Influence of vitamin C moderate dose supplementation on exercise-induced lipid peroxidation, muscle damage and inflammation

Effetti di una moderata integrazione di vitamina C su perossidazione lipidica, infiammazione e danno muscolare indotti dall’esercizio fisico

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SUMMARY

Aim. The purpose of this study was to evaluate the effect of moderate dose vitamin C supplementation on exercise-induced lipid peroxidation, muscle damage and inflammation.

Methods. Sixteen healthy untrained male individuals participated in a 30-min exercise at 75% VO2max. Subjects were randomly assigned to one of two groups: 1) placebo (P) and 2) vitamin C (VC: 500 mg vitamin C). Blood samples were obtained prior to supplementation (baseline), 2 h after supplementation (immediately pre-exercise), immediately, 2 and 24 h after exercise. Plasma levels of vitamin C, total antioxidant capacity (TAC), creatine kinase (CK), malondialdehyde (MDA), total leukocytes, neutrophils, lymphocytes, interleukin-6 (IL-6), and CRP were measured.

Results. With supplementation, plasma vitamin C concentration increased significantly only in the VC group (P<0.05). TAC decreased significantly just in P group, 2 and 24 h after exercise (P<0.05). Although MDA levels were similar between groups at the baseline, only in the P group it increased significantly after exercise (P<0.05). CK increased immediately and 2 h after exercise in both groups and 24 h after exercise just in placebo group compared with pre-exercise (P<0.05). Markers of inflammation (total leukocytes, neutrophils, CRP and IL-6) increased significantly in response to the exercise in both groups (P<0.05).

Conclusion. In conclusion, it seems that vitamin C acute moderate dose supplementation affects exercise-induced lipid peroxidation and muscle damage, but not inflammatory markers.

Key words: Dietary supplements - Exercise - Ascorbic acid - Inflammation.

RIASSUNTO

Obiettivo. Obiettivo del presente studio è stato quello di esaminare l’effetto di una moderata integrazione di vitamina C sulla perossidazione lipidica, sull’infiammazione e sul danno muscolare indotti dall’esercizio fisico.

Metodi. Sessanta individui di sesso maschile non allenati hanno eseguito un esercizio di 30 minuti al 75 % del VO2max. I soggetti sono stati assegnati in maniera casuale in uno di due gruppi: 1) placebo (P) e 2) vitamina C (VC: 500 mg di vitamina C). I campioni ematici sono stati raccolti prima della somministrazione dell’integratore (baseline), 2 ore dopo (immediatamente prima dell’esercizio), immediatamente, 2 e 24 ore dopo l’esercizio. Sono stati poi misurati i livelli plasmatici di vitamina C, capacità antiossidante totale (TAC), creatinchinasi (CK), malondialdeide (MDA), leucociti, neutrofili e linfociti totali, interleuchina-6 (IL-6), e PCR.

Risultati. Con l’integrazione, la concentrazione plasmatica di vitamina C è aumentata in maniera significativa solo nel gruppo VC (P<0.05). La TAC è diminuita in maniera significativa solo nel gruppo P, 2 e 24 ore dopo l’esercizio (P<0.05). Sebbene i livelli basali di MDA fossero simili tra i gruppi, l’IMDA è aumentata in maniera significativa solo nel
Physical exercise may increase accumulation of free radicals and induce oxidative stress as a response to the increased oxygen consumption. Free radicals or, more generally, reactive oxygen/nitrogen species (RONS) are products of normal cellular metabolism. RONS are well known for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. The excess RONS can damage cellular lipids, proteins, or DNA, impairing their normal function. Evidence for increased RONS production during and following exercise is provided by numerous investigations noting an increase in various oxidative stress biomarkers following both acute aerobic and anaerobic exercise. It was claimed that adaptations to oxidative stress mediated by free radicals play an important role in maintaining cell viability in tissues routinely subjected to repeated stresses (e.g., muscle following regular exercise, etc.). In fact, some investigators have found that regular exercise could also exert protective and beneficial effect on oxidative stress related disease. Nevertheless, there is an agreement that acute and intense bout of exercise may induce malicious increase in RONS which may lead to cell and organ damage in lack of sufficient defensive antioxidative system. Therefore, some investigators believe that supplementation with antioxidants may be one intervention to reduce oxidative stress in such conditions.

