

THE ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF BERGAMOT (CITRUS BERGAMIA) IN PROFESSIONAL ATHLETES DURING ENDURANCE TRAINING

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ABSTRACT

Introduction: many studies have shown that endurance sports athletes have a biochemical profile of inflammation and high oxidative stress, due to the high load of training. Recent studies have shown that bergamot, in addition to the already known lipid-lowering properties, also has anti-inflammatory and antioxidant properties. The aim of the study was to determine the effects of bergamot juice on biomarkers of inflammation and oxidative stress in professional athletes who practice cross-country skiing.

Methods: 10 international-level cross-country skiing male athletes were enrolled in the study and as a control group we enrolled 10 national-level cross-country skiing male athletes. We performed a blood sample during the unloading phase of the athletic training (T0) and we dosed high sensitivity C-reactive protein (hsCRP) and oxidized low-density lipoprotein (oxLDL). The international-level athletes took 500 ml/day of bergamot juice, which instead the athletes of the control group did not take. Afterwards, the athletes started the training load period and after 30 days of intense training (T1) we re-evaluated the same blood parameters. The two groups had the same training patterns, but athletes of group I sustained a significantly higher volume of training sessions than the athletes of group II.

Results: In group I there was a significant reduction in hsCRP ($p < 0.05$), while there was no significant increase in oxLDL despite the greater training load. In group II, on the other hand, there was no significant change in hsCRP levels between T0 and T1, while there was a significant increase in oxLDL ($p < 0.05$).

Conclusions: Our data showed that supplementation with bergamot juice in professional cross-country skiing athletes could reduce inflammation and oxidative stress due to the intense training. More studies with a higher number of subjects will be needed to understand better the role of bergamot in this category of athletes.

Keywords: cross-country skiing, bergamot, oxLDL, hs-CRP; inflammation, oxidative stress.

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Introduction

Inflammation is a physiological event aimed at restoring organic homeostasis and participates in many fundamental functions of the human body such as the immune response against infectious agents; however, when the inflammation is excessive and protracted over time it contributes to the development of many chronic diseases⁽¹⁾. Dysregulation of immune system cells and inflammatory mediators is the basis

of the development of systemic inflammation which can have consequences: tissue injury, remodeling of the extracellular matrix, angiogenesis and fibrosis in inflamed tissues which ultimately cause dysfunction of the organs affected by such processes⁽²⁾.

It is well known that one of the mechanisms that can trigger inflammation is excessive oxidative stress. Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and the ability of cells to buffer ROS and repair cellular

damage caused by oxidative stress. Oxidative stress is involved in many physiological processes (aging and exercise) and in the pathophysiology of conditions such as: systemic inflammation, cardiovascular diseases, neurodegenerative diseases, tumors⁽³⁾. Many studies have recently focused on the role of oxidative stress in sports. It is well known that muscle contractions that are produced during intense physical activity and protracted over time lead to the production of high levels of ROS in skeletal muscle⁽⁴⁾.

Several papers have shown that a pathological imbalance of inflammation and oxidative stress can have extremely harmful effects on the health of athletes^(5,6). In fact, it has been demonstrated that there is a correlation between oxidative stress and the increased risk of early atherosclerosis and muscle injuries in professional athletes⁽⁷⁻⁹⁾. It is also known that the greater the workload of athletes, the greater the possibility of having a negative imbalance towards a more oxidized and inflamed profile. In fact, endurance athletes are those with the most considerable risk of developing systemic inflammation and oxidative stress with negative health consequences. Several studies have also shown that in addition to the health of athletes, oxidative stress also affects the performance of these elite athletes, and if not properly kept under control, it can lead to overtraining^(10,11). Cross-country skiing is fully part of the group of endurance sports⁽¹²⁾ and even the highest level athletes of this discipline can face overtraining⁽¹³⁾.

When the skeletal muscle is activated, it releases specific cytokines called myokines, which play a role both in the immune system and in the metabolism of adipose tissue. It is well documented in the literature that a moderate amount of exercise has a systemic anti-inflammatory effect^(14,15). At the same time, a systemic inflammatory response can be triggered by excessive exercise, a condition that is very common in professional athletes. During periods of intense training, it is important to maintain the right balance between the pro and anti-inflammatory response to maintain the health and performance of the athlete^(16,17).

