Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance[1–3]

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ABSTRACT

Background: Exercise practitioners often take vitamin C supplements because intense muscular contractile activity can result in oxidative stress, as indicated by altered muscle and blood glutathione concentrations and increases in protein, DNA, and lipid peroxidation. There is, however, considerable debate regarding the beneficial health effects of vitamin C supplementation.

Objective: This study was designed to study the effect of vitamin C on training efficiency in rats and in humans.

Design: The human study was double-blind and randomized. Fourteen men (27–36 y old) were trained for 8 wk. Five of the men were supplemented daily with an oral dose of 1 g vitamin C. In the animal study, 24 male Wistar rats were exercised under 2 different protocols for 3 and 6 wk. Twelve of the rats were treated with a daily dose of vitamin C (0.24 mg/cm^2 body surface area).

Results: The administration of vitamin C significantly ($P = 0.014$) hampered endurance capacity. The adverse effects of vitamin C may result from its capacity to reduce the exercise-induced expression of key transcription factors involved in mitochondrial biogenesis. These factors are peroxisome proliferator–activated receptor coactivator 1, nuclear respiratory factor 1, and mitochondrial transcription factor A. Vitamin C also prevented the exercise-induced expression of cytochrome C (a marker of mitochondrial content) and of the antioxidant enzymes superoxide dismutase and glutathione peroxidase.

Conclusion: Vitamin C supplementation decreases training efficiency because it prevents some cellular adaptations to exercise. *Am J Clin Nutr* 2008;87:142–9.

KEY WORDS Free radicals, $\dot{V}O_2$max, antioxidant enzymes, antioxidant supplements, exercise, exhaustion, vitamins, gene expression, hormesis, reactive oxygen species

INTRODUCTION

Acute physical exercise induces augmented generation of reactive oxygen species (ROS) in muscle and in other organs (1–3). Because of that, it has been generally accepted over the past 20 y that increasing the concentrations of antioxidants within a muscle cell should provide greater protection against these oxidizing agents and should reduce fatigue (4–7). However, the functional significance of exercise-induced oxidative stress is open to discussion. Results from several laboratories indicate that ROS are signals that serve to up-regulate the expression of a number of genes (8, 9). Thus, ROS can exert favorable effects and can be involved in the process of training adaptation. Up-regulation of endogenous antioxidant systems in response to regular training exerts beneficial effects in the prevention of chronic disease processes (10) and has also been related to longevity in flies (11) and mice (12).

The maximal capacity to take up, transport and utilize oxygen during exercise is $\dot{V}O_2$max (13). Endurance is defined as the time limit of a person's or animal's ability to maintain a specific power level during a running protocol (14). Large-scale epidemiologic studies of humans with and without cardiovascular disease show that low aerobic exercise capacity is an stronger predictor of mortality than are other established risk factors, such as diabetes, smoking, hypertension, or chronic obstructive pulmonary disease (15–18). These observations are consistent with the role of impaired regulation of mitochondrial function as an important mechanism for low aerobic capacity (19). The relations among $\dot{V}O_2$max, muscle oxidative capacity, endurance capacity, and maximal aerobic workload capacity have been discussed for years (20). Davies et al (21) concluded that muscle oxidative capacity (ie, the mitochondrial content of muscle) was a major determinant of endurance capacity, whereas $\dot{V}O_2$max was only indirectly related to endurance capacity but was directly related to exercise intensity. In eukaryotic cells, mitochondrial biogenesis requires gene products from 2 physically separated genomes—one contained within the organelle and the other contained within the nucleus. Peroxisome proliferator–activated receptor co-activator 1 (PGC-1) is a recently identified coactivator of nuclear receptors. It powerfully induces mRNA expression for important nuclear transcription factors such as nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription

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factor A (mTFA). Mitochondrial biogenesis is usually estimated by the change in the content of a typical marker protein, such as cytochrome C (22).

The aim of the present study was to explore the effects of vitamin C administration on training-induced increases in $\dot{V}O_2$max and endurance capacity and on the skeletal muscle mitochondrial biogenesis in both rats and humans. The results of the present study show that supplementation with vitamin C does not improve but partially decreases the improvement in $\dot{V}O_2$max associated with exercise training in rats and in humans. Moreover, it very significantly hampers endurance capacity in animals, as a result of the decrease in mitochondriogenesis that is normally associated with exercise training.

SUBJECTS AND METHODS

Men

Fourteen healthy sedentary men volunteered for this study. We considered as sedentary a person with a $\dot{V}O_2$max < 43 mL · min^{-1} · kg^{-1} (14, 23) or with a physical activity index of <2000 kcal/wk, which was evaluated with a questionnaire (24). All men were nonsmokers and free of any known illness as ascertained by questionnaire. Men taking any form of vitamin supplementation were excluded.