Interkeukine (IL-6) is one of the cytokines, most often classified as an inflammatory cytokine. IL-6 production is modulated by carbohydrate availability in muscles, which functions as an energy sensor. It is released from contracting muscles in large amounts and exerts its endocrine effect on adipose tissue, inducing lipolysis and gene transcription in abdominal subcutaneous fat. IL-6 also known as exercise factor and belongs to myokine family. In addition, immune cells are mobilized and activated during exercise in response to muscle damage and also via the actions of stress hormones (catecholamines, glucocorticoids, CRF) and cytokines (IL-6, TNF-α).

The exercise-induced oxidative stress is mediated by reactive oxygen/nitrogen species (RONS), are products of normal cellular metabolism. RONS are well known for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. The excess RONS can damage cellular lipids, proteins, or DNA, impairing their normal function. Evidence for increased RONS production during and following exercise is provided by numerous investigations noting an increase in various oxidative stress biomarkers following both acute aerobic and anaerobic exercise. It was claimed that adaptations to oxidative stress mediated by free radicals play an important role in maintaining cell viability in tissues routinely subjected to repeated stresses (e.g., muscle following regular exercise, etc.). In fact, some investigators have found that regular exercise could also exert protective and beneficial effect on oxidative stress related disease.

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echolamines, growth hormone, cortisol) which are released in response to increased metabolic demands and core temperature during exercise. Exercise-induced oxidative stress is another factor that affects cytokine production. Oxidative stress may result from oxidative reactions within skeletal muscle as well as from muscle damage. During exercise, endogenous antioxidant enzymes and dietary antioxidant supplements can potentially attenuate cytokine production by directly neutralizing RONS and/or inhibiting the activity of redox-sensitive signal transduction pathways.

The available evidence suggests that the ingestion of large amounts of vitamin C offers some protection against lipid peroxidation. As vitamin C is water-soluble, availability may be increased with a single dose, and there may be no need for prolonged supplementation. In our previous study, an administration of a single high dose (1000 mg) vitamin C supplementation 2 h before one bout of exercise could influence lipid peroxidation and muscle damage. However, high dose supplementation of vitamin C has some side effects such as iron poisoning and kidney stones. Furthermore, moderate single dose supplementation may not interfere with necessary adaptive processes.

The purpose of the present study was to determine whether exercise-induced lipid peroxidation and inflammation could be affected by single moderate dose (500 mg) vitamin C supplementation in untrained men participating in a 30-min running at 75% VO_{2max}.

Materials and methods

Subjects and supplementation

This was a double-blind, placebo-controlled study. Sixteen healthy, non-smoking, untrained young men were recruited for participation in this study. All subjects who took part in this study had approval from Guilan university Advisory Committee. Study was based on the Declaration of Helsinki. All participants were informed verbally and in writing about the nature and demands of study, and subsequently completed a health history questionnaire and gave their written informed consent. Subjects were free of vitamin/mineral supplementation for six weeks prior to the study. The subjects were randomly and evenly divided into a supplemented (VC) group and a placebo (P) group. Physical inmunitarie are mobilized and attivad during the esercizio fisico, in risposta al danno muscolare e mediante le azioni degli ormoni dello stress (catecolamine, ormoni della crescita, cortisolo), i quali sono rilasciati in risposta a un aumento del fabbisogno metabolico e della temperatura basale corporea durante l’esercizio fisico. Lo stress ossidativo indotto dall’esercizio fisico è un altro fattore che si ripercuote sulla produzione di citochine. Lo stress ossidativo può derivare da reazioni ossidative nei muscoli scheletrici, oltre che dal danno muscolare. Durante l’esercizio fisico, gli eccessi antiossidanti endogeni e gli integratori alimentari di antiossidanti possono potenzialmente attenuare la produzione di citochine, neutralizzando direttamente le RONS e/o inibendo l’attività dei pathway di trasduzione del segnale redox-sensitive.

