CLINICAL SCIENCE

Organic grape juice intake improves functional capillary density and postocclusive reactive hyperemia in triathletes

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OBJECTIVE: The aim of this study was to evaluate the effect of organic grape juice intake on biochemical variables and microcirculatory parameters in triathlon athletes.

INTRODUCTION: The physiological stress that is imposed by a strenuous sport, such as a triathlon, together with an insufficient amount of antioxidants in the diet may cause oxidative imbalance and endothelial dysfunction.

METHODS: Ten adult male triathletes participated in this study. A venous blood sample was drawn before (baseline) and after 20 days of organic grape juice intake (300 ml/day). Serum insulin, plasma glucose and uric acid levels, the total content of polyphenols, and the erythrocyte superoxide dismutase activity were determined. The functional microcirculatory parameters (the functional capillary density, red blood cell velocity at baseline and peak levels, and time required to reach the peak red blood cell velocity during postocclusive reactive hyperemia after a one-min arterial occlusion) were evaluated using nailfold videocapillaroscopy.

RESULTS: Compared with baseline levels, the peak levels of serum insulin ($p = 0.02$), plasma uric acid ($p = 0.04$), the functional capillary density ($p = 0.003$), and the red blood cell velocity ($p < 0.001$) increased, whereas the plasma glucose level ($p < 0.001$), erythrocyte superoxide dismutase activity ($p = 0.04$), and time required to reach red blood cell velocity during postocclusive reactive hyperemia ($p = 0.04$) decreased after organic grape juice intake.

CONCLUSION: Our data showed that organic grape juice intake improved glucose homeostasis, antioxidant capacity, and microvascular function, which may be due to its high concentration of polyphenols. These results indicate that organic grape juice has a positive effect in endurance athletes.

KEYWORDS: Endurance athletes; Nailfold videocapillaroscopy; Glucose homeostasis; Antioxidant capacity; Microcirculatory function.

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INTRODUCTION

The triathlon is a strenuous sport that includes three modalities: swimming, cycling, and running. Physiological stress and muscle damage may occur during training and competitions.[1] Studies indicate that continuous exercise and a heavy training load improve enzymatic antioxidant protection[2] and promote vasodilatation of the cutaneous microcirculation in humans[3,4] possibly because of augmented nitric oxide bioactivities in the dermal microcirculation.[4] These observations have considerable physiological impact and may indicate that endurance training modifies the thermoregulatory control of skin blood flow and reduces the risk of cardiovascular disease in athletes.

Circulatory adaptations are important for exercise maintenance in athletes.[4] However, several mechanisms can destroy or disrupt these adaptations. For instance, the production of intracellular reactive oxygen species may increase because of the alteration of the metabolic oxidation rate in the organism.[1] To avoid oxidative damage, reactive oxygen species are counteracted by a sophisticated antioxidant defense system that includes enzymatic scavengers, such as superoxide dismutases (SOD), catalases, and peroxidases, as well as several other nonenzymatic low molecular weight molecules, such as glutathione and uric acid, which are synthesized by cells, and ascorbate, carotenoids and polyphenols, which are acquired from the diet.[5] Additionally, a paradox seems to arise when the antioxidant capacity is unbalanced, and enhanced oxidative stress may lead to cellular damage and endothelial dysfunction.[6]

Grape juice is a relevant source of polyphenolic compounds.[7] Organic agriculture, which does not use pesticides during cultivation, yields a higher content of secondary metabolites.[8] Polyphenol-rich beverages prevent platelet aggregation,[9] increase plasma antioxidant capacity,[10] improve hyperglycemic control,[11] and protect against cardiovascular diseases.[12] Taken together, these factors improve microvascular endothelial function.[2,3,4] However, no data are available on the effects of organic grape juice (OGJ) on glucose homeostasis, antioxidant capacity and cutaneous microcirculatory function in athletes. The aim of this study was to evaluate the effects of the inclusion of OGJ in the diet of triathletes on glucose homeostasis, antioxidant status, and cutaneous microcirculatory function.

METHODS

Subjects

Male triathletes of the same team ($n = 13$) were selected. After passing the exclusion criteria (use of vitamin and/or mineral supplements), ten triathletes participated in the study. These athletes were adults (34 ± 7 years) and had been members of the team for the previous five years. Information concerning the time (hours) of weekly exercise practice was obtained using individual structured interviews. During the study, the athletes were instructed not to change their dietary pattern except for exclusion of grapes and their derivatives from their daily intake. They were also instructed to maintain their rigorous weekly training program (22 ± 3 h/w; 30 km of cycling, 7 km of running and 2 km of swimming per day).