Before the experimental tests, men were initially assessed for $\dot{V}O_2$max, and their height, weight, body mass index (in kg/m^2), and body surface were calculated. None of these characteristics differed significantly between the 2 groups. A summary of the men's characteristics is given in **Table 1**.

Men were randomly divided into 2 experimental groups: exercised ($n = 9$) and exercised but treated with a daily dose of vitamin C (1 g) ($n = 5$). Both groups of men were instructed to take the vitamin C at 0900 every day throughout the study.

All men were informed verbally and in writing about the nature of the study, including all potential risks. Written informed consent was obtained from all participants. The Ethics Committee of the University of Valencia granted ethical approval.

Methods

The maximal exercise test was performed on a magnetic brake bicycle ergometer (Ergomedic 828; Monark, Varberg, Sweden). Basal values of oxygen uptake were obtained for all men after a 5-min rest period. The men next performed a 3-min warm-up at an intensity of 80 W. The exercise started, after a 3-min rest, with a load corresponding to 2 metabolic equivalent tasks with load increments of 2 metabolic equivalent tasks every 3 min (25). For the entire test, the men were instructed to keep a constant pace of 70 rpm. At exhaustion, they continued pedalling against a resistance of 50 W for 3 min, unless complications occurred. The highest VO_2 value obtained in any 10-s period was taken as $\dot{V}O_2$max; it was considered to be achieved by the following endpoint criteria: *1*) a heart rate at maximal exercise higher than 90% of the predicted heart rate maximal for the subject's age (26), *2*) a maximal respiratory exchange ratio ≥ 1.15; *3*) a plateau of oxygen consumption despite increasing workload; and *4*) a blood lactate concentration > 10 mmol/L. Expired fractions of oxygen and carbon dioxide were averaged over each 30-s period and analyzed via an on-line open circuit spirometry system (Oxicon Pro; Jaeger, Wurzburg, Germany). The volume and composition of the expired gases collected were expressed in standard terms (standard temperature and pressure, dry). Heart rate was measured continuously by using a heart rate monitor (Polar Sportstester PE 3000; Electro KY, Kempele, Finland) fitted to the chest at the V5 position, and averaged over 5-s intervals.

TABLE 1
Characteristics of the men before the training program[1]

	Trained group ($n = 9$)	Trained and vitamin C–supplemented group ($n = 5$)
Age (y)	30.8 ± 5.7	28.8 ± 1.2
Height (cm)	180.0 ± 0.1	175.0 ± 0.1
Weight (kg)	75.6 ± 11.9	77.1 ± 5.4
BMI (kg/m^2)	23.3 ± 2.7	25.2 ± 1.9

[1] All values are $\bar{x}$ ± SD. No significant differences between means were found (2-tailed Student's t test).

Experimental design

Each man participated in 2 experimental tests (before training and after 8 wk of training). Men arrived at the laboratory between 1000 and 1100. They were instructed to follow their usual dietary pattern before the test and to avoid alcohol in the preceding 24 h. Men were also required to record their dietary intake for the 24 h before the test and to repeat the same diet for the second visit. All men were required to avoid any strenuous exercise for 72 h before participation in the test. After the initial $\dot{V}O_2$max test, all men underwent a program of regular exercise on a static bicycle on 3 d/wk for 8 wk. The intensity of the training increased from 65% to 80% $\dot{V}O_2$max over the course of the experiement, at a rate of a 5% increase every 2 wk. The duration of the training was 40 min.

Two blood samples were collected after an overnight fast. The samples were collected 1 d before the $\dot{V}O_2$max tests were performed. The men took their usual supplement at 0900, or 2 h before the blood draw.

Animals

Thirty-six adult (3-mo-old) male Wistar rats weighing ≈300 g were randomly divided into 6 equal groups. All animals were fed a rodent maintenance diet (Global diet 2014l; Harlan Teklad, Madison, WI).

For real-time reverse transcriptase–polymerase chain reaction (RT-PCR) experiments, 18 of the animals were divided into 3 groups (ie, untrained, trained, and trained with vitamin C supplementation) of 6 animals each. The rats in this study were trained for 3 wk. For the Western blot studies and performance experiments ($\dot{V}O_2$max and endurance capacity), the other 18 animals were also divided into the 3 groups. The rats in this study were trained for 6 wk. (The details of the training characteristics are found in the Exercise protocol section.)

The experimental protocol was approved by the Committee on Ethics in Research of the Faculty of Medicine of the University of Valencia.