Several authors, in this regard, have shown that supplementation with natural anti-inflammatory and antioxidant products can contribute to the health and performance of these athletes^(18,19). At the same time, recent studies have shown the usefulness of monitoring the blood levels of some biomarkers of inflammation such as high sensitivity C-reactive protein (hsCRP)⁽²⁰⁾ and oxidative stress such as oxidized low-density lipoprotein (oxLDL)⁽²¹⁾ in order to keep

under control the pathophysiological responses of the athletes, also in case of supplementation with antioxidants, and consequently modulate the training loads.

Bergamot is a citrus fruit of the genus *Citrus* (scientific name *Citrus Bergamia*). The fruit has a yellow peel and is the size of an orange. Although originating in Southeast Asia, 80% of the bergamot is produced in Calabria (Southern Italy). Bergamot has a peculiar composition of flavonoids, in particular it is rich in neohesperidin and naringin. Scientific literature has shown that bergamot has several positive health effects, including the reduction of cholesterol, systemic inflammation and oxidative stress⁽²²⁻²⁵⁾. However, there is little scientific data on the role of bergamot in professional athletes. The purpose of the study was to verify whether the intake of 500 ml per day of bergamot juice can modulate the inflammatory response and oxidative stress in cross-country skiers engaged in intense training.

Methods

Study design

10 international-level cross-country male athletes (experimental group or group I) and 10 national-level cross-country male ski athletes (control group or group II) were enrolled for this study in the Division of Sports Medicine of the University of Rome Tor Vergata. Before being enrolled in the study, the athletes were subjected to a medical evaluation by a multidisciplinary team made up of cardiologists, sports doctors and nutritionists in order to exclude that any pathologies or drug intake could interfere with the results of this study. All the enrolled athletes were in good health and none of them smoked, had pathological conditions or took drugs. Clinical assessments were made for all athletes in one day. All athletes were in possession of the sports medical certification for their category in accordance with national regulations. The study was approved by the local ethics committee (ID number 41.17) and all subjects signed an informed consent. The study was conducted in accordance with the declaration of Helsinki. The characteristics and the main anthropometric parameters of the two groups are shown in Table 1.

The 30-days study was carried out in 2 phases of athletic training: the first phase (T0) represented by the end of an unloading period for both groups and the second phase (T1) during a loading period for both groups. The two groups had the same training patterns, but athletes of group I, because of their

preparation for international competitions, sustained a significantly higher volume of training sessions than the athletes of group II. The physical activity carried out was quantified in METs and the 2011 version of the Compendium of Physical Activities was used as a reference for the classification of physical activities⁽²⁶⁾.

	Group I	Group II
Age (years)	26,5 ± 3,2	28,1 ± 2,8
Stature (cm)	178,6 ± 4,5	170,9 ± 3,2
Weight (kg)	71,5 ± 6,9	71,7 ± 6,7
BMI (kg/m ²)	23,5 ± 3,9	23,3 ± 3,5

Table 1: Age, stature, weight and BMI of the two study groups.

After the first visit (T0), the athletes of the group I started taking 500 ml of bergamot juice (100% pure bergamot juice) per day for the duration of the study (30 days). On the other hand, the athlete of group II did not take bergamot juice. Both groups had the same diet and none of the athletes changed the common diet during the study phases.

Blood samples were collected through venipuncture at T0 and after 30 days of intense training (T1). The samples were immediately centrifuged, separated and stored at -80 ° C for future analysis in the Department of Laboratory Medicine of the University Tor Vergata. The blood samples were collected in the morning with the athletes in a rest condition. The biomarkers considered for this study were the high sensitivity C-reactive protein (hsCPR) and oxidized low-density lipoprotein (oxLDL). HsCPR was determined with an immunoassay using the Dimension Vista Siemens platform (Siemens Healthcare GmbH, Marburg, Germany). The circulating oxLDL level was measured by enzyme-linked immunosorbent assay using the mAb-4E6 antibody (MercoDia AB, Uppsala, Sweden), according to the customer protocol.

Statistic analysis

All data were analyzed for their distribution properties. The results are presented as mean ± standard deviation. Comparisons between groups were performed using paired sample t-test.