Experimental design

The Ethical Committee of the Universidade do Estado do Rio de Janeiro in Brazil (COEP070/2007) approved the study protocol. The study consisted of a 20-day intervention pilot trial in a selected group of ten adult male elite triathletes living and training in the same city and environment (Rio de Janeiro, Brazil) who agreed to follow the study design under the same training conditions.

Triathletes consumed 300 ml of OGJ daily for 20 days. OGJ was produced by a Brazilian industry from *V. labrusca*, which is a variety of Bordeaux and is cultivated in southern Brazil. The total content of polyphenols in OGJ (5.32 mg/ml) was determined using high-performance liquid chromatography (HPLC).[7]

Sample collection

Fasting (12 h) blood samples were obtained after 20 h of exercise in the periods before (baseline) and after 20 days of OGJ intake. Blood samples (10 ml) were collected by venous puncture into tubes with or without heparin as an anticoagulant. Blood samples with heparin were centrifuged at $1{,}800\times g$ for 10 min to separate the plasma and blood cells. After the removal of the buffy coat, erythrocytes were washed three times with ice-cold 0.9% NaCl, and the cells were lysed with an equal volume of ice-cold deionized water. Aliquots of plasma, serum, and erythrocytes were stored at –20℃ until analysis.

Laboratory assays

Serum insulin was measured using a radioimmunoassay with a commercial kit (MP, Biomedicals, USA). Plasma glucose was determined by enzymatic method (Gold Analisa, catalog number 434, RJ, Brazil), plasma uric acid was determined by colorimetric analysis (Gold Analisa, catalog number 451, RJ, Brazil) and the total content of polyphenols was determined by Folin–Ciocalteu method employing galic acid as standard as previously described.[13] The superoxide dismutase activity in erythrocytes (E-SOD) was determined with a commercial kit based on the enzymatic colorimetric method (Stressgen, catalog number 900-157, MI, USA).

The intra-assay variation coefficient for all measurements was lower than 5%. All of the assays were run in duplicate except for the assay measuring serum insulin levels, which was run in triplicate. The homeostasis model assessment for the insulin resistance (HOMA2-IR) index was obtained using the HOMA calculator v.2.2.2 (2009).[14]

Anthropometric measurements

The total body mass was measured to the nearest 0.1 kg using a portable scale. The height was determined to the nearest 0.5 cm using a stadiometer. The subcutaneous body fat was assessed using the skin-fold measurement technique with a Lange skinfold caliper. The localization of ten sites (pectoral, midaxillar, subscapular, abdominal, suprailiac and iliospinal muscles, triceps, biceps, quadriceps and calf) and the execution of measurements (in triplicate) were performed by a trained anthropometrist, as previously described.[15] The percent body fat was calculated.[16] The fat-free mass was estimated by subtracting the fat mass from the total body mass.

Dietary assessment

The dietary habits of triathletes were assessed using a 24-h recall for three days applied before and during the study, including two weekdays and one weekend day. Food pictures and rulers were provided to help estimate the amount of food and beverages consumed. A well-trained dietitian verified and quantified the food records. The dietary nutrient analysis was conducted using the Avanutri software version 3.1.5 (AVANUTRI, RJ, Brazil).

Microvascular Function Assessment

Nailfold videocapillaroscopy was performed by the same observer and analyzed according to a standardized and well-validated methodology using the fourth finger of the left hand in a temperature-controlled environment (24℃).[17] All of the subjects had fasted for 12 h and abstained from exercise for 20 h. Using the Cap Image software,[18] the following microvascular parameters were determined: (a) the functional capillary density (FCD), which is the number of capillaries/mm^2 with red blood cell flux, was evaluated using a magnification of 250x in a 3-mm area of the distal row of capillaries in three different areas; (b) the diameters of afferent, efferent, and apical capillaries were determined based on morphological parameters; (c) the red blood cell velocity at rest (RBCV) and its peak after an one-min arterial occlusion ($RBCV_{max}$) were determined; and (d) the time taken to reach $RBCV_{max}$ ($TRBCV_{max}$) was noted. With the exception of the FCD, the microvascular parameters were measured at a magnification of 680x before and after the postocclusive reactive hyperemia (PORH) response. FCD, RBCV, $RBCV_{max}$, and $TRBCV_{max}$ are considered functional parameters. Before performing the RBCV measurements on an individual capillary loop, a pressure cuff (1-cm wide) was placed around the proximal phalanx and connected to a mercury manometer. The basal RBCV was measured three times, and the intra-assay variation coefficient was 17.1%. Each variable was tested once at PORH. The nailfold videocapillaroscopy exam was repeated on nine subjects on different days, and the inter-assay variation coefficient of the tested functional parameters ranged from 2.0% to 9.0%.

