Chronic (-)-Hydroxycitrate Administration Spares Carbohydrate Utilization and Promotes Lipid Oxidation during Exercise in Mice

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ABSTRACT (-)-Hydroxycitrate (HCA) is an active ingredient that is extracted from the rind of the Indian fruit, Garcinia cambogia, which is available as an herbal supplement and is used to lose weight. In this study, the acute and chronic effects of HCA on energy metabolism were examined in male Std ddY mice. Mice were placed into metabolic chambers and administered 10 mg HCA or water (control) orally. Serum free fatty acid levels were significantly higher 100 min after administration in the HCA group, but the respiratory exchange ratio was not different from that in the control group. The concentration of glycogen in the gastrocnemius muscle was higher in the HCA group 16 h after administration, and in a separate study, the maximum swimming time until fatigue was slightly longer (P = 0.21) than that in the control group on d 1. The difference was significant on d 3 after 3 d of HCA or water administration. Other mice were administered 10 mg HCA or water orally twice a day for 25 d. On d 26, they were placed into metabolic chambers after administration and allowed to rest for 1 h, followed by 1 h of running at 15 m/min. Respiratory gas was monitored. The respiratory exchange ratio was significantly lower in the HCA group during both resting and exercising conditions. These results suggest that chronic administration of HCA promotes lipid oxidation and spares carbohydrate utilization in mice at rest and during running. J. Nutr. 130: 2990–2995, 2000.

KEY WORDS: • (-)-hydroxycitrate • endurance exercise • carbohydrate utilization • lipid oxidation

A number of dietary supplements are used by athletes to increase the efficiency of training (e.g., protein supplements) and to complement the loss of water, electrolytes or carbohydrate during exercise (e.g., some types of sports drinks) (Applegate and Grivetti 1997). Athletes expect that these dietary supplements might compensate for a lack of training. These dietary supplements may enhance the effects of training. A number of studies revealed that the most important effect of endurance training may be the sparing effect of glycogen utilization during exercise through an increase in the ability of skeletal muscle to oxidize lipids (Maughan 1998, Williams 1998).

Costill et al. (1978) and Ivy et al. (1979) suggested that caffeine has a positive ergogenic effect. They proposed that the effect is achieved by an elevation in catecholamines, which enhance fat oxidation either by increasing free fatty acid (FFA) levels or muscle triacylglycerol lipolysis (Graham and Spriet 1991). Plasma FFA were elevated 1 h after caffeine ingestion in some studies (Powers et al. 1983, Sasaki et al. 1987). The failure to demonstrate consistently an increase in FFA may be a result of increased uptake by the active muscle. A number of supplements such as capsaicin and its analogs (Kim et al. 1997, 1998a, and 1998b), medium-chain triglycerides (Matsumoto et al. 1996) and Nanpao, a traditional Chinese medicine (Saito et al. 1998), have been reported to increase endurance performance. However, caffeine is a substance prohibited by International Olympic Committee, medium-chain triglycerides increase the serum ketone body concentration, causing a sense of fatigue (Matsumoto et al. 1996) and an effective dose of capsaicin (10 mg/kg body) is too pungent for human use.

Recently, (-)-hydroxycitrate (HCA) has been reported to affect lipid metabolism (Kriketos et al. 1999, McCarty 1994 and 1995). HCA is an active ingredient that is extracted from the rind of the Indian fruit, Garcinia cambogia (Lewis and Neelakantan 1965); it is available as an herbal supplement and decreases adipose tissue weight after ingestion for a few weeks (Chee et al. 1977, Greenwood et al. 1981, Rao and Sakariah 1988). HCA is a competitive inhibitor of ATP:citrate lyase (EC 4.5.3.8), inhibits fatty acid synthesis and reduces appetite in rodents (Watson et al. 1969). Whether HCA administration affects lipid oxidation and endurance exercise performance is unclear. We speculated that HCA may aid in aerobic performance, but no research exists concerning such an effect. The purpose of this study was to investigate the possibilities of HCA as a dietary supplement to increase endurance exercise performance.