The Shapiro-Wilk test will be used to verify data distribution. A p-value of <0.05 was considered statistically significant. Statistical analyzes were performed using IBM SPSS 21.0 for Windows (IBM Corp., Armonk, New York, USA).

Results

The training patterns were similar for the two groups of professional athletes. However, the athletes of group I, in consideration of their preparation for international competitions, have performed higher training volumes than the athletes of the group II. The analysis of the data showed, in fact, that at T1 the difference in training volume quantified in METs per week between group I and group II was significant ($p < 0.05$) (Table 2).

	Group I	Group II
METs per week at T0	9651,2 ± 1057,2	6322,5 ± 842,1 **
METs per week at T1	16732,6 ± 1729,3	10756,4 ± 1102,3 *

Table 2: Physical activity expressed in METs carried out per week in the two study groups at T0 and T1.

* $p < 0.05$, **not significant

In the group I there was a significant reduction in hsCPR ($p < 0.05$) at T1. Instead, there has not been a significant variation in oxLDL levels between T0 and T1, despite the higher training volume (Table 3).

	T0	T1
hsCPR (mg/dl)	0,17 ± 0,08	0,06 ± 0,02*
oxLDL (pg/ml)	387,92 ± 27,6	408,8 ± 19,1**

Table 3: hsCPR and oxLDL of group I at T0 and T1.

* $p < 0.05$, **not significant

In the group II the data did not demonstrate a significant difference in hsCPR values. Instead, a significant increase in oxLDL has been demonstrated (Table 4).

	T0	T1
hsCPR (mg/dl)	0,11 ± 0,08	0,09 ± 0,06**
oxLDL (pg/dl)	347,76 ± 31,6	374,14 ± 26,95*

Table 4: hsCPR and oxLDL of group II at T0 and T1.

* $p < 0.05$, **not significant

Discussion

The scientific literature has shown that intense physical activity can trigger excessive inflammation and oxidative stress and this condition can be detected by some laboratory parameters such as hsCPR and oxLDL⁽²⁷⁻²⁹⁾.

The data of our study showed that in the athletes of group I at T1 there was a reduction in inflammation, which was demonstrated by the significant reduction in hsCPR (Figure 1).

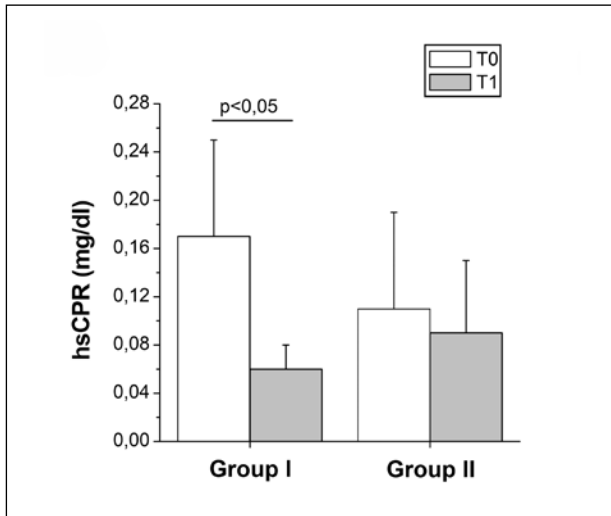


Fig. 1: Difference in hsCRP (mg/dl) levels between T0 and T1 in Group 1 and Group 2.

Therefore, there was a reduction in the inflammatory state despite the fact that the training volume increased, compared to the unloading phase. On the other hand, in group II there was no significant change in hsCPR between T0 and T1. These experimental data suggest that the supplementation with bergamot juice, which the athletes of group I took, could reduce inflammation levels. This phenomenon could be explained by the already known anti-inflammatory properties of bergamot⁽²²⁻²⁴⁾. In group I from T0 to T1 there was no significant change in oxLDL levels, while in group II from T0 to T1 there was a significant increase in oxLDL (Figure 2). Interestingly, oxLDL levels did not increase in group I as opposed to group II, although the athletes of group I performed higher training volumes. These data therefore suggest that bergamot juice could have a protective antioxidant effect in these athletes⁽²²⁻²⁴⁾.