Supplementation of rats with vitamin C

Vitamin C was administered orally to persons and to rats. The dose of vitamin C in the animal study was calculated by taking into account the animal's body surface area (BSA). BSA has been recommended as the main basis for drug dosage, because the rate of metabolism or redistribution of a drug is proportional to the

metabolic rate, which in turn reflects heat losses that are generally proportional to the surface area (27). The dose used for the men in the present study was 1 g/d, which is equivalent to 0.06 mg/cm^2 BSA, and which is the dose commonly used by sport practitioners. The dose administered to rats was 500 mg/kg body wt, which is equivalent to 0.24 mg/cm^2 BSA. In relation to BSA, this dose is only ≈4-fold that used in humans. We gave this high dose of vitamin C to the animals because it has proved to be very effective as an antioxidant in previous studies (4).

Ascorbic acid concentrations

A blood tube containing lithium heparin was centrifuged at 760 × *g* for 20 min at room temperature, and an aliquot of plasma (0.6 mL) was immediately added to 0.6 mL of 10% metaphosphoric acid (Sigma Chemicals Co Ltd, Poole, United Kingdom), mixed by vortex, and then immediately stored at −70 °C. Analysis using HPLC according to the method of Mohr and Stocker (28) was conducted later.

Exercise protocol

Endurance-trained animals were exercised 5 d/wk on an animal treadmill (Model 1050 LS Exer3/6; Columbus Instruments, Columbus, OH) at 75% $\dot{V}O_2$max. We followed a modification of the protocol of Davies et al (21). The first day's training session was 25 min long. The duration of each work period was increased by 5 min/d until, on the last day of week 3, rats were required to run for a full 85 min.

The group of animals trained for 6 wk were maintained at 85 min exercise/d for the final 6 wk of the study with only a modification of the running speed (30 m/min at a grade of 15%). The untrained group was exercised at the same speed for only 10 min every 3 d for the entire 3- or 6-wk period. Exercise motivation was provided for all rodents by means of an electronic shock grid at the treadmill rear. Both groups were fed an ad libitum laboratory diet. Twenty-four hours after the last training session, an endurance test was administered to each rat. Exercise endurance capacity was assessed during a run to exhaustion at 26.8 m/min at a grade of 15%. As each endurance test progressed, animals experienced increasing difficulty in matching the pace of the treadmill: This resulted in a rising frequency of landings on the electrical shock grid at a rear of the continuous belt. The endpoint for every test was marked by a rat's inability to return to the treadmill belt from the shock grid and by the rat's incapacity to right itself when placed supine on a flat surface. The time to exhaustion was recorded for each rat.

All of the animals were also given a graded intensity treadmill test to determine $\dot{V}O_2$max. After an initial 2 min at 15% grade and 26.8 m/min, treadmill speed was increased by 6.7 m/min every 2 min until the animal failed to maintain the intensity of the exercise. The maximal running speed was considered the maximal aerobic workload capacity of the animal (20).

After the tests, animals were given 48 h of complete rest before being killed for skeletal muscle recovery and analysis. During these 2 d, the animals were still supplemented with the same dose of vitamin C.

Rats were anesthetized with 50 mg sodium pentobarbital/kg by intraperitoneal injection. Blood and the soleus and gastrocnemius muscles were obtained by quick removal. The muscles were freeze-clamped immediately and stored at −80 °C until used. Rats were killed by an overdose of the anesthetic.

Immunoblot analysis

Aliquots of muscle lysate (40–60 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Proteins were then transferred to nitrocellulose membranes, which were incubated overnight at 4 °C with appropriate primary antibodies: cytochrome C, mTFA, NRF-1, PGC-1, and α-actin (Santa Cruz Biotechnology Inc, Santa Cruz, CA). Thereafter, membranes were incubated with a secondary antibody for 1 h at room temperature. Specific proteins were visualized by using the enhanced chemiluminescence procedure as specified by the manufacturer (Amersham Biosciences, Piscataway, NJ). Autoradiographic signals were assessed by using a scanning densitometer (BioRad, Hercules, CA).

Real-time reverse transcriptase–polymerase chain reaction

RNA was isolated from rat muscles by using the QuickPrep Total RNA extraction kit (Amersham Biosciences) as described by the manufacturer. Quantitative real-time RT-PCR was performed using the Tth DNA polymerase kit (Roche Diagnostics–Boehringer Mannheim, Mannheim, Germany) as described by the manufacturer. Real-time quantification of the enzymes relative to glyceraldehyde-3P-dehydrogenase mRNA was performed with a SYBR Green I assay (Invitrogen Corp, Carlsbad, CA) and evaluated using the iCycler detection system (BioRad). The threshold cycle (Ct) was determined, and the relative gene expression was expressed as follows: fold change = 2^(−ΔΔCt). The specific primers (5′ to 3′) used were CGTGCTCCCACACATCAATC and TGAACGTCACCGAGGAGAAG for manganese–superoxide dismutase (Mn-SOD); GACATCAGGAGAATGGCAAG and CATCACCAAGCCAATACCAG for glutathione peroxidase (GPx); GTATGCTAAGTGCTGATGAA and GGGTTTGGAGGGTGAGAT for NRF-1; AGTTCATACCTTCGATTTTC and TGACTTGGAGTTAGCTGC for mTFA; and CCTGGAGAAACCTGCCAAGTATG and GGTCCTCAGTGTAGCCCAAGATG for the housekeeping gene *GAPDH*.