MATERIALS AND METHODS

Animals and diets. Seven-week-old male Std ddY mice (Japan SLC, Hamamatsu, Japan) were used. They were housed in standard cages.
(33 × 23 × 12 cm; 6 mice/cage) under controlled conditions of temperature (22 ± 0.5°C), humidity (50%) and lighting (lights on from 1800 to 0600 h). They were given free access to water and a nonpurified commercial diet (type MF; Oriental Yeast, Tokyo, Japan) containing 5 (g/kg diet): water, 70; protein, 240; fat, 51; fiber, 32; carbohydrates, 545. All animals received humane care as outlined in the NIH guidelines (NRC 1985) and by the Kyoto University Animal Care Committee. HCA used in this study was in free acid form and was provided by Nippon Shinyaku (Kyoto, Japan). In all experiments, HCA was dissolved in distilled water and administered orally as a 0.48 mol/L solution.

**Apparatus.** The gas analyzer used for the assessment of metabolic rate in the mice consisted of six acrylic metabolic chambers, CO2, and O2 analyzers (model RL-600, AlcoSystem, Tokyo, Japan), and a switching system (model AN16-A-S, AlcoSystem, Tokyo, Japan) to sample gas from each metabolic chamber. Each metabolic chamber had a 125.4 cm² floor and was 6.5 cm in height. Room air was pumped through the chambers at a rate of 1.0 L/min. Expired air was dried in a cotton thin column and then directed to an O2/CO2 analyzer. Air from each chamber was sampled for 60 s. During the last 1 s, the O2 and CO2 concentrations were measured 100 times and the average was used for the calculation of oxygen consumption and the respiratory exchange ratio (RER). Therefore, the data for each chamber could be obtained every 7 min and the data were stored on a spreadsheet.

The oxygen consumption (VO2), carbon dioxide exhaustion (VCO2), RER, carbohydrate oxidation and lipid oxidation were calculated using the following equations:

\[
VO_2 = \left[\frac{FN_2}{FN_1} \times FIO_2 - FE_2O_2\right] \times VT \times 10
\]

\[
VCO_2 = \left[FE_2O_2 - FICO_2\right] \times VT \times 10
\]

\[
RER = \frac{VCO_2}{VO_2}
\]

Carbohydrate oxidation = \(4.51 \times RER - 3.18 \times VO_2\)

Lipid oxidation = \(1.67 \times (1 - RER) \times VO_2\)

where \(FEN_2\) is the concentration of nitrogen in the exhaust air, \(FIN_2\) is the concentration of nitrogen in the room air, \(FE_2O_2\) is the concentration of oxygen in the exhaust air, \(FIO_2\) is the concentration of oxygen in the room air, \(FECO_2\) is the concentration of carbon dioxide in the exhaust air, \(FICO_2\) is the concentration of carbon dioxide in the room air and \(VT\) is the air flow through the chamber corrected to standard temperature pressure dry.

The swimming system used in the experiment for the measurement of the swimming time of mice to exhaustion was an adjustable-swimming system (model Simplex II, Columbus, OH) for running of mice. The system consisted of three running lanes of equal dimensions (28.8 × 4.7 × 4 cm) and each lane was encased within a metabolic chamber designed to measure respiratory gas. An electric air fan provided mixing of the air. The system could therefore monitor respiratory gases of up to three mice independently.

**Experimental design**

**Experiment 1: Single HCA treatment and metabolic variables.** After a 1-wk preliminary period, 60, 54 and 12 mice were used to measure the effects of a single oral HCA treatment on respiratory gas, serum variables and glycogen accumulation, respectively. Mice (n = 60) were separated into three groups with equal body weights; each mouse was placed into a metabolic chamber designed to measure respiratory gas. Mice were prohibited access to the diet and water from 0930 h. At 1000 h, they were administered orally 100 (10 mg) or 300 μL (30 mg) of a 0.48 mol/L HCA solution, or water, and the respiratory gas was analyzed for 2 h after the administration. To measure serum variables, another 54 mice were separated into three groups, prohibited access to the diet and administered HCA orally as described above. They were killed by decapitation either 30 or 100 min after the administration. Blood was rapidly collected from the neck, and the concentrations of serum glucose, FFA and triglyceride were measured. To investigate the effect of HCA on glycogen accumulation, 12 other mice were separated into three groups as described above, given free access to the commercial diet after administration of the agent and killed by decapitation 16 h later. The gastrocnemius muscle and liver were rapidly removed, frozen in liquid N₂, weighed and analyzed for the determination of tissue glycogen.

