Oral vitamin C administration Decreases Training Efficiency and Muscle Mitochondrial Biogenesis in Rats

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ABSTRACT

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. The present study was designed to study the effect of vitamin C on training efficiency in rats. Thirty male Albino rats were exercised under protocol for 6 weeks. Ten of the rats were treated with a daily dose of 500 mg/kg body weight of vitamin C (0.24 mg/cm² body surface area). The administration of vitamin C 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 nuclear respiratory factor 1 and mitochondrial transcription factor A. Vitamin C also prevented the exercise-induced expression of the antioxidant enzymes superoxide dismutase and glutathione peroxidase. The study showed that Vitamin C supplementation decreases training efficiency because it prevents some cellular adaptations to exercise.

INTRODUCTION

Acute physical exercise induces augmented generation of reactive oxygen species (ROS) in muscle and in other organs(1-3). It has been generally accepted over the past 20 years 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 could 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 VO$_2$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 a stronger predictor of mortality than are other established risk factors, such as diabetes, smoking, hypertension, or chronic obstructive pulmonary disease$^{(15,16,17,18)}$. 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 VO$_2$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 (i.e. the mitochondrial content of muscle) was a major determinant of endurance capacity, whereas VO$_2$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 co-activator of nuclear receptors. It powerfully induces mRNA expression for important nuclear transcription factors such as nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (mTFA)$^{(22)}$.

The aim of the present study was to explore the effects of vitamin C administration on training-induced increases in VO$_2$max and endurance capacity and on the skeletal muscle mitochondrial biogenesis in rats.

**SUBJECTS & METHODS**

**Animals:**
Thirty healthy adult male Albino rats weighing 180-220 grams obtained from the animal house of Faculty of Science, Minia University. They were kept in galvanized iron cages under standardized conditions to achieve acclimatization. All animals were fed a rodent maintenance diet and given free water ad-libitum. They were randomly divided into 3 equal groups.

- Group 1: untrained (10 rats).
- Group 2: trained (10 rats).
- Group 3: trained with vitamin C supplementation (10 rats).

The rats in the study were trained for 6 weeks. The study was performed in accordance with the guidelines for the care and use of laboratory animal$^{(23)}$.

**Supplementation of rats with vitamin C:**
Vitamin C was administered orally to rats. The dose administered to rats was 500 mg/kg body wt, which is equivalent to 0.24 mg/cm$^2$ body surface area$^{(24)}$. That dose is 4-fold that in humans. That high dose of vitamin C was given to the animals because it has proved to be very effective as an antioxidant in previous studies$^{(4,25)}$.

**Exercise protocol:**
Endurance-trained animals were exercised 5 d/week on an animal treadmill (Model 1050 LS Exer3/6;
Columbus Instruments, Columbus, OH) at 75% \( \text{VO}_2 \text{max} \). A modification of the protocol of Davies et al.,(21) was followed. The first day’s training session was 25 minutes long. The duration of each work period was increased by 5 minute/day until, on the last day of the 3rd week, rats were required to run for a full 85 minutes. The group of animals trained for 6 weeks were maintained at 85 minutes exercise/day for the final 6 weeks of the study with only a modification of the running speed (30 m /minutes at a grade of 15%). The untrained group was exercised at the same speed for only 10 minutes every 3 days for the entire 6-weeks period. Exercise motivation was provided for all rats by means of an electronic shock grid at the treadmill rear. 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/minutes at a grade of 15%. As each endurance test progressed, animals experienced increasing difficulty in matching the pace of the treadmill. 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 \( \text{VO}_2 \text{max} \). After an initial 2 minutes at 15% grade and 26.8 m/minutes, treadmill speed was increased by 6.7 m/min every 2 minutes 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 hours of complete rest before being killed for skeletal muscle recovery and analysis. During these 2 days, the animals were still supplemented with the same dose of vitamin C. Rats were anesthetized with 50 mg sodium pentobarbital/kg by intraperitoneal injection. Sufficient blood samples for biochemical analysis were taken from the venous plexus localized in the orbit behind the eye ball using capillary pipette. Rats were killed by an overdose of the anesthetic. The soleus and gastrocnemius muscles were obtained by quick removal. The muscles were freeze-clamped immediately and stored at -80 °C until used.

