Erik W. Faria, Daryl L. Parker. JEPonline. 2002;5(4):35-40. The purpose of this study was to examine the effect and efficacy of dietary supplementation with cytochrome C, a reputed performance enhancer, on physiological and biochemical parameters. One female and eighteen trained male endurance runners participated in a double-blind, placebo (PL), crossover design study. The effect of oral ingested cytochrome C (C-C) supplementation on VO_2peak , blood lactate (La^-), ventilatory anaerobic threshold (T_{van}), run time to exhaustion, heart rate (HR), and rated perceived exertion (RPE) was examined. Analysis of the data revealed no significant effect of oral ingested cytochrome C on VO_2peak or run time to exhaustion. There were no significant differences between treatments for La^- levels at VO_2 peak, following a submaximal 13 min run or the 3 mile run ($p>0.05$). T_{van} was not found to be significantly different between PL and C-C trials ($p>0.05$) and no significant difference was noted for HR at T_{van} between trials ($p>0.05$). No significant difference was observed between treatments for the subject's psychological perception as measured by RPE. These findings demonstrate that oral ingestion of 800 mg/day for 6 d and an additional 1600 mg of cytochrome C one hour prior to exercise has no significant measurable effect on VO_2 peak, La^- , T_{van}, run time to exhaustion, HR, and RPE.

Key Words: Ergogenic Aids, Nutrition, Dietary Supplements, Aerobic Exercise

INTRODUCTION

Cytochrome C consists of a single polypeptide chain of 194 amino acids and a covalently attached heme group. It functions indirectly to influence the consumption of oxygen in the living cell (1). Accordingly, cytochrome C shuttles electrons from cytochrome reductase to cytochrome oxidase, the final components in the electron/proton transport chain. In doing so, it contributes to the build-up of a proton electrochemical gradient derived by the

vectorial transfer of electrons between cytochrome C and molecular oxygen. The resulting electro-chemical gradient is used elsewhere, for instance in the synthesis of adenosine triphosphate (ATP).

Research has repeatedly shown that endurance exercise results in an increase in the presence of cytochrome C activity in skeletal muscle (2,3,4). Consistent with previous studies of endurance training in animals and humans, recent work by Houle-Leroy et al. (5) demonstrate increased cytochrome C activity in response to endurance training in mice. There is substantial evidence confirming that the physiological consequence of increased levels of cytochrome C is an augmentation in the capacity to oxidize pyruvate and fatty acids and to generate ATP (3,5,6).

Given the metabolic role of cytochrome C, the oral ingestion of cytochrome C has been touted to enhance oxidative phosphorylation and ATP synthesis at the cellular level. The question remains whether supplementation of cytochrome C without an increase in total presence and content of mitochondrial mass to support cytochrome C function will enhance exercise performance. Entrepreneurs who market homeopathically prepared nutraceuticals “ENDURO CAPS™ for muscular endurance” postulate that ENDURO CAPS™, which contain trimethylglycine (a vegetable substance), calcium gluconate (an emulsifier and buffer) and cytochrome C (main ingredient), will facilitate exercise performance including: 1) increase muscular endurance by maximizing oxygen carrying capacity; 2) reduce blood lactate accumulation; and, 3) raise the anaerobic threshold by forestalling the accumulation of blood lactate.

These advertised alleges, however, are based solely upon the theoretical ergogenic potential of cytochrome C when orally ingested. Previous research has examined ergogenic effects of supplemental combinations of inosine, vitamin E, cytochrome C, and coenzyme Q10 (7) and cytochrome C combined with inosine (8). To date, no scientific investigation has examined the effect exclusively of a high dosage of oral ingested cytochrome C on the exercise performance in trained subjects. Therefore, the purpose of this investigation was to examine the effect and efficacy of oral ingestion of cytochrome C on peak oxygen uptake ($\text{VO}_{2\text{peak}}$), blood lactate (La^-), ventilatory anaerobic threshold (T_{van}), heart rate (HR), rated perceived exertion (RPE) and run time to exhaustion.