Statistical analysis

Results are expressed as means ± SDs. Normality of distribution was checked with the Shapiro-Wilk test, and homogeneity of variance was tested by Levene's statistics. In humans (for $\dot{V}O_2$max) and in animals (for running time and $\dot{V}O_2$max), a repeated-measures 2-factor analysis of variance was performed. Repeated measures were performed for training (before training compared with after training); the second factor was the treatment (vitamin C supplementation or nonsupplementation). The main effect of training was tested with a 2-tailed Student's *t* test.

For the real time RT-PCR and the Western blot analysis, we used a one-factor analysis of variance and post hoc Bonferroni's comparisons to evaluate statistical differences. The level of statistical significance was set at $P < 0.05$. We used SPSS software (version 13.0.1; SPSS Inc, Chicago, IL) for all statistical analyses.

RESULTS

Vitamin C administration significantly increased the plasma concentrations of the antioxidant in both men and rats

We measured the plasma ascorbic acid concentrations in both animals and humans. The vitamin C–supplemented groups of

both men and rats had significantly ($P = 0.009$) higher plasma vitamin C concentrations in both experimental models. Plasma ascorbic acid concentrations increased from 43.1 ± 12.5 μmol/L to 130.6 ± 56.8 μmol/L in the group of supplemented animals ($n = 5$; $P = 0.009$).

In the unsupplemented group, we found no significant change in plasma ascorbic acid concentrations. The values after the first and second extractions were 45.3 ± 10.7 and 50.2 ± 9.2 μmol/L, respectively. We also measured ascorbic acid concentrations in blood plasma from men, which increased from 43.7 ± 13.1 μmol/L before supplementation to 166.4 ± 21.0 μmol/L after supplementation ($n = 5$, $P < 0.001$). However, we found no significant increase in the unsupplemented group. The values after the first and second extractions were 44.1 ± 10.7 and 47.8 ± 7.5 μmol/L, respectively. Although the doses in the animal and human study were different (*See* Subjects and Methods), the percentage of increase in the plasma concentrations of vitamin C in the supplemented groups and in controls did not differ significantly in either experimental model.

Vitamin C administration significantly hampers endurance capacity in rats and does not improve $\dot{V}O_2$max associated with training in rats and in humans

Training significantly ($P = 0.004$) increased the maximal running time in rats (**Table 2**), from 99.2 ± 6.6 min in untrained rats to 284.3 ± 105.9 min in trained rats. However, this increase was significantly ($P = 0.014$) prevented by daily supplementation with vitamin C. In the supplemented animals, the running time increased only 26.5%, from 101.2 ± 9.7 min to 128.0 ± 44.7 min. Although we found a dramatic effect of vitamin C on endurance time in animals, we did not find the same effect on $\dot{V}O_2$max. We performed a $\dot{V}O_2$max test before and after the training period (6 wk) and found a significant ($P = 0.05$) increase in $\dot{V}O_2$max of 17.0% after 6 wk of training in the unsupplemented group and an increase in $\dot{V}O_2$max of 4.7% in the supplemented group. The differences were not significant.

In the human study, we found an almost-identical result: $\dot{V}O_2$max increases of 22.0% in the unsupplemented group of men and 10.8% in the supplemented group after 8 wk of training. These differences were not significant, but there were data from only 5 men, and thus the study may not have been adequately powered to find a difference.

ROS formed in exercise activated the expression of antioxidant enzymes in skeletal muscle, but vitamin C administration prevents the activation

The group of animals trained for 3 wk had significantly ($P = 0.02$) higher mRNA concentrations of 2 antioxidant enzymes, Mn-SOD and GPx, in their skeletal muscle after the training. However, this increase was prevented by supplementation with vitamin C, as is shown in **Figure 1**. Thus, supplementation with an antioxidant vitamin hinders the adaptation of these enzymes to training.