**Experiment 2. HCA treatment and maximum swimming time.** Mice (n = 18) were forced to swim to exhaustion at a flow rate of 8 L/min twice at 3-d intervals during the preliminary period of 1 wk and then were divided into three groups. They were orally administered 100 (10 mg) or 300 (30 mg) μL of a 0.48 mol/L HCA solution or 100 μL of water (control) at ~1700 h and given free access to the commercial diet and water. Starting the next day, mice swim until fatigued for 20 min daily, and the maximum swimming time was measured. Mice were administered HCA solutions orally every day after swimming (at ~1700 h).

**Experiment 3. Chronic HCA treatment and lipid oxidation.** Mice (n = 18) were divided into two groups so that the mean body weights were equal in both groups. They were orally administered 100 μL of a 0.48 mol/L HCA (10 mg) or water (control) twice a day (first administration, from 1000 to 1100 h; second administration, from 1700 to 1800 h) for 25 d. Mice had free access to food and water; body weight and food intake were measured every day. After the final administration at d 26, each mouse was placed into a treadmill chamber and allowed to rest for 1 h, followed by a 1-h run at the speed of 15 m/min; respiratory gas was monitored. On d 27, they were killed by decapitation and the gastrocnemius and quadriceps muscles, epididymal and perirenal adipose tissues, liver, spleen, heart and kidneys were removed, and tissues were weighed.

**Biochemical analyses.** The muscle glycogen content was measured spectrophotometrically by a method using enzymatic techniques as described elsewhere (Passonneau and Lauderdale 1974). Briefly, after hydrolysis of the muscle sample in 0.6 mol/L HCl at 100°C for 2 h, the glucose residues were determined with a commercial kit (Glucose CII Test Wako, Wako Pure Chemical, Osaka, Japan).

Blood was collected from the severed neck veins; serum was obtained by centrifugation (3000 × g for 10 min) and stored at −80°C until analysis. Serum glucose, FFA and triglyceride were measured with commercial kits (Glucose CII, NEFA C, triglyceride G Test Wako, Wako Pure Chemical, Osaka, Japan).

**Statistical analysis.** Data are presented as means ± SEM. All of the statistical analyses were performed by StatView version 4.5 (SAS Institute, Cary, NC). Body weight, organ weights, serum variables, glycogen concentrations and maximum swimming time until fatigue were analyzed by ANOVA, and post-hoc comparisons were made using Fisher’s test. In Experiment 1, carbohydrate and lipid oxidation, and the RER were analyzed by two-way repeated-measures ANOVA, and post-hoc comparisons were made using Fisher’s test. In Experiment 3, comparisons between the controls and the HCA group were made by two-way repeated-measures ANOVA and were analyzed independently for at rest and during running. Differences were considered significant at P < 0.05.

**RESULTS**

**Single HCA treatment and metabolic variables (Experiment 1).** The RER was greater than in controls in the group administered 30 mg HCA, but not in that administered 10 mg, at 20 and 30 min (Fig. 1A). Oxygen consumption did not differ among the groups (Fig. 1B). Carbohydrate oxidation (Fig. 2B) was greater and lipid oxidation (Fig. 2A) was lower than in controls at 20 and 30 min after administration in the group administered 30 mg, but not in that administered 10 mg. Serum FFA concentrations 100 min after administration were 0.473 ± 0.026, 0.560 ± 0.022 and 0.483 ± 0.025
mmol/L in mice administered 0 (control), 10 and 30 mg HCA, respectively. There was a significant difference between the control and 10-mg HCA groups. There were no significant differences in serum FFA concentrations 30 min after administration. Serum glucose and triglycerides were not different from controls in either experimental group at either 30 or 100 min after administration.