Statistical analysis

The data were represented as the mean $\pm$ standard deviation. After grape juice intake, FCD, RBCV, $TRBCV_{max}$, and plasma polyphenols were not normally distributed and were log_{10}-transformed before statistical analysis. After OGJ intake, changes in biochemical variables and microcirculatory parameters from baseline were evaluated using the paired *t*-test. The acceptable level for statistical significance was established at $p<0.05$. The statistical analysis was conducted using Sigma Stat software (3.5, Ashburn, USA).

RESULTS

The baseline total body mass, percent fat mass, and fat-free mass were 71 $\pm$ 4.0 kg, 10 $\pm$ 3.4%, and 63.4 $\pm$ 3.9 kg, respectively, and they did not change during the study. The mean energy (4121 $\pm$ 1407 kcal) and macronutrient (62 $\pm$ 25%, 15 $\pm$ 5% and 23 $\pm$ 5% for carbohydrates, proteins, and lipids, respectively) intake were adequate[19] and similar throughout the study.

OGJ intake led to an increase in total plasma polyphenol concentration after 20 days of OGJ intake ($p = 0.048$). Although the difference was small, this result has clinical relevance because polyphenols have a low bioavailability, and the higher plasma concentration after OGJ showed adherence to OGJ intake in the athlete's diet.

During the study, all athletes were normouricemic (<0.42 μmol/l),[20] normoglycemic (<5.6 mmol/l)[21] and were not insulin-resistant (Table 1). After 20 days of OGJ intake, the fasting plasma glucose concentration decreased ($p<0.001$), and the fasting serum insulin concentration increased ($p = 0.03$). However, HOMA did not change. The plasma uric acid concentration increased ($p = 0.01$) and E-SOD activity decreased ($p = 0.03$) (Table 1) in relation to the antioxidant status.

After OGJ intake, FCD ($p<0.001$) and $RBCV_{max}$ ($p<0.001$) increased, whereas $TRBCV_{max}$ decreased ($p<0.05$) compared with respective baseline levels (Table 2).

DISCUSSION

Physical exercise and polyphenol intake may increase antioxidant capacity[2,10] and cardiovascular health,[12,22] including the health of the cutaneous vascular bed.[4] To our knowledge, the present study is the first one to show that OGJ intake for short period may improve glucose homeostasis, antioxidant capacity and microcirculatory parameters in elite triathletes.

Circulatory adaptation in response to intense exercise is an important determinant of an athlete's performance. It is well recognized that routine exercise improves microcirculatory parameters in locally active muscle and in the whole organism[4,22] by promoting vasodilatation that is observed in the cutaneous microcirculation,[23,24] glucose uptake,[25] and the endogenous antioxidant status.[26] Exogenous antioxidants that are provided by an antioxidant-rich diet can interact with endogenous antioxidants and reduce the risk of cellular injury that occurs following intense exercise.

Improving the antioxidant capacity by polyphenol intake may be determined using different biomarkers of oxidative stress.[27] E-SOD is a cytosolic antioxidant enzyme that is responsible for superoxide anion dismutation into oxygen and hydrogen peroxide[28] and is sensitive to the intake of polyphenols in humans.[10,29] Uric acid is the principal component of plasma antioxidant capacity[30] as a potent scavenger of peroxyl and hydroxyl radicals, especially during metabolic stress.[31]

In the present study, the increased plasma uric acid concentration was possibly due to the fructose concentration in OGJ, which accelerates purine metabolism.[32] Decreased E-SOD activity may be caused by the reduction of intra- and extracellular oxidative imbalances. The consumption of grapes and its derivatives has been associated with lower levels of plasma glucose.[26] In the present study, we observed a reduction in fasting plasma glucose and an increase in serum insulin in athletes after OGJ intake. These results may be mediated by different mechanisms, such as the insulin-mimetic effects of polyphenols, including the increased transport and oxidation of glucose as well as glycogen synthesis,[33] the resveratrol-mediated activation of sirtuin 1,[34] which improves insulin sensitivity and increases

Table 1 - Biochemical parameters for glycemic homeostasis and antioxidant status in triathletes before (baseline) and after 20 days of organic grape juice intake (n = 10).