ROS formed in exercise activated mitochondrial biogenesis in skeletal muscle, but vitamin C administration prevents the activation

The group of animals trained for 3 wk had significantly ($P = 0.027$) higher skeletal muscle protein concentrations of PGC-1 after training (**Figure 2**), which was followed by a subsequent increase in the mRNA concentrations of NRF-1 and mTFA. Supplementation with vitamin C prevented all of these effects (**Figure 3**A). The changes in mRNA were followed by changes in the protein concentrations of these nuclear transcription factors that were evident in the group of rats trained for 6 wk. NRF-1

TABLE 2
Training-induced increases in maximum oxygen uptake ($\dot{V}O_2$max) in men and in $\dot{V}O_2$max and maximal endurance time in rats and the effect of vitamin C administration[1]

	n	Before training	After training	Absolute difference	Relative difference	*P*[2]
					%	
$\dot{V}O_2$max						
Men (mL · kg^{-1} · min^{-1})						
Not supplemented	9	38.2 ± 1.1[3]	46.6 ± 4.1	8.2 ± 2.9	22.0	NS
Vitamin C–supplemented	5	41.2 ± 5.1	45.6 ± 7.0	4.4 ± 4.2	10.8	
P for effect of training[4]			0.019			
Animals (m · min^{-1})						
Not supplemented	6	54.4 ± 4.5	63.7 ± 9.6	9.3 ± 6.9	17.0	NS
Vitamin C–supplemented	6	56.3 ± 9.0	58.9 ± 9.0	2.7 ± 2.8	4.7	NS
P for effect of training			0.005			
Endurance capacity						
Animals (min)						
Not supplemented	6	99.2 ± 6.6	284.3 ± 105.9	185.2 ± 107.1	186.7	0.014
Vitamin C–supplemented	6	101.2 ± 9.7	128.0 ± 44.7	26.8 ± 47.2	26.5	
P for effect of training			0.004			

[1] Human study: $\dot{V}O_2$max improvement after 8 wk of training in sedentary men; effect of vitamin C administration in trained ($n = 9$) and in trained and vitamin C–supplemented ($n = 5$) men. Rat study: $\dot{V}O_2$max and maximal endurance time improvement for endurance-trained animals in 6 wk; effect of vitamin C administration in trained ($n = 6$) and trained and vitamin C–supplemented ($n = 6$) rats. In both studies, differences were checked for statistical significance by a repeated-measures 2-factor ANOVA.

[2] Training × treatment interaction.

[3] $\bar{x}$ ± SD (all such values).

[4] Before training compared with after training.

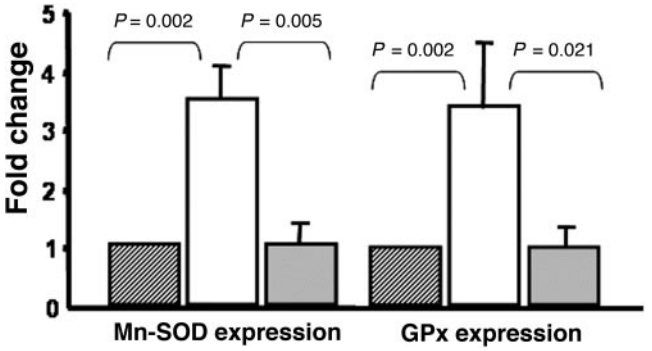

FIGURE 1. Mean (±SD) gene (mRNA) expression of manganese-superoxide dismutase (Mn-SOD) and glutathione peroxidase (GPx), measured by real-time reverse transcriptase–polymerase chain reaction in skeletal muscle samples of untrained rats (▨; $n = 6$), rats trained for 3 wk (□; $n = 6$), and rats trained for 3 wk but treated with vitamin C (▩; $n = 6$). Training induced up-regulation of Mn-SOD and GPx is prevented by vitamin C administration. Threshold cycles (Ct) were analyzed by the 2^(−ΔΔCt) method. Fold change is expressed in relation to the control group (untrained). A one-factor ANOVA and post hoc Bonferroni's comparisons were used to identify significant differences.

and mTFA protein concentrations were significantly ($P = 0.048$) higher in trained than in untrained animals (Figure 3B). However, in animals treated with vitamin C, training did not result in any significant change in the concentrations of these transcription factors. The early molecular events initiated after 3 wk of training were sustained when the training period was extended.

Vitamin C administration during training decreases the protein concentrations of markers of mitochondrial content

Mitochondrial content can be estimated by the change in the content of a typical marker protein, such as cytochrome C (20, 22). Cytochrome C protein concentrations are significantly ($P = 0.40$) higher in trained than in untrained animals (**Figure 4**). However, in animals treated with vitamin C, training did not result in any significant change in the concentration of cytochrome C.