The concentration of glycogen in the gastrocnemius muscle 16 h after administration was 4.31 ± 0.22, 5.65 ± 0.45 and 4.36 ± 0.30 mg/g wet tissue in mice fed 0 (control), 10 and 30 mg HCA, respectively. There was a significant difference between the control and 10-mg HCA groups. Liver glycogen concentration did not differ among groups (data not shown).

**HCA treatment and maximum swimming time (Experiment 2).** In the group administered 10 mg HCA, the increase in maximum swimming time on d 1 was slightly longer (P = 0.21) than that of the control group. The increase on d 3 was significantly longer than that of the control group after 3 d of treatment (Fig. 3, P < 0.05).

**Chronic HCA treatment and lipid oxidation (Experiment 3).** Administration of 10 mg HCA twice a day for 25 d did not affect food intake (Fig. 4, histogram). There were no differences in body weight among the groups before treatment, but after 3 wk, the HCA group was 1.2 g lighter than the control group (Fig. 4, line, P = 0.34). Epididymal adipose (P = 0.24) and perirenal adipose (P = 0.18) tissue weights tended to be lower, whereas relative liver (3.84 ± 0.47 vs. 4.25 ± 0.18%) and spleen (0.24 ± 0.06 vs. 0.31 ± 0.03%) weights were significantly greater in HCA-treated mice than in controls.

On d 26, the metabolic rate was monitored after HCA administration. HCA solution (100 μL of 0.48 mol/L, 10 mg) was given 60 min before the start of the running exercise (0 min in Fig. 5). Thirty minutes after administration, the RER began to decrease in mice administered HCA but not in the control group. The RER in the control group and HCA group was 0.86 ± 0.01 and 0.82 ± 0.02 at 1 h after administration, respectively (P < 0.05, two-way repeated-measures ANOVA when at rest, Fig. 5). The RER increased abruptly by 0.05 in both groups after the start of the running exercise (at 65 min in Fig. 5), but decreased gradually after the belt speed reached the set value of 15 m/min (at 75 min in Fig. 5). The RER reached ~0.8 at the end of the running period in both groups and was significantly lower in the HCA-administered group throughout the running period (P < 0.01, two-way repeated ANOVA during running). Oxygen consumption of mice in both groups gradually decreased to 80 mL/kg body during rest in the metabolic chamber and increased to 100–120 mL/kg body during the running period (data not shown).

Lipid oxidation, calculated from the RER and oxygen con-

![FIGURE 1](image1.png)  
**FIGURE 1** Effects of a single (-)-hydroxycitrate (HCA) treatment on (A) the respiratory exchange ratio and (B) oxygen consumption in mice (Experiment 1). Each mouse was housed in a metabolic chamber and orally administered 10 or 30 mg HCA or water at time 0 when at rest, and respiratory gas was collected. Values are means ± SEM, n = 20. Values at a time point without common letters are significantly different, P < 0.05; NS, P ≥ 0.05.

![FIGURE 2](image2.png)  
**FIGURE 2** Effects of a single (-)-hydroxycitrate (HCA) treatment on (A) lipid and (B) carbohydrate oxidation in mice (Experiment 1). Each mouse was housed in a metabolic chamber and orally administered 10 or 30 mg HCA or water at time 0 when at rest, and respiratory gas was collected. Values are means ± SEM, n = 20. Values at a time point without common letters are significantly different, P < 0.05; NS, P ≥ 0.05.
sumption, tended to be lower in the control group compared with the HCA group for 60 min before the start of exercise (P < 0.07, two-way repeated-measures ANOVA, Fig. 6). Lipid oxidation during the first 20 min of running was higher in the HCA group than in the control group (P < 0.0001 during running, two-way repeated-measures ANOVA, Fig. 6A). Although there was no difference in carbohydrate oxidation before the start of the running period, carbohydrate oxidation during the 60-min running period was lower in the HCA group than in the control group (P < 0.0001, two-way repeated-measures ANOVA, Fig. 6B).