RT–polymerase chain reaction:
RNA was isolated from rat muscles by using Trizol RNA extraction (Life Technologies, Rockville, Md). Reverse transcriptase and the first PCR were combined and the second PCR was done using the following specific primers which are CGTGCTCCCACACATCAATC and TGAACGTCACCCGAGGAGAAG for manganese superoxide dismutase (Mn-SOD); GACATCAGGAGAATGGC and CATCACCAAGCC AATACCAG for glutathione peroxidase (GPx); GTATGCTAAGTGGCTGATGAA and GG GTTTGGAGGGTGAGAAG for NRF-1 and AGTTCATACCTTCGATTTTC and TGACTTG AGTTAG CTGC for mTFA yielding products under the following conditions: one cycle of 5 min at 94°C, 30 cycles of 30 s at 94°C and 40 s at 72 °C and final extension
of 7 min at 72°C using DNA thermal cycler 480 (Perkin Elmer, Cetus) for 30 minutes.

Gel electrophoresis detection: five micro liters buffer was added to 20µl of the PCR product. The amplified gene was analyzed by electrophoresis in 1.2 % agarose gel using standard method. The Ethidium bromide stained gel was examined under ultraviolet light. The size of the bands was assessed by direct comparison with standard molecular weight markers (26).

Statistical Analysis:
Results were expressed as mean ± standard deviation (S.D.). Students's t-test was used. P value of less than 0.05 was considered significant.

RESULTS

Vitamin C administration significantly hampers endurance capacity and does not improve VO\textsubscript{2}max associated with training in rats:
Training significantly ($P<0.004$) increased the maximal running time in rats (Table 1), from 98.3±6.1 minutes in untrained rats to 279.3±14.4 minutes in trained rats. However, that increase was significantly ($P<0.004$) prevented by daily supplementation with vitamin C. In the supplemented animals, the running time increased only 15.5%, from 103.1±5.4 minutes to 118.6±10.1 minutes. Although we found a dramatic effect of vitamin C on endurance time in animals, we did not find the same effect on VO\textsubscript{2}max. We performed a VO\textsubscript{2}max test before and after the training period (6 weeks) and found a significant ($P<0.005$) increase in VO\textsubscript{2}max of 33.6% after 6 weeks of training in the un-supplemented and an increase in VO\textsubscript{2}max of 3 % in the supplemented group.

Table (1): Training-induced increases in maximum oxygen uptake in VO\textsubscript{2}max and maximal endurance time in rats and the effect of vitamin C administration

<table>
<thead>
<tr>
<th></th>
<th>Before training (n=10)</th>
<th>After training (n=10)</th>
<th>Relative difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VO\textsubscript{2}max</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (untrained)</td>
<td>49.3±1.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No supplementation</td>
<td>55.3±3.1</td>
<td>88.9±6.1**</td>
<td>33.6</td>
</tr>
<tr>
<td>Vitamin C supplementation</td>
<td>56.3±2.2</td>
<td>59.3±1.6</td>
<td>3</td>
</tr>
<tr>
<td><strong>Endurance capacity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (untrained)</td>
<td>49.3±1.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No supplementation</td>
<td>98.2±6.1</td>
<td>279.4±14.4*</td>
<td>181.2</td>
</tr>
<tr>
<td>Vitamin C supplementation</td>
<td>103.1±5.4</td>
<td>118.6±10.1*</td>
<td>15.5</td>
</tr>
</tbody>
</table>

* $P<0.004$  ** $P< 0.005$
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 6 weeks had higher mRNA concentrations of 2 antioxidant enzymes, Mn-SOD and GPx, in their skeletal muscle after the training. However, that increase was prevented by supplementation with vitamin C, as shown in Figure 1. Thus, supplementation with an antioxidant vitamin hinders the adaptation of these enzymes to training.