L’evidenza disponibile suggerisce che l’assunzione di grandi quantità di vitamina C offra una certa protezione contro la perossidazione lipidica. Poiché la vitamina C è idrosolubile, la sua disponibilità può essere aumentata con una dose singola e potrebbe non essere necessario un regime di integrazione prolungato nel tempo. Nel nostro precedente studio, la somministrazione di una singola dose elevata (1000 mg) di vitamina C due ore prima di una sessione di allenamento era in grado di influenzare la perossidazione lipidica e il danno muscolare. Tuttavia, un’elevata dose di vitamina C ha alcuni effetti collaterali come l’avvelenamento da ferro e i calcoli renali. Inoltre, una moderata integrazione a dose singola potrebbe non interferire con i necessari processi adattativi.

Obiettivo del presente studio è stato quello di determinare se l’inflammazione e la perossidazione lipidica indotte dall’esercizio fisico possono essere influenzate da una moderata integrazione di vitamina C a dose singola (500 mg) in uomini non allenati partecipanti a una corsa da 30 minuti al 75% del VO_{2max}.

Materiali e metodi

Soggetti e integrazione

Si è trattato di uno studio in doppio cieco, controllato con placebo. Sessanta uomini giovani, sani e non allenati, hanno preso parte allo studio. Tutti i soggetti che hanno preso parte allo studio hanno ricevuto l’approvazione da parte del Comitato consultivo dell’Università di Guilan. Lo studio è stato condotto in accordo ai principi della dichiarazione di Helsinki. Tutti i partecipanti sono stati informati a voce e per iscritto.
characteristics and skinfold measurement taken from four sites (biceps, triceps, subscapular and suprailliac) were similar in both groups (Table I). The supplementation consisted of capsule containing 500 mg of vitamin C, whereas the placebo consisted of similar capsule containing 500 mg of lactose. Capsule was administered orally 2 h before exercise.

Preliminary measurements

The Bruce protocol was used for the VO2max test. Subjects ran on a treadmill beginning at a moderate pace; every three-min the grade and intensity was increased until exhaustion. This was performed at least two weeks before the main trial.22

Experimental design and procedures

On the day of the test, subjects arrived at the laboratory after an overnight fast of at least 10 h. A venous blood sample was taken after subjects had been standing for at least 15-min, after which subjects consumed a light standardized meal (two boiled eggs) and a tablet of vitamin C (500 mg) or placebo (lactose) and rested for 2 h. After rest, venous blood sample was taken. following a 10-min warm-up consisting of running at 50% VO2max (5-min) and stretching (5-min), subjects ran on treadmill for 30 min at 75% VO2max. Blood samples were taken immediately, 2 and 24 h after exercise.

Blood sampling and analysis

Approximately 8 mL of whole-blood was withdrawn at each time point, and 3 mL of whole-blood was added to tubes containing ethylenediaminetetra-acetic acid (EDTA) as an anticoagulant. Two small aliquot of EDTA-treated blood were removed for determination of differential Leukocyte using Cell counter (K-1000 Sysmax, Japan). An aliquot of EDTA-treated blood (1.5 mL) was subsequently centrifuged at 3000 g for 15 min (4 °C) to obtain

della natura e delle richieste dello studio, hanno compilato un questionario sulla loro storia clinica e hanno fornito il loro consenso informato. I soggetti non avevano assunto nessuna integrazione di minerali/vitamina C nelle 6 settimane antecedenti lo studio. I soggetti sono stati divisi in maniera casuale e uniforme in un gruppo con integrazione di vitamina C (VC) e in un gruppo con assunzione di placebo (P). Le caratteristiche fisiche e le misurazioni delle pliche cutanee prese da quattro punti (bicipitale, tricipitale, sottoscapolare e soprailiaco) erano simili in entrambi i gruppi (Tabella I). L’integrazione consisteva di una capsula contenente 500 mg di vitamina C, mentre il placebo consisteva di una capsula simile contenente 500 mg di lattosio. La capsula è stata assunta per via orale due ore prima dell’esercizio.