METHODS

Study population

One female and eighteen male endurance runners (40.2 ± 13.4 yr; 176.6 ± 7.6 cm; 76.6 ± 8.0 kg) who trained 40 to 70 mi/ week, served as subjects. All subjects were informed of the purpose, protocol, and procedures of the study, which was approved by the University Committee for the Protection of Human Subjects. A signed written informed consent was obtained, prior to testing, from each subject.

Study design

The research design, with some modifications, was adopted from the work of Williams et al. (8). A double-blind, placebo, crossover design was employed. Following a learning trial to familiarize the subjects with treadmill running, the subjects underwent two experimental trials, each comprised of three test runs. Trials were exactly 2 weeks apart, and each subject reported to the laboratory at the same time of day. Subjects completed, on the same day, three separate laboratory treadmill tests, with the ingestion of a placebo (PL) or cytochrome C (C-C). Each trial included a submaximal 13 min treadmill run (13 SMR), a 3-mile run (3 MR), and a VO_2 peak test (VP). Subjects were randomly assigned to either the experimental or placebo treatment for each trial run. Knowledge of the subjects' trial orders was kept confidential by a pharmacist not involved in the conduct of the study. All test runs were performed on a Quinton 18-65 treadmill (Quinton Instruments, Seattle, Washington). Trial test protocol

Subjects prepared for each test trial by taper training a day or two prior to testing and followed their normal pre-race nutritional practices. Before testing, body weight was measured and after resting a few minutes, a 44 μ L blood sample via a digit puncture was drawn. Blood samples were assayed in duplicate for lactate using a Yellow Springs Instruments Model 2300 analyzer (Yellow Springs, Ohio).

Following a short rest, the subject ran on the treadmill for 13 min, with a 3 min warm-up at a speed of 6 mi/hr and 10 min at an individually desired speed; the 13 SMR protocol was held constant for each trial. An RPE, using the Borg 20-point scale (9) was obtained by hand signals at minutes 3, 8, and 13.

Immediately following the 13 SMR, a second blood sample was drawn. Following a 15-min rest, the subject then performed the 3 MR. For the 3 MR, the speed of the treadmill was gradually increased to an individually pre-determined race pace, and timing was initiated at that point for both the experimental trials. Split times were announced for the first 0.25 mile, each 0.5 mile, each mile, and next to last 0.25 mile. During the first 3 MR subjects were allowed to request, by hand signals, that the speed of the treadmill be adjusted to their desired race pace. The first 3 MR pace was duplicated, in order to keep the exercise intensity constant, at the second trial 3 MR test.

Minute ventilation (V_E), oxygen uptake (VO_2), and carbon dioxide production (VCO_2) were measured over 15 s intervals using an automated open-circuit SensorMedics (Yorba Linda, California) metabolic gas analysis system. Minute averaged values were used for data analysis. The pneumotachometer was calibrated before every test using a standard 3-L syringe. Gas analyzers were calibrated prior to and following each test using known certified calibration gases. An exercise adapted 5-lead electrocardiogram was monitored throughout the test on a SensorMedics electrocardiograph. RPE was queried prior to each split time. Immediately following the 3 MR, a third blood sample was drawn.

Following a 30-min rest interval, the subject performed the maximal run test to ascertain VO_{2peak} . A modified Balke protocol was used, with the treadmill speed held constant and the grade increased every 2 min. The initial speed was 8.0 mi/hr and the initial grade was 0%, increased to 5% after the first 2 min and 2.5% every 2 min thereafter. Subjects ran to volitional exhaustion. Oxygen uptake peak was determined as the highest VO_2 reached during exercise. Criteria used to assure that VO_{2peak} was reached included an increase of less than 2 ml/kg/min in VO_2 between two successive increasing workloads, a respiratory exchange ratio >1.0 , the attainment of maximal heart rate ($HR < 5$ beats/min), and volitional exhaustion (10). RPE was obtained every min and at exhaustion. Maximal run time was recorded when the subject was unable to maintain the treadmill pace. The final blood sample was drawn immediately following this test (11). Post-exercise finger tip blood samples were analyzed for lactate level.