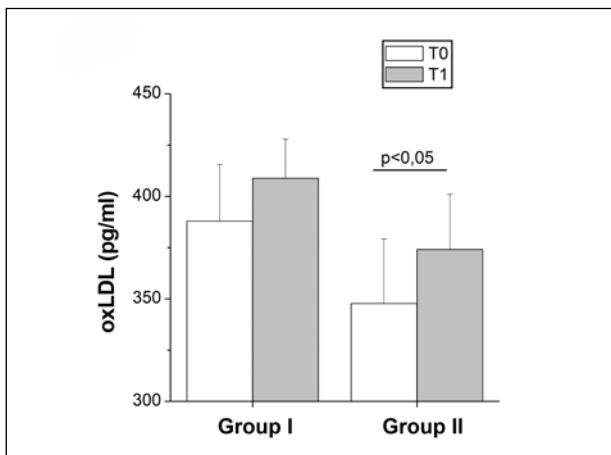


Fig. 2: Difference in oxLDL (pg/ml) levels between T0 and T1 in Group 1 and Group 2.

Many studies have shown that intense physical activity can trigger a considerable increase in the biomarkers of inflammation and oxidative stress in professional athletes⁽³⁰⁻³²⁾. For this reason several authors have experimented various natural antioxidant compounds as supplementation in this category of athletes⁽³³⁻³⁵⁾. For example, hydroxytyrosol, a derivative of olive oil, has proven effective in reducing oxLDL levels in professional soccer players⁽⁹⁾ and amateur athletes⁽³⁶⁾. OxLDL are considered to be one of the major players in the formation of atherosclerotic plaque⁽³⁷⁻⁴⁰⁾ and their increase in the blood has been correlated with early atherosclerosis in professional athletes⁽⁴¹⁻⁴⁴⁾.

In general, the reduction of oxidative stress and systemic inflammation biomarkers with natural products is one of the objectives of researchers to first safeguard the health and then also the performance of elite athletes. Our data showed that in group I athletes, there was a significant reduction in hsCPR levels, and therefore in the inflammatory state, despite the 30 days of training. On the other hand, always in group I our data did not show a significant increase in oxLDL and therefore in the oxidative state between T0 and T1. In scientific literature it is reported that in the intense training phases, professional athletes have a significant increase in oxLDL. Instead, in group II data did not show a change in hsCPR levels, but statistical analysis showed a significant increase in oxLDL levels, according to the data from the scientific literature^(9,45).

Our data could be explained with a beneficial effect of bergamot on the inflammatory and oxidative state in the athletes of group I, despite the training phase and even more interesting despite the greater load of training volume compared to group II. By comparing the two groups, thereby, the athletes of group I had a reduction in hsCPR and did not have a significant increase in oxLDL, which we would have expected, despite the higher training load. The intake of 500 ml/day of natural bergamot juice during the loading phase of training contributed to reducing the levels of inflammation and oxidative stress biomarkers in these athletes, compared to group II athletes, who carried out a lower training load and have not taken bergamot juice.

These data are extremely interesting because several studies have shown that an increase in inflammation and oxidative stress in athletes is connected with a greater risk of muscle inju-

ry, overtraining and cardiovascular disease^(8,11,41). Modulating the inflammatory and oxidative response with natural and lawful products in accordance with the directives of the World Anti-Doping Agency is certainly a topic of great scientific interest to allow athletes with an international profile to be able to compete with greater safety for their health. To the best of our knowledge, this is the first study evaluating the effects of bergamot on hsCRP and oxLDL in professional cross-country skiers during endurance training

Conclusions

The large training volumes to which endurance athletes are subjected such as cross-country skiing favor the onset of oxidative stress and inflammation (46-49). The administration of bergamot juice in cross-country skiers of the international level during the loading period of the athletic preparation had a protective effect on the values of hsCPR and oxLDL. On the contrary, in national-level athletes who have not taken bergamot juice, an increase in oxLDL blood values has been documented. Therefore, bergamot juice has shown anti-inflammatory and antioxidant action in cross-country skiing athletes during endurance training. More studies with a greater number of subjects and perhaps with athletes of different sports will be necessary to confirm our data and better to understand the effects of bergamot on professional athletes.

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