Misurazioni preliminari

Il protocollo di Bruce è stato utilizzato per il test del VO2max. I soggetti hanno effettuato una corsa su un tapis roulant a passo moderato; ogni tre minuti il grado e l’intensità sono stati aumentati fino alla spossatezza. Tale test è stato realizzato almeno 2 settimane prima della prova principale.22

Disegno sperimentale e procedure

Il giorno del test, i soggetti sono arrivati al laboratorio dopo un digiuno notturno di almeno 10 ore. Un campione di sangue venoso è stato prelevato dopo che i soggetti erano rimasti in posizione eretta per almeno 15 minuti, dopo che i soggetti avevano consumato un leggero pasto standardizzato (due nove bollite e una compressa di vitamina C (500 mg) o placebo (lattosio) e aver riposato per due ore. Dopo il riposo, è stato prelevato il campione di sangue venoso. Dopo 10 minuti di riscaldamento consistente di una corsa al 50% del VO2max (5 minuti) e di stretching (5 minuti), i soggetti hanno effettuato una corsa sul tapis roulant per 30 minuti al 75% del VO2max. I campioni ematici sono stati prelevati immediatamente, 2 e 24 ore dopo l’esercizio.
plasma, for sample vitamin C analysis, 0.03 mL of distilled water and 0.06 mL of 10% metaphosphoric acid (Merek, Germany) were added to 0.03 mL of plasma and vortexed in a 1.5-mL centrifuged tube for ~10 s. The suspension was placed over ice for at least 10-min and sheltered from strong light. The mixture was then centrifuged at 23000 g for 10 min at 4 °C. A 0.05 mL sample of supernatant was immediately injected to HPLC (Jasco, Japan) to determine vitamin C concentration of plasma.

Serum was obtained by allowing whole blood (~4 mL) to clot for 20-min, followed by chilled centrifugation (4 °C) at 3000 g. Serum creatine kinase (CK) were determined at 37 °C using commercially available methods (Roche Hitachi-911, Germany and Japan). Serum IL-6 and TAC was analyzed using a commercially available solid-phase high-sensitivity ELISA (Dynex, uS). CRP was measured by a nephelometric procedure (Binding, England). For MDA measurement, an aliquot portion of 0.05 mL serum was added to 0.25 mL of 0.1 M TCA and to 0.7 mL of distilled water. Then the sample was centrifuged at 4500 g for 5 min and used for HPLC (Jasco, Japan) analysis.

Statistical analysis

Results are expressed as means±SEM, and P<0.05 was considered statistically significant. An independent two-way analysis of variance with repeated measures was used to compare results between treatments and over time. Where significant f ratios were found, a Tukey Honest Significant Difference test was used to determine location of variance. When there were only single comparisons, a Student’s t-test with Bonferroni correction for correlated data was used to determine whether any differences between treatments existed.

Results

Antioxidant markers

Baseline resting plasma vitamin C concentrations were not different between groups (figure 1). Two hours after supplementation, plasma vitamin C significantly increased in VC group (P<0.05). Vitamin C concentrations decreased over the course of exercise in the VC group but were still significantly higher immediately and 2 h after exercise (P<0.05). However, 24 h after

Prelino e analisi dei campioni di sangue

Circa 8 ml di sangue interno sono stati prelevati in ogni punto temporale e 3 ml di sangue interno sono stati aggiunti alle provette contenenti acido etilenediaminetetraacetico (EDTA) come anti-coagulante. Due piccole aliquote di sangue trattato con EDTA sono state rimosse per il conteggio differenziale dei leucociti utilizzando una conta-cellule (K-1000 Sysmex, Giappone). Un’aliquota di sangue trattato con EDTA (1.5 ml) è stata successivamente centrifugata a 3000 g per 15 minuti (4 °C) per ottenere il plasma. Per l’analisi della vitamina C nel campione, 0.03 ml di acqua distillata e 0.06 ml di acido metatarsico al 10% (Merek, Germania) sono stati aggiunti a 0.03 ml di plasma e sottoposti a vortex in una provetta centrifugata da 1,5 ml per ~10 s. La sospensione è stata posta su ghiaccio per almeno 10 minuti e tenuta al riparo dalla luce intensa. La miscela è stata centrifugata a 23000 g per 10 minuti a 4 °C. Un campione di supernatante da 0.05 ml è stato immediatamente iniettato nell’HPLC (Jasco, Giappone) per determinare la concentrazione plasmatica di vitamina C.