DISCUSSION

Vitamin C modulates endurance capacity but not maximal oxygen uptake after training

The maximal rate of oxygen consumption ($\dot{V}O_2$max) increased significantly after 8 wk of training in both the nonsupplemented men (22.0% increase) and the men supplemented with vitamin C (10.8% increase). In 1999, Nielsen et al (29) found no effect of antioxidant supplementation on $\dot{V}O_2$max in triathletes. We found a very similar result in our animal study—ie, a significant increase in $\dot{V}O_2$max after 6 wk of training in both the nonsupplemented (17.0% increase) and the vitamin C–supplemented (4.7% increase) groups. Endurance capacity is dependent mainly on the mitochondrial content of skeletal muscle (muscle oxidative capacity), not on the cardiovascular factors previously mentioned (20). For obvious ethical reasons, we could not perform an endurance laboratory test in our volunteers. Thus, to determine the effect of the antioxidant administration and exercise in the mitochondrial muscle content, we performed another series of experiments in rats. We divided our animals into 2 training groups: endurance-trained for 3 wk and endurance-trained for 6 wk. Six weeks is approximately the period required to achieve a new steady state mitochondrial content in response to endurance training (15), although changes in mitochondrial protein and mRNA content can be apparent at much earlier time points (22). In our study, endurance-trained rats showed a clear increase (186.7%) in their endurance capacity. However, the administration of vitamin C dramatically decreased this adaptation to only 26.5%. This finding is in keeping with a previous study in which it was shown, using endurance-trained rats, that $\dot{V}O_2$max increased only 14% despite a 100% increase in muscle oxidative capacity (21). One of the main conclusions from that study was that the mitochondrial content of muscle is a major

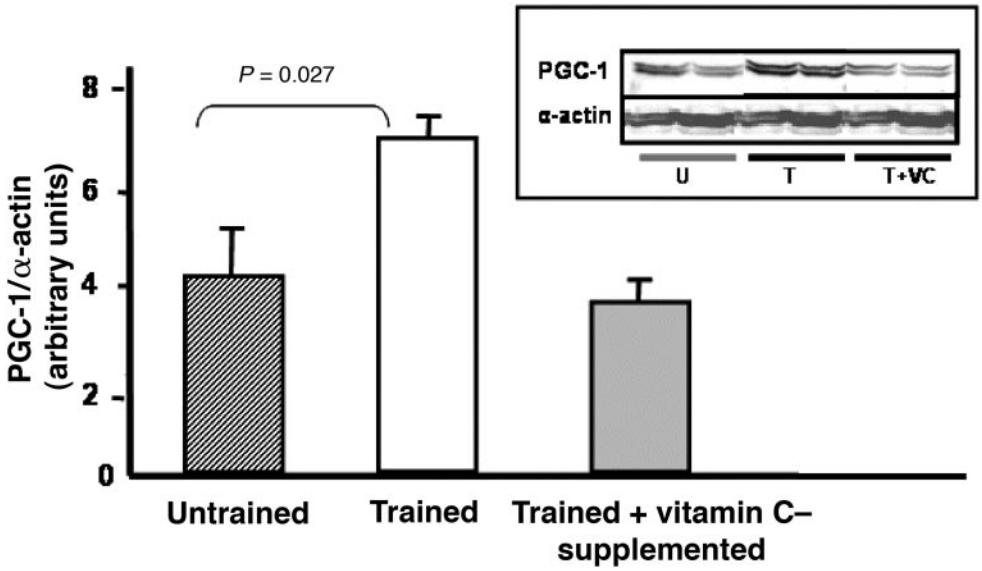

FIGURE 2. Mean (±SD) results of Western blot analysis of peroxisome proliferator–activated receptor co-activator 1 (PGC-1) in the cytosolic fraction of skeletal muscle in untrained rats ($n = 6$), rats trained for 3 wk ($n = 6$), and rats trained for 3 wk but treated with vitamin C ($n = 6$). Training induces the expression of PGC-1, but vitamin C administration prevents it. A one-factor ANOVA and post hoc Bonferroni's comparisons were used to identify significant differences.

