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Physical activity increases the bioavailability of flavanones after dietary aronia-citrus juice intake in triathletes

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ABSTRACT

Control and triathlete volunteers ($n = 8$ and $n = 15$, respectively) were given 400 mL and 200 mL of aronia-citrus juice (AC-juice), respectively. The 24 h urine samples were hydrolysed to determine the flavanones concentration by UPLC-QqQ-MS/MS. The flavanones metabolites in both groups of volunteers were glucuronides, sulfates, and sulfo-glucuronides, and the total excretion of flavanones increased fivefold in the triathletes compared with the control volunteers. The increase of ninefold in the homoeriodictyol of triathletes compared to control volunteers may suggest the overactivation of the microbiota metabolism caused by physical exercise. No differences concerning the bioavailability were detected between men and women in controlboth groups. The AC-juice could provide synergistic effects on health due to the increase in the bioavailability of flavanones, avoiding the deleterious effects caused by the overdosage of nutritional supplements.

1. Introduction

In the past decade, a number of clinical trials based on dietary interventions have been performed to establish the bioefficiency of distinct subclasses of polyphenols (Kay, 2010). The applicability of polyphenols to the athlete's world and their health benefits remain scarcely addressed (Trombold, Barnes, Critchley, & Coyle, 2010). In order to gain a further insight into the relationship between training, nutrition, and health, a variety of nutritional supplements have been developed to increase the physical outcome of the training programs regardless of the natural option of fruit juices with bioactive components (Trombold et al., 2010).

Citrus juices are known for their high content in flavonoids, especially flavanones (400–600 mg L^{-1}) (Gil-Izquierdo, Gil, & Ferreres, 2002; Gil-Izquierdo, Gil, Ferreres, & Tomás-Barberán, 2001). These compounds are mostly attached to rhamnoglucosides which need to be removed by the colon microflora in order to be absorbed (Silberberg et al., 2006). Flavanones have shown a more permanent systemic level, due to the enterohepatic cycle, which allows the re-excretion of metabolites, by bile, and their reabsorption in the small intestine or colon, and therefore, a longer residency at physiological level (Manach, Morand, Gil-Izquierdo, Bouteloup-Demange, & Rémésy, 2003).

In recent years, research in this field has focused on the augmentation of flavanones bioavailability, by different mechanisms, in order to increase the health-promoting properties of citrus juices. The combination of aronia (*Aronia melanocarpa*) with citrus juices could provide synergistic effects of flavanones plus anthocyanins, among other bioactive compounds (Habauzit et al., 2011). However, as far as we are aware, the effect of physical activity on the bioavailability of the target compounds (flavanones) from aronia-citrus juices (AC-juice) remains unknown.

The aim of the present study was to identify the circulating flavanones metabolites after the intake of AC-juice, and compare their bioavailability in triathletes with that found in control volunteers. In addition, the flavanones excretion was also evaluated, for a week before and after AC-juice intake, in triathletes.

2. Methods and materials

2.1. Chemicals

Naringenin, eriodictyol, homoeriodictyol, hesperetin, and isosakuranetin were purchased from Extrasynthèse (Genay, France). Hesperetin 7-*O*-glucuronide was synthesised in our lab according to the method described by Boumendjel, Blanc, Williamson, and Barron (2009). All LC–MS grade solvents were obtained from J.T. Baker (Phillipsburg, NJ). Formic acid and chlorhydric acid were purchased from Panreac (Barcelona, Spain). The β-glucuronidase,

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type H2 from *Helix pomatia* was obtained from Sigma–Aldrich (St. Louis, MO).

2.2. AC-juice

The juice composition is based on a mixture of citrus juice (95%) with 5% of *A. melanocarpa* juice. The content in nutrients and caloric supply of the AC-juice that control volunteers and triathletes consumed is summarised in Tables 1 and 2, respectively, detailing the percentage of contribution of the juice to the total diet.

2.3. Calculation of the training loads in triathletes

The quantification of training programs is addressed to evaluate their effects on physiological adaptation and subsequent performance (Borresen & Lambert, 2009). Currently, mathematical models are suitable for the quantification of the training loads. Indeed, the training impulse proposed by Banister and Calvert (1980) and its subsequent revision are recognized as valid for the quantification of training efforts. However, the evolution of sports performance has led to specific models for some exercises (Lucia et al., 2006).

In our work, the training load quantification was performed using the 'Objective Load Scale' (ECOs) developed by Cejuela Anta and Esteve-Lanao (2011). The training load that supports a triathlete is an indication of their performance level. The training loads developed by the triathletes in the present work were similar to those found in other studies of endurance athletes (Lucia et al., 2006; Rodríguez-Marroyo et al., 2003).