Il siero è stato ottenuto permettendo al sangue interno (~4 ml) di coagulare per 20 minuti, seguito dalla centrifugazione a freddo (4 °C) a 3000 g. La creatininosina (CK) sierica è stata determinata a 37 °C utilizzando metodi commercialmente disponibili (Roche Hitachi-911, Germany e Giappone). L’IL-6 e la CAT sieriche sono state analizzate utilizzando un dosaggio immunoenzimatico in fase solida (ELISA) ad alta sensibilità (Dynex, uS). La PCR è stata misurata mediante una procedura nefelometrica (Binding, Inghilterra). Per la misurazione dell’MDA, una parte aliquota di 0.05 ml di siero è stata aggiunta a 0.25 ml di acido tricloroaetico (0,1 M) e a 0,7 ml di acqua distillata. Il campione è stato quindi centrifugato a 4500 g per 5 minuti e utilizzato per l’analisi con HPLC (Jasco, Giappone).

Analisi statistica

I risultati sono espressi come medie±ESM e p<0.05 è stato considerato statisticamente significativo. Un’analisi della varianza a due vie per misure ripetute è stata utilizzata per confrontare i risultati tra i trattamenti e nel tempo. Laddove sono stati rilevati rapporti F significativi, un test HSD di Tukey è stato utilizzato per determinare il luogo della varianza. Quando vi erano solo confronti singoli, un test t di Student con correzione di Bonferroni per i dati correlati è stato utilizzato per determinare se esistessero differenze tra i trattamenti.

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the exercise, plasma vitamin C concentrations were almost similar to the baseline values in both groups. Baseline resting serum TAC was not different between groups (Table II). TAC increased immediately after exercise in placebo group (P>0.05) and decreased 2 and 24 hours after exercise when compared with baseline values (P<0.05). In VC group, TAC increased after supplementation and continued to increase after exercise and after 2 h, returning to baseline values after 24 h. Neither of differences were significant between the two groups (P>0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Baseline (-2)</th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
<th>2h</th>
<th>24h</th>
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<tbody>
<tr>
<td>TAC (u/mL)</td>
<td>P</td>
<td>10±1.10</td>
<td>11.21±0.80</td>
<td>11.4±1.00</td>
<td>8.5±0.70†</td>
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<td></td>
<td>VC</td>
<td>7.9±1.70</td>
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<tr>
<td>MDA (nmol/mL)</td>
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<td>6563±769</td>
<td>7729±860</td>
<td>10713±864</td>
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<tr>
<td></td>
<td>VC</td>
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<td>6638±445</td>
<td>8188±554</td>
<td>9913±755</td>
<td>5700±392</td>
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<td>Neutrophils (/µL)</td>
<td>P</td>
<td>3507±483</td>
<td>4440±720</td>
<td>5161±658</td>
<td>8267±751</td>
<td>3015±376</td>
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<td>Lymphocytes (µL)</td>
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<td>4149±347</td>
<td>4825±355</td>
<td>7483±701</td>
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<tr>
<td>CRP (mg/dL)</td>
<td>P</td>
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<td>1650±1219</td>
<td>2077±216</td>
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<tr>
<td></td>
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<td>2.76±0.33</td>
<td>2.86±0.23</td>
<td>2.96±0.40</td>
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</table>
Markers of lipid peroxidation and muscle damage

Serum malondialdehyde (MDA) is shown in Table II. Serum concentrations of MDA increased 2 h after exercise only in placebo group (P<0.05). There were not significant differences between groups for MDA over the course of exercise (P>0.05). Serum creatine kinase (CK) is shown in figure 2. CK increased above baseline values after exercise in both groups. The increase of CK was significant immediately and 2 h after exercise in both groups as well as 24 h after exercise only in placebo group (P<0.05). There were no differences between groups for CK over the course of the experiment (P>0.05).