DISCUSSION

This study was designed to investigate the effects of HCA administration on endurance exercise in mice. The major findings were as follows: 1) serum FFA were increased 100 min after a single oral administration of 10 mg HCA with no change in the RER; 2) administration of 10 mg HCA promoted the accumulation of glycogen in skeletal muscle 16 h after administration; 3) maximum swimming time until fatigue was significantly increased after 3 days of oral administration; and 4) lipid oxidation was increased and carbohydrate was spared during light running exercise after chronic administration.

Because acute administration of 10 mg HCA did not affect the RER but increased serum FFA concentration 100 min after administration, lipid metabolism may be promoted and carbohydrate utilization could be spared. Also, the glycogen concentration of gastrocnemius muscle became significantly higher in these mice 16 h after administration. Hellerstein and Xie (1993) reported that administration of 0.263 mmol/(kg·d) [1.64 mg/(d·mouse)] increased liver glycogen accumulation in rats administered HCA and refed glucose intravenously (Hellerstein and Xie 1993).

In this study, in mice chronically administered 10 mg HCA twice a day, the RER was significantly lower during the 60-min running period (P < 0.01). Lipid oxidation was significantly greater and carbohydrate oxidation was significantly less in these mice during the early stages of running (P < 0.05). During the transition from rest to moderate-intensity exercise, the muscle shifts from using primarily circulating FFA to using a blend of FFA, extramuscular glucose and muscle glycogen (Ahlborg et al. 1974, Bergstrom et al. 1967, Wahren et al. 1971). Carbohydrate storage in the body is limited compared with the energy storage in adipose tissue, and sparing of carbohydrate during exercise is therefore beneficial for competition. A diet resulting in greater utilization of lipids may affect carbohydrate utilization at the early stage of exercise and result in an increased capacity for endurance exercise. For example, capsaicin or its analog caused epinephrine secretion and increased lipid oxidation 2 h after administration; thus, the mice that were given capsaicin or its analog 2 h before the start of exercise had significantly greater endurance capacity (Kim et al. 1997 and 1998b). In this study, the RER in mice
fed HCA gradually began to decrease from 30 min before the start of exercise, and the differences between the two groups continued for the first 15 min of running. The enhancement of lipid oxidation at the early stage of exercise could lead to increased endurance exercise capacity. For example, in high fat diet–adapted rats, muscle glycogen was spared during the early stages of prolonged exercise (Nakamura et al. 1998), and the running time until exhaustion was longer than that in rats adapted to a low carbohydrate diet (Miller et al. 1984). In subjects ingesting a caffeine solution before the start of running, glycogen utilization was spared for the first 15 min of exercise and endurance exercise time until fatigue was longer than in subjects who ingested dextrose (Spriet et al. 1992).

The RER in the HCA group continued to decrease, and there was a significant difference between the control mice and HCA-treated mice during the 60-min rest (preexercise) period after administration (Fig. 5). The divergence of the RER of the two groups with time was not considered to be due to a transition from the fed to the food-deprived state of mice. HCA was given 60 min before the start of exercise and mice had free access to food until treatment was given. Chronic administration did not reduce food intake, and there were no differences in the RER among the groups 30 min after HCA administration (at 30 min in Fig. 5). Hence, we considered that the divergence of the RER was due to HCA. Chronic HCA administration might have increased energy expenditure during the 3-wk experimental period in this study. In studies from the University of South Carolina, subjects receiving 750 mg/d (d person), equivalent to 0.375 mg/d (d mouse) lost 4–5 kg weight during the 8-wk study period (Conte 1993). In another recent study, rats fed a high (75%) carbohydrate diet and supplemented with HCA (52 mmol HCA/kg 70% dextrose diet, 65 kJ/g) for 28 d had a 12.6% increase in 24-h energy expenditure with no change in the RER (Vasseli et al. 1998).

Numerous studies have shown that endurance exercise performance is augmented by the promotion of lipid oxidation. There are several mechanisms by which HCA may promote lipid oxidation. HCA could be acting through stimulation of the autonomic nervous system, a direct effect on metabolism in specific tissues or organs, or effects on energy substrate flux and availability. Promotion of lipid oxidation by administration of HCA may be attributed to the activity of carnitine palmitoyltransferase (CPT-I), which plays an important role in regulating the flux of long-chain fatty acids into mitochondrial oxidative metabolism in mammalian tissues (Ruderman et al. 1999). CPT-I activity is completely inhibited by malonyl-CoA, whose production is suppressed by HCA.