![Figure 1: 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, rats trained for 6 weeks and rats trained for 6 weeks but treated with vitamin C.](image1)

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

The group of animals trained for 6 weeks had higher skeletal muscle mRNA concentrations of NRF-1 and mTFA which were followed by changes in the protein concentrations of these nuclear transcription factors. Supplementation with vitamin C prevented all of these effects (Figure 2).

![Figure 2: Training-induced up-regulation of nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (mTFA) is prevented by vitamin C administration.](image2)
DISCUSSION

The maximal rate of oxygen consumption (VO\textsubscript{2max}) increased significantly after 8 weeks of training in both the non-supplemented men and the men supplemented with vitamin C\textsuperscript{(20)} \textsuperscript{1}. Nielsen et al.\textsuperscript{(27)} found no effect of antioxidant supplementation on VO\textsubscript{2max} in athletes, a similar result was found in our animal study. A significant increase in VO\textsubscript{2max} after 6 weeks of training in both the non-supplemented (33.6 % increase) and the vitamin C–supplemented (3 % increase) groups was found. Endurance capacity is dependent mainly on the mitochondrial content of skeletal muscle (muscle oxidative capacity), not on the cardiovascular factors previously mentioned\textsuperscript{(20)} \textsuperscript{1}. Thus, to determine the effect of the antioxidant administration and exercise in the mitochondrial muscle content, we performed another series of experiments in rats. Six weeks of training is approximately the period required to achieve a new steady state mitochondrial content in response to endurance training\textsuperscript{(15)}, although changes in mitochondrial protein and mRNA content can be apparent at much earlier time points\textsuperscript{(22)} \textsuperscript{1}. In the current study, endurance-trained rats showed a clear increase in their endurance capacity. However, the administration of vitamin C dramatically decreased that adaptation. Such finding is in agreement with a previous study in which it was shown, using endurance-trained rats, that VO\textsubscript{2max} increased only 14% despite a 100% increase in muscle oxidative capacity\textsuperscript{(21)}. One of the main conclusions from that study was that the mitochondrial content of muscle is a major determinant of endurance capacity, whereas the maximal aerobic workload capacity appears to be regulated by VO\textsubscript{2max}\textsuperscript{(21)}. We offer a molecular explanation for that result (i.e. 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 mitochondrial pathway: NRF-1 and mTFA. 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 pro-oxidant in vivo. These pro-oxidative reactions of vitamin C readily occur in vitro, and it has been shown that they also may have relevance in vivo\textsuperscript{(28)}. A high intake of iron along with ascorbic acid could increase in vivo lipid per-oxidation of LDL and therefore, could increase the risk of atherosclerosis\textsuperscript{(29)}. However, another study showed that, in iron overloaded plasma, ascorbic acid acts as an antioxidant and prevents oxidative damage to lipids in vivo\textsuperscript{(30)}. 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\textsuperscript{(9,31)}. 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(13). We report here that exercise training causes an increase in the expression of antioxidant enzymes, which is prevented by the administration of vitamin C.

A major conclusion that can be drawn from our experiment 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. It is known that exercise can modify the rates of several of steps, leading to mitochondrial biogenesis. An understanding of how exercise can produce that effect could help us decide whether exercise is beneficial for patients suffering from mitochondrial disorders, as well as a variety of metabolic diseases(32).

The second major conclusion that can be drawn from our experiment is that supplementation with vitamin C lowers training efficiency. Endurance capacity is directly related to the mitochondrial content. That variable is seriously hampered by antioxidant supplementation, whereas VO_{2max}, which is dependent also on the cardiovascular system adaptations, is not significantly affected. Such 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 VO_{2max}(33).