Markers of inflammation and IL-6

The effect of participation in the 30-min exercise at 75% VO2max on circulating total leukocytes, neutrophils and lymphocytes counts, as well as CRP concentrations are shown in Table II. Total leukocytes and neutrophils counts, as well as CRP concentrations were significantly increased in both groups after exercise, returning to pre-exercise values 24 h later (P<0.05). There were no significant differences for lymphocytes counts between groups.

In the placebo group, the levels of IL-6 were significantly higher than in the vitamin C group 2 and 24 h after exercise (P<0.05). There were no significant differences between groups for serum IL-6 concentrations over the course of the experiment (P>0.05).

figure 2.—Serum CK before and after exercise. Values represent means ± SEM (N.=8). *Values in placebo and vitamin C groups above pre-exercise (P<0.05). # Values in placebo group above pre-exercise (P<0.05). VC: vitamin C; P: placebo; -2: baseline; PE: post-exercise.

figure 3.—Serum IL-6 concentrations. Values represent means ± SEM (N.=8). *Values in placebo and vitamin C groups above baseline and pre-exercise (P<0.05). VC: vitamin C; P: placebo; -2: baseline; PE: post-exercise.
phocytes in both groups after exercise compared to pre-exercise (P>0.05). There were no differences between groups for total leukocytes, neutrophils and lymphocytes counts as well as CRP over the course of the experiment (P>0.05). Serum IL-6 is shown in figure 3. IL-6 concentrations increased after exercise (P<0.05) and decreased to almost pre-exercise levels 24 h after exercise in both groups. There were no detectable differences between the placebo and VC groups (P>0.05).

Discussion

The main aim of this study was to investigate whether moderate dose vitamin C supplementation 2 h before exercise, would influence the lipid peroxidation, muscle damage and inflammatory responses following 30-min running at 75% VO_{2max}.

Acute supplementation of VC could increase plasma vitamin C levels 2 h after supplementation. Therefore, it seems that high dose and prolonged supplementation of VC is unnecessary and moderate acute dose vitamin C can increase vitamin C plasma concentration nearly same as prolonged and high dose supplementations. Following acute supplementation of VC, total antioxidant capacity (TAC) increased in VC group immediately after exercise although it was not significant. Two hours after exercise TAC decreased significantly just in placebo group and continued to decrease until 24 h later showing possible effect of VC supplementation in VC group after 2 h.

MDA was significantly blunted after exercise in VC group whereas in placebo group MDA increased significantly 2 h after exercise (Table II). The result is in agreement with our previous study and Ashton but not Thompson and Davidson and Gleeson. The effect of vitamin C on MDA as a marker of lipid peroxidation is possibly due to fitness level or training status of our participants. It is possible that untrained individuals may be more responsive to antioxidant supplementation than endurance-trained athletes. Some but not all studies indicate that endurance training improves endogenous antioxidant defense system. Watson et al. pointed out that the plasma level of lipid soluble antioxidants in trained subjects is higher than normal untrained subjects with the same diet regimen denoting higher level of protection against lipid peroxidation.

Non sono state osservate differenze rilevabili tra il gruppo placebo e il gruppo VC (P>0.05).

Discussione

Obiettivo del presente studio è stato quello di valutare se una modesta integrazione di vitamina C, due ore prima dell’esercizio, possa influenzare la perossidazione lipidica, il danno muscolare e le risposte infiammatorie in seguito a un’corsa di 30 minuti al 75% del VO_2max.

L’integrazione acuta di VC potrebbe aumentare i livelli di vitamina C nel plasma 2 ore dopo l’integrazione. Però, sembra che una dose elevata e una prolungata integrazione di VC sia inutile, mentre una dose moderatamente acuta di vitamina C può aumentare la concentrazione plasmatica di vitamina C per la stessa durata di una dose elevata. In seguito a un’integrazione acuta di VC, la capacità antiossidante totale (CAT) è aumentata nel gruppo VC immediatamente dopo l’esercizio, sebbene non in maniera significativa.

Due ore dopo l’esercizio la CAT è diminuita in maniera significativa solo nel gruppo placebo ed è continuata a diminuire fino a 24 ore dopo, mostrando il possibile effetto dell’integrazione della VC nel gruppo VC dopo 2 ore.