Chronic consumption of a high fat diet has been reported to increase endurance exercise performance (Lambert et al. 1994, Starling et al. 1997). A single or short-term feeding of a high fat diet elevated the serum FFA concentration but did not increase endurance exercise performance (Okano et al. 1996 and 1997, Whitley et al. 1998). It is thought that the continuously high serum FFA concentration during chronic consumption of a high fat diet could increase lipid oxidation capacity. In this study, the serum FFA concentration was significantly elevated by single administration of 10 mg HCA, but the RER and endurance exercise performance were unaffected (our pilot study). Mice chronically administered 10 mg HCA had an enhanced lipid oxidation capacity during exercise (Fig. 5, 6).

Recently, Kriketos et al. (1999) did not detect any effect of HCA administration on lipid oxidation in men during either rest or moderately intense exercise on a cycle ergometer. However, in those studies, subjects received a daily dose of 3.0 g per person (nearly equal to 1.5 mg/d (d mouse)) for 3 d, which is a much smaller dose than that used in other studies. Also, their experimental period of 3 d was quite short compared with the other studies. Chee et al. (1977) observed that the rate of hepatic fatty acid synthesis was depressed by a single intraperitoneal injection of 0.4 mmol HCA [83.2 mg/d (d mouse)] or a single meal containing 52.6 mmol/kg diet [32.8 mg/d (d mouse)] in rats and chickens. Rao et al. (1988) reported that rats fed a diet containing 2 g/kg HCA for 15 d had significantly less epididymal fat. Our present study was based on pilot studies conducted in our laboratory, in which we used a dose of HCA that ranged from 5 to 30 mg/d (d mouse), taking into consideration the results of the studies by Chee et al. (1977) and Rao et al. (1988).

Acute administration of 30 mg HCA increased the RER, in contrast to the decrease due to chronic administration of 10 mg HCA. In addition, because 30 mg HCA reduced food intake by 87% (pilot study), we judged that the dose of 30 mg was not suitable for investigation of the effect of HCA on...
endurance exercise performance, and did not examine the effect of chronic administration of 30 mg HCA.

Acute administration of 30 mg HCA increased the RER and depressed lipid oxidation for the first 3 h after administration. The mechanism responsible for the observed increase in RER is uncertain. One possible mechanism may be due to a buffering action of serum. For example, serum lactate concentration is high at the end of the intense exercise. The blood pH is constantly adjusted within a relatively narrow range, and carbon dioxide is thus liberated from the carbonate in the blood in order to adjust pH when lactic acid accumulates in the blood. The liberated carbon dioxide is exhaled from the lungs, increasing the RER.

Similarly, after administration of 30 mg HCA, HCA in blood could cause the liberation of carbon dioxide from the carbonate in the blood in order to adjust pH, and the liberated carbon dioxide expired from the lungs might result in an increase in the RER. We contend that the effect is negligible when a smaller dose (10 mg) of HCA is administered. It should be noted that HCA used in the previous experiments was not a lactone but a free acid. Another possible mechanism is the stress-induced release of epinephrine or noradrenaline. We observed that mice orally administered 30 mg (300 μl of 0.48 mol/L) HCA repeatedly rubbed their faces with their hands. The endocrinological changes in the mice were not evaluated here. RER is increased by an infusion of the stress hormones, cortisol, epinephrine and glucagon, in amounts designed to simulate plasma levels seen in patients after trauma (Weisman et al. 1996).

In summary, oral administration of 10 mg HCA elevated serum FFA concentration and increased muscle glycogen concentration in trained mice at rest. Mouse that were chronically (twice daily for 25 d) administered HCA had a significantly reduced serum FFA concentration in mice at rest. Mice that were chronically (twice daily for 25 d) administered HCA repeat-

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**LITERATURE CITED**


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