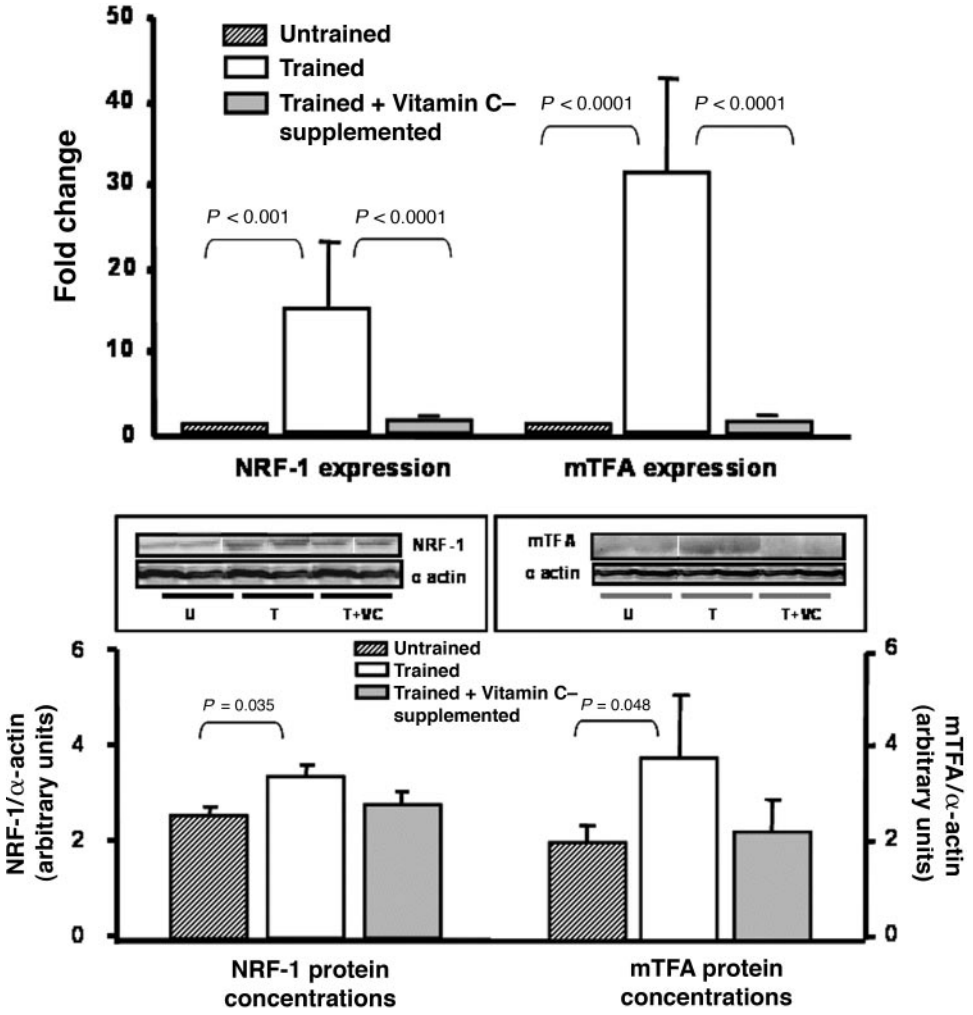

FIGURE 3. Training-induced up-regulation of nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (mTFA) is prevented by vitamin C administration. *A*: Mean (±SD) gene (mRNA) expression of NRF-1 and mTFA, measured by real-time reverse transcriptase–polymerase chain reaction in skeletal muscle samples of untrained rats ($n = 6$), rats trained for 3 wk ($n = 6$), and rats trained for 3 wk but treated with vitamin C ($n = 6$). Threshold cycles (Ct) were analyzed by the 2^(−ΔΔCt) method. Fold change is expressed in relation to the untrained (control) group. A one-factor ANOVA and post hoc Bonferroni's comparisons were used to identify statistical differences. *B*: Mean (±SD) results of Western blot analysis of NRF-1 and mTFA in the cytosolic fraction of skeletal muscle in untrained rats ($n = 6$), rats trained for 6 wk ($n = 6$), and rats trained for 6 wk but treated with vitamin C ($n = 6$). A one-factor ANOVA and post hoc Bonferroni's comparisons were used to identify significant differences.

determinant of endurance capacity, whereas the maximal aerobic workload capacity appears to be regulated by $\dot{V}O_2$max (21). We offer a molecular explanation for this result (ie, that vitamin C decreases exercise-induced mitochondrial biogenesis and the antioxidant capacity in skeletal muscle). We have found that exercise training up-regulates the following mitochondriogenic pathway: PGC-1→ NRF-1→ mTFA→ cytochrome C. All of these adaptations are prevented by vitamin C administration.

When supplementing with vitamin C, there is the possibility that it may act as a prooxidant in vivo. These prooxidative reactions of vitamin C readily occur in vitro, and it has been shown that they also may have relevance in vivo (30). A high intake of iron along with ascorbic acid could increase in vivo lipid peroxidation of LDL and therefore could increase the risk of atherosclerosis (31). However, another study showed that, in iron-overloaded plasma, ascorbic acid acts as an antioxidant and prevents oxidative damage to lipids in vivo (32). In the present study, we measured different variables of oxidative stress, eg, blood glutathione oxidation and plasma malondialdehyde, in rats and men (data not shown); we did not find an indication of an in vivo prooxidant effect of vitamin C in any of the experimental groups.