Antioxidant supplementation is very popular among athletes, but data showing any beneficial effects on muscle function of that 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 weeks) caused unfavorable effects on endurance performance(34). 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(35,36). Coombes et al.(37) reported that, in the muscles of un-fatigued rats, supplementation with vitamin E and lipoic acid depressed muscle tetanic force at low stimulation frequencies. One year later, it was shown that supplementation of racing greyhounds with one g vitamin C/day for 4 weeks significantly lowered their speed(38). Intense exercise increased lipid peroxidation, decreased Bcl-2 expression and induced an antioxidant response in lymphocytes. Supplementation with moderate levels of antioxidant vitamins reduced exercise-induced oxidative damage, but without blocking the cellular...
adaptation to exercise\(^{(39)}\). Exercise-induced oxidative stress ameliorates insulin resistance and causes an adaptive response promoting endogenous antioxidant defense capacity. Supplementation with antioxidants may preclude these health-promoting effects of exercise in human\(^{(40)}\). 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, 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. The results of the present study show that supplementation with vitamin C does not improve but partially decreases the improvement in VO\(_{2}\)\(_{\text{max}}\) associated with exercise training in rats. Moreover, it very significantly hampers endurance capacity in animal as a result of the decrease in mitochondrialgenesis that is normally associated with exercise training.

**REFERENCES**


تناول فيتامين ج بالغ يقلل الكفاءة التدريبية والحوية الجينية الميتوكوندريدية للعضلات في الفئران

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غالباً ما يتناول ممارسو الرياضة فيتامين ج أثناء التمرين لأن النشاط العضلي القوي ينتج عنه عملية كبدية
والتي ينتج عنها تغير تركيز الجلوكاتونين وكذلك زيادة في البروتينات والحمض النووي والدهون نتيجة للأكسدة
في العضلات والدم. ولكن هناك شك في النتائج الصحية من تناول فيتامين ج الإضافي أثناء ممارسة الرياضة.
لهذا قمنا بإجراء دراسة تأثير فيتامين ج على الكفاءة الترموينية في الفئران.
تم أخذ 30 فئران سليم وقسمتهم إلى 3 مجموعات كل مجموعة تتكون من 10 فئران. المجموعة
الأولى لم ت تعرض إلى أي تمارين، أما المجموعة الثانية فقد تعرضت تحت بروتوكل تمرين تحمل لمدة 6
أسابيع كما تم إضافة فيتامين ج للمجموعة الثالثة أثناء أداء التمارين.
وجد أن تناول فيتامين ج أثناء التمرين قد أدى إلى تقليل الحمل الترمويني في المجموعة الثالثة.
كذلك وجد أن التمرين قد أدى إلى رفع الجري الأقصى في الفئران بدرجة ذات دلالة إحصائية من
3.3±6.1 في المجموعة التي لم تعرض للتمرين إلى 27.9±4.2 في الفئران التي
ادت التمارين.
ولكن هذه الزيادة قد قلت بدرجة ذات دلالة إحصائية في المجموعة الثالثة التي تناولت فيتامين ج كما أدى
فيتامين ج إلى تأثير سبي على حدة التحمل في الفئران.
وهذا التأثير الغير متوقع لفيتامين ج ربما نتج من قدرته على تقليل إنتاج عوامل النسيج الذي تفرز أثناء
التمرين. وأيضاً قد تؤثر على منع إنتاج بعض الأحماض المشعة الإكسدة وهي سوبر أكسيد ديمتريوتنز
والمجولاتونين و بير أوكسيدن. إذا تم استخدام طريقة التفاعل المباشر الكامل لدراسات إنتاج ازيم
Mn-SOD، NFR-I & mTFA في الفئران في الثلاث مجموعات، وكذلك دراسات إنتاج GPx،
و من نتائج البحث وجد أن تناول فيتامين ج قد أدى إلى تقليل إنتاج كل من الأحماض المتعال والحمض النسيج في
المجموعة الثالثة.
وإذا فإن هذه الدراسة قد أوضح أن تناول فيتامين ج قد أدى إلى تقليل الكفاءة الترموينية عن طريق منع
بعض الكيفات التي تحدث داخل الخلايا أثناء التمرين.