The previously-developed method used allowed the quantification of the training loads in the sport of triathlon (swim, bike, run, and transitions), which are determined by the difficulty to maintain technique, delayed muscle soreness, typical workout density, and energy cost of each separate sport. The values of daily and weekly trainings were determined and summarised to assess the training load (ECOs) of each volunteer, depending on their physical characteristics and the intensity of the training program (the ECO data presented in this work are the average of the individual ECOs of the triathletes). Briefly, and from a general point of view, intensity is considered exponentially –not linearly– with the aim of levelling off the total training stress for a given performance level. The volume is quantified by time and this allows a better comparison of different performance levels and terrain conditions (pavement, uneven laps) (Cejuela Anta & Esteve-Lanao, 2011).

2.4. Study design

Two clinical assays were developed with two intervention groups, control population (non-trained volunteers) ($n = 8$, 4 women and 4 men) and triathletes ($n = 15$, 5 women and 10 men). Both groups were non-smokers and did not receive any medication during the experimental procedure. Women were not in menstrual days during the study. The control population consumed two glasses of juice while triathletes ingested a glass of juice according to their schedule, supervised by the nutritionist and the training load (ECO).

Table 1
Dietary characteristics and caloric intake of the control volunteers group (% of contribution of the juice to the diet has been detailed between brackets).

	Daily intake	AC-juice intake (400 mL)
Carbohydrates (g)	214.0	36.0 (16.8%)
Sugars (g)	38.1	13.3 (34.9%)
Proteins (g)	129.8	1.8 (1.4%)
Fats (g)	55.7	0.1 (0.2%)
Iron (mg)	12.5	0.5 (4.2%)
Vitamin C (mg)	162.4	154.8 (95.3%)
Vitamin E (mg)	5.0	0.2 (3.0%)
Vitamin A (μg)	220.1	3.0 (1.4%)
Total polyphenols (mg)	115.5	115.5 (100.0%)
Flavanones (mg)	86.1	86.1 (100.0%)
Flavones (mg)	29.4	29.4 (100.0%)
Energy intake (kcal)	1857.9	152.0 (8.2%)

Table 2
Dietary characteristics, caloric intake, and training loads of the triathlete volunteers group (% of contribution of the juice to the diet has been detailed between brackets).

	Diet control	Diet + juice intake	AC-juice intake (200 mL)
Carbohydrates (g)	326.1	344.1	18.0 (5.2%)
Sugars (g)	121.3	127.9	6.6 (5.2%)
Proteins (g)	133.7	134.6	0.9 (0.6%)
Fats (g)	113.7	113.7	0.1 (0.1%)
Iron (mg)	20.9	21.2	0.3 (1.2%)
Vitamin C (mg)	178.7	256.1	77.4 (30.2%)
Vitamin E (mg)	21.0	21.1	0.1 (0.4%)
Vitamin A (μg)	2970.0	2971.5	1.5 (0.1%)
Total polyphenols (mg)	64.6	122.4	57.8 (47.2%)
Flavanones (mg)	45.0	88.1	43.1 (48.9%)
Flavones (mg)	0.7	15.4	14.7 (95.5%)
Flavan-3-ols (mg)	8.4	8.4	–
Flavonols (mg)	8.9	8.9	–
Isoflavones (mg)	1.6	1.6	–
Energy intake (kcal)	2820.0	2896.0	76.0 (2.6%)
Training loads			
ECOs	2400	2500	

In the first assay, the bioavailability and metabolism were compared between a control group of non-trained volunteers and the group undergoing strenuous and chronic exercise in order to know the influence of the sports performance on the nature, occurrence and excretion of flavanone metabolites in urine. The control volunteers followed a diet without polyphenol-based food and products derived, and the only source of flavonoids was that provided by the AC-juice. The diet for triathletes developed by the nutritionist provided a balanced contribution of carbohydrates, proteins, fats, vitamins and microelements where the plant-foods (included those containing flavanones) were ingested when required. In detail, control volunteers and triathletes (2500 ECOs of training load (Cejuela Anta & Esteve-Lanao, 2011) consumed a diet with equal intake of flavanones provided by juice (86.1 mg) and by juice + diet (88.1 mg), respectively for a week (Tables 1 and 2).

In the other assay, the objective was to know the evolution of the bioavailability and metabolism of flavanones between a low charge of training and a period of strenuous chronic exercise for two weeks. The triathletes followed during the first week a control diet containing 45.0 mg of flavanones (2400 ECOs) and 88.1 mg of flavanones (provided by a glass of juice + diet) (2500 ECOs) during the second week (Table 2). The study was approved by the Bioethics Committee of University Hospital of Murcia and all participants gave their written informed consent to participate in the dietary intervention study.

Volunteers' urines were collected for 24 h, starting from 8.00 a.m with the intake of the juice up to 8.00 a.m. the following day. They were immediately frozen at −80 °C until their analysis. Flavanones concentration was determined after total hydrolysis of urine with urine β-glucuronidase (sulfatase activity) (Habauzit et al., 2011; Manach et al., 2003).

2.5. UPLC-QqQ-MS/MS analysis

The flavanones metabolites in urines were analysed using a UPLC–MS/MS (Agilent Technologies, Waldbronn, Germany) with