	Baseline	After grape juice intake
Glucose (mg/dl)	89.4 $\pm$ 8.9	67.1 $\pm$ 11.2*
Insulin (μU/ml)	13.3 $\pm$ 2.7	16.9 $\pm$ 3.7*
HOMA2-IR	1.7 $\pm$ 0.1	1.9 $\pm$ 0.4
Uric acid (μmol/l)	0.3 $\pm$ 0.07	0.4 $\pm$ 0.06*
E-SOD (U/mg protein)	27.8 $\pm$ 6.3	24.3 $\pm$ 2.5*
Plasma polyphenols (mg of galic acid/ml)	0.2 $\pm$ 0.03	0.3 $\pm$ 0.04*

* $p \leq 0.05$ by paired t-test. HOMA2-IR: homeostasis model assessment for insulin resistance; E-SOD: superoxide dismutase activity in erythrocytes.

Table 2 - Microcirculatory parameters of triathletes before (baseline) and 20 days after OGJ intake (n = 10).

Microcirculatory parameters	Baseline	After grape juice intake
Functional capillary density (n/mm^2)	10.7 $\pm$ 2.3	14.9 $\pm$ 2.7 *
Afferent diameter (μm)	17.6 $\pm$ 2.9	17.9 $\pm$ 2.0
Apical diameter (μm)	23.7 $\pm$ 4.1	23.7 $\pm$ 1.4
Efferent diameter (μm)	19.8 $\pm$ 4.7	20.9 $\pm$ 3.3
RBCV (mm/s)	0.3 $\pm$ 0.05	0.3 $\pm$ 0.01
$RBCV_{max}$ (mm/s)	0.3 $\pm$ 0.02	0.4 $\pm$ 0.01*
$TRBCV_{max}$ (mm/s)	5.9 $\pm$ 2.8	4.0 $\pm$ 1.1 *

* $p \leq 0.05$ by paired t-test. FCD: functional capillary density; RBCV: red blood cell flux velocity at rest; $RBCV_{max}$: red blood cell flux velocity after 1-min arterial occlusion; $TRBCV_{max}$: time required to reach peak red blood cell velocity.

the number of mitochondria in hepatocytes, and increased GLUT-4 expression in skeletal muscle.[35]

Nailfold videocapillaroscopy is a highly reproducible noninvasive method that can be useful to provide quantitative information on skin microcirculation.[17] Microcirculatory function is strongly influenced by body mass,[17] diabetes, and oxidative status.[36] Altered microvascular structures may contribute to insulin resistance by limiting the rate of glucose delivery and insulin-mediated glucose uptake.[37] These alterations may also reduce athletic performance.

In the present study, microcirculatory parameters (FCD, $RBCV_{max}$, and $TRBCV_{max}$) were altered after OGJ intake. A significant increase in FCD indicated an improvement of tissue nutrition. The postocclusive reactive hyperemia response (PORH), which is normally used to induce capillary recruitment, is thought to be determined at the level of small arterioles and to be independent of the autonomic nervous system. After the onset of reperfusion, blood flow sharply increases, followed by a gradual return to its baseline level, and is influenced by the accumulation of vasodilator metabolites (including nitric oxide) and the formation of reactive oxygen species, which are normally washed out or destroyed by circulating blood and smooth muscle cell reactivity. During reperfusion, the myogenic response, which is mediated by the rapid stretching of microvascular smooth muscle cells, is responsible, at least partially, for the return of blood flow to its baseline values. The role of the endothelium in modulating the myogenic response remains controversial. In the current study, we detected an increase in $RBCV_{max}$ and a decrease in $TRBCV_{max}$ after OGJ intake. These results clearly indicate an amelioration of PORH, which is accompanied by a decrease in its duration. These results may be caused by improvements in glucose homeostasis that in turn improve endothelial function through reduced peroxynitrite formation, restored eNOS expression and, therefore, increased production and availability of NO.

In conclusion, our results suggest that the consumption of organic red grape juice, which is a source of polyphenols, may benefit the antioxidant capacity, glucose homeostasis and microcirculatory parameters in endurance athletes. Although limited by the small sample size, our results are consistent with other studies and may suggest that the consumption of grape juice can positively affect an athlete's health and reduce the risk of cardiovascular diseases. Further studies are required to clarify the role of polyphenols in glucose homeostasis and the antioxidant capacity based on the microvascular parameters in athletes using different modalities. Clinical trials using OGJ may be used to investigate the possible benefits of OGJ on athletic performance and health.

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