Ventilatory anaerobic threshold (T_{van}) was observed following the VP incremental test and determined from computer plots. These plots were examined by three investigators familiar with T_{van} determination and required agreement by at least two of the three individuals. The criteria for determination of T_{van} were: 1) an increase in V_E/VO_2 without a concomitant increase in V_E/VCO_2 , and 2) a sudden upward nonlinear increase in V_E (12,13). Verbal encouragement was given equally for all subjects under both treatments by the same investigator.

Dietary Supplementation

Since cytochrome C is a protein substance containing ironporphyrin prosthetic groups. When taken orally its digestion, absorption, and metabolism are likely to be in concert with dietary protein and its iron conceivably re-enters the body's iron store (14). If oral ingested cytochrome C is denatured by gastric proteolytic enzymes it is unlikely to enter the mitochondria (15). A resin coat (enteric coated) was added to each capsule to prevent possible premature denaturation.

Treatment capsules contained 400 mg of cytochrome C, 100 mg of trimethylglycine, and 50 mg of calcium gluconate. The product recommended dose was followed, where ingestion occurred at a rate of two capsules (800 mg of cytochrome C) each morning for six days prior to testing and two additional capsules one hour before each test trial. All subjects were instructed to maintain their current nutritional and activity program and to avoid initiating a new exercise mode or schedule. The seven-day total dosage of cytochrome C was 6400 mg. This dosage represented an approximate four-fold ingestion volume compared to that of Snider et al. (7) who administered 6430 mg of cytochrome C over a 28-day period. The placebo consisted of calcium citrate (Citrical) and was identical in appearance. Supplements were placed in generic capsules coded for identification by a cooperating pharmacist and were packaged in identical containers which were numbered and letter coded. Subjects self-administered the capsules. Knowledge of the contents of coded supplements was retained from the subjects and investigators until the completion of the study.

Statistical analyses

Data are reported as mean \pm SD. Statistical comparisons were made between and within the C-C and PL trials utilizing ANOVA with repeated measures. The Tukey HSD post-hoc test was then utilized to reveal the source of any significant results using an alpha level of $p>0.05$.

RESULTS

To determine whether the oral ingestion of cytochrome C resulted in a change in the variables examined, the parameters were measured during the 13 SMR, 3 RM and VP exercise trials. There was no significant ($p>0.05$) difference observed between C-C and PL trials for VO_2 , HR, V_E , La^- , and RPE for the 13 SMR and 3 RM trials. (Table 1).

Table 1. Comparison of mean \pm SD exercise variables for submaximal 13 min run (13 SMR) and 3-mile run (3 MR) for cytochrome C (C-C) and placebo (PL) trials.

Variable	13-SMR		3-RM	
	C-C	PL	C-C	PL
VO_2 (ml/kg/min)	39.76 \pm 4.5	39.50 \pm 6.4	44.21 \pm 4.4	56.50 \pm 6.6
HR (b/min)	147 \pm 6.0	141 \pm 8.0	165 \pm 8.0	162 \pm 8.0
V_E (l/min)	76.4 \pm 13.2	78.1 \pm 14.3	90.6 \pm 15.0	91.8 \pm 14.8
La^- (mmol/L)	2.5 \pm 1.2	2.2 \pm 0.8	3.1 \pm 1.5	2.2 \pm 1.7
RPE	10 \pm 2.0	10 \pm 2.2	12 \pm 2.0	12 \pm 2.0

Table 2. Comparison of mean \pm SD exercise variables for $\text{VO}_{2\text{peak}}$ Test (VP) for cytochrome C (C-C) and placebo (PL) trials.

Variable	VP	
	C-C	PL
$\text{VO}_{2\text{peak}}$ (ml/kg/min)	55.80 \pm 8.8	56.50 \pm 9.2
HR (b/min)	181 \pm 8.0	177 \pm 9.0
V_E (l/min)	136.10 \pm 26.1	140.0 \pm 20.1
La^- (mmol/L)	7.69 \pm 1.7	7.88 \pm 2.2
RPE	15 \pm 2.0	15 \pm 2.2
$\text{VO}_2 T_{\text{van}}$ (ml/kg/min)	48.44 \pm 7.8	50.22 \pm 7.6
HR T_{van} (b/min)	167 \pm 11.0	165 \pm 8.0
Time to Exhaustion (min)	8.7 \pm 1.4	8.29 \pm 1.6

To examine whether cytochrome C enhanced aerobic capacity, criterion measures of $\text{VO}_{2\text{peak}}$, run time to exhaustion, heart rate, V_E , blood lactate, and T_{van} were observed. There was no significant ($p>0.05$) difference observed between C-C and PL treatments for any of the parameters examined. (Table 2).