L’MDA era significativamente attenuata dopo l’esercizio nel gruppo VC, mentre nel gruppo placebo l’MDA era aumentata in maniera significativa 2 ore dopo l’esercizio (Tabella II). Il risultato è in linea con il nostro precedente studio e con Ashton, ma non con Thompson e Davidson e Gleeson. L’effetto della vitamina C sull’MDA come marcato re di perossidazione lipidica è probabile dovuto al livello di fitness o allo stato di allenamento dei nostri partecipanti. È possibile che soggetti non allenati siano maggiormente responsivi all’integrazione di antiossidanti rispetto ad atleti allenati nella resistenza. Alcuni studi indicano che l’allenamento della resa migliore il sistema endogeno di difesa antiossidante. Watson et al. hanno evidenziato che i soggetti allenati abbiamo un maggiore livello plasmatico di antiossidanti solubili nei lipidi rispetto ai soggetti non allenati che seguono lo stesso regime nutrizionale, indicando un maggiore livello di protezione contro la perossidazione lipidica nei soggetti allenati. Secondo questi e altri studi similari, un significativo aumento nel livello di MDA si verifica in seguito a un esercizio intenso sarebbe attendibile in soggetti non allenati con un basso livello di capacità antiossidante rispetto ai soggetti allenati. Inoltre, l’effetto attenuativo dell’integrazione antiossidan-
in trained subjects. According to this and similar studies, a significant increase in serum MDA level following acute exercise could be expected in untrained subjects which have low level of antioxidant capacity in compare with trained subjects. Also, the tempering effect of antioxidant supplementation on lipid peroxidation and consequently lower level of serum MDA in untrained group seems reasonable.

CK, as a marker of muscle damage, increased immediately and 2 h after exercise in both groups, returning to pre-exercise values after 24 h only in VC group (figure 2). It seems that VC supplementation has been able to blunt serum CK similar to MDA. The reason for vitamin C influence on CK is probably due to inhibition of lipid peroxidation in VC group. According to some researches, lipid peroxidation may lead to membrane permeability and the escape of muscle constituents such as CK.

It has been proposed that antioxidant supplementation could prevent exercise-induced oxidative stress and protect against both oxidative damage and inflammation. In present study, there were not significant differences in total leukocytes, Neutrophils and lymphocytes counts as well as CRP concentrations between groups.

Depending on the protocol of exercise, the degree of increase in serum IL-6 is different. After marathon races, serum level of IL-6 increases more than 100 times whereas after 30-min of downhill running at a gradient of -18% it elevated only nearly three times. In our study IL-6 increased nearly two times in both groups. Discrepancy in the level of the IL-6 changes are likely due to various protocols of physical exercise which induce different types of energy metabolism, mainly depending on energy expenditure, calories intake, duration and intensity of exercise. In the present study, vitamin C has not been able to affect IL-6. Our result was in agreement with Thompson and Davidson but not Thompson. There are several possible explanations for the lack of effect of VC supplementation on IL-6 plasma level in the present study.

In our study, absolute change in plasma IL-6 was too low (nearly two times). The lack of any effect of vitamin C in the present study could be due to probably minimal uptake of vitamin C into leukocytes and muscle tissue, where it might be expected to modify cytokine production. Another explanation for this finding is that blood glucose levels regulate changes in IL-6 and cortisol during exercise while vitamin
C has less effect. Furthermore, it is likely that vitamin C has not been able to modulate IL-6 because the increase did not achieve some form of critical threshold level.

Conclusions

In summary, acute moderate dose vitamin C supplementation, 2 h before exercise increased plasma concentrations of vitamin C before and after exercise and affected MDA after exercise compared to placebo group. Vitamin C did not show any effect on IL-6 and inflammatory markers such as total leukocytes, neutrophils, lymphocytes and CRP. As a result, moderate dose vitamin C supplementation possibly alleviated lipid peroxidation and muscle damage but not inflammatory responses after 30-min running at 75% VO_{2max}. Furthermore, because of the same influence on vitamin C concentration and possible side effects of high dose supplementation, it seems that there is no need to a higher dose intake of vitamin C before exercise.

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