Free radicals as signals in muscle cell metabolism: potential interference by antioxidant vitamins

It is important to consider that free radicals are not always damaging to cells; in many cases, they serve as signals to adapt muscle cells to exercise via modulation of gene expression (9, 33). We have found that training causes an increase in 2 major antioxidant enzymes (Mn-SOD and GPx) in skeletal muscle. We were surprised to see that vitamin C prevents these beneficial effects of training. On the basis of the paradigm that enzymatic antioxidant systems such as Mn-SOD and GPx provide a first-line defense against ROS, it is expected that exercise may induce these protective mechanisms. Moderate exercise increases life span and decreases disability in rats (12) and humans (15). We report here that exercise training causes an increase in the

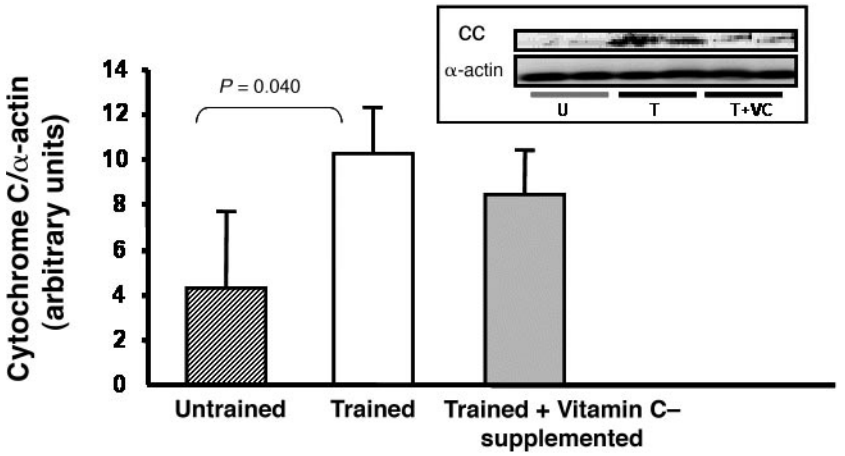

FIGURE 4. Western blot analysis of cytochrome C (CC) in the cytosolic fraction of rat skeletal muscle in untrained rats ($n = 6$), rats trained for 6 wk ($n = 6$), and rats trained for 6 wk but treated with vitamin C ($n = 6$). Training induces the expression of CC, but the administration of vitamin C prevents it. A one-factor ANOVA and post hoc Bonferroni's comparisons were used to identify significant differences.

expression of antioxidant enzymes, which is prevented by the administration of vitamin C.

Moderate exercise as an antioxidant

A major conclusion that can be drawn from our experiments is that exercise itself is an antioxidant, because training increases the expression of 2 antioxidant enzymes related with longevity—namely, SOD and GPx. We provide evidence that the continuous presence of small stimuli, such as low concentrations of ROS, in fact induces the expression of antioxidant enzymes as a defense mechanism. Low concentrations of radicals may be considered to be beneficial, because they act as signals to enhance defenses, rather than being deleterious, as they can be when they are at higher concentrations.

Antioxidant vitamins impair training efficiency

The second major conclusion that can be drawn from our experiments is that supplementation with vitamin C lowers training efficiency. Endurance capacity is directly related to the mitochondrial content. This variable is seriously hampered by antioxidant supplementation, whereas $\dot{V}O_2$max, which is dependent also on the cardiovascular system adaptations, is not significantly affected. This information is helpful for nutritionists who must prepare diets for athletes whose performance is dependent on their endurance capacity. It should be taken into account that some of the world's best marathon runners exhibit rather modest measures of $\dot{V}O_2$max (34). Antioxidant supplementation is very popular among athletes, but data showing any beneficial effects on muscle function of this type of widespread practice are elusive. In fact, several reports have shown deleterious effects of antioxidant treatment. As early as 1971, it was shown that vitamin E supplementation (400 IU/d for 6 wk) caused unfavorable effects on endurance performance (35). In 1996 and 1997, a Scandinavian journal published 2 reports showing the deleterious effects of ubiquinone-10 supplementation on the performance of humans after a high-intensity training program (36, 37). In 2001, Coombes et al (38) reported that, in the muscles of unfatigued rats, supplementation with vitamin E and α-lipoic acid depressed muscle tetanic force at low stimulation frequencies. One year later, it was shown that supplementation of racing greyhounds with 1 g vitamin C/d for 4 wk significantly slowed their speed (39). Taking into account that a high fitness level is associated with a lower risk of premature death from any cause, the effect of vitamin C administration on endurance capacity has important implication for nutritionists, physicians, and exercise trainers and practitioners. Thus, the common practice of taking vitamin C supplements during training (for both health-related and performance-related physical fitness) should be seriously questioned.

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