DISCUSSION

The current study was undertaken to examine the effect and efficacy of oral ingested cytochrome c as an ergogenic aid to aerobic running performance. Advertisements claim that ENDURO CAPS™, the primary ingredient of which is cytochrome C, supplementation increases the flow of oxygen to the working muscle thereby enhancing endurance and forestalling the production of blood lactate. Further, the allegation is sustained that by delaying the production of blood lactate the anaerobic threshold and run time to exhaustion will increase. Research is cited to support these claims, however, none of these studies investigated the oral ingestion of cytochrome C supplementation (16,17). The primary findings of the present investigation demonstrate that the oral ingestion of 6400 mg of cytochrome C during a 7 d period does not enhance aerobic running performance as measured by run time to exhaustion, anaerobic threshold, and blood lactate level. These results confirm and extend the findings Snider et al. (7) who measured cycling time to fatigue at 70% of VO_2 max following 90 minutes of running at 70% of VO_2 max in highly trained triathletes who had consumed, for 4 weeks, a combination of cytochrome C and several other possible ergogenic substances (i.e., inosine, vitamin E, and coenzyme Q10). Similarly, Williams et al. (8) observed that the ingestion of cytochrome C in combination with inosine did not improve running performance.

Booth and Holloszy (17) found a significant increase in total work performed following a cytochrome C increase in vivo during training. In contrast to these findings, oral supplementation of cytochrome C had no measurable effect, during the VP test, on run time to exhaustion. If the asserted effect of cytochrome C were realized the rate of flux through the electron transport system could positively affect the availability of oxidatively derived ATP during exercise thereby extending exercise duration. Moreover, a higher VO_2 peak value would be expected if the mitochondrial level of cytochrome C was increased. To that end, Holloszy (18) and Booth and Narahara (19) demonstrated the coupling of cytochrome C increase with enhanced aerobic capacity. However, the data from the present investigation provide no convincing evidence of the oral ingestion effect or efficacy of cytochrome C. In this regard, there is no direct evidence that increasing cytochrome C without also enhancing total mitochondrial mass increases the maximal rate of VO_2 . It can be argued that the total presence and content of the entire mitochondrial mass is essential to support cytochrome C function.

Research does, however, reveal increased cytochrome C levels in skeletal muscle induced by means other than oral ingestion. In this regard, it is noteworthy that recent evidence indicates a role of calcium and protein kinase C in inducing increases in cytochrome C gene expression (3). Further, cytochrome C has been shown to increase in the white region of muscle in response to 4 wk of 5-aminoimidazole-4-carboxamide-1 β -ribofuranoside (AICAR) injections leading to chronic 5'-AMP-activated protein kinase (20). It is important to mention that the increase in cytosolic calcium during excitation-contraction coupling and the rise in plasma fatty acids that accompanies prolonged exercise bouts also appears to be important in inducing the increase in mitochondrial enzymes. The authors remark that these effects of exercise were not mimicked by the AICAR injections (20).

Conclusions

In conclusion, the main effect of oral ingestion of cytochrome C on aerobic run time to exhaustion was not statistically demonstrable and the parameters observed for treatment trials were not different from those of the placebo trials. These findings clearly demonstrate that the supplementation of oral cytochrome C (800 mg/d for 6 d and 1600 mg on d 7) fails to enhance aerobic run performance to exhaustion, T_{van} , VO_2 peak, or alter blood lactate accumulation during submaximal and maximal exercise. Within the limitations of this study, the use of oral ingested cytochrome C supplementation as an ergogenic aid for the enhancement of aerobic exercise performance remains unsupported.

Address for Correspondence: Dr. Irvin E. Faria, 3731 Dell Road, Carmichael, CA 95608; Phone: (916) 944-3305; E-mail: irvfaria@saclink.csus.edu

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