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One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans☆

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Abstract

Strawberries are an important fruit in the Mediterranean diet because of their high content of essential nutrients and beneficial phytochemicals, which seem to exert beneficial effects in human health. Healthy volunteers were supplemented daily with 500 g of strawberries for 1 month. Plasma lipid profile, circulating and cellular markers of antioxidant status, oxidative stress and platelet function were evaluated at baseline, after 30 days of strawberry consumption and 15 days after the end of the study. A high concentration of vitamin C and anthocyanins was found in the fruits. Strawberry consumption beneficially influenced the lipid profile by significantly reducing total cholesterol, low-density lipoprotein cholesterol and triglycerides levels (−8.78%, −13.72% and −20.80%, respectively; $P<.05$) compared with baseline period, while high-density lipoprotein cholesterol remained unchanged. Strawberry supplementation also significant decreased serum malondialdehyde, urinary 8-OHdG and isoprostanes levels (−31.40%, −29.67%, −27.90%, respectively; $P<.05$). All the parameters returned to baseline values after the washout period. A significant increase in plasma total antioxidant capacity measured by both ferric reducing ability of plasma and oxygen radical absorbance capacity assays and vitamin C levels (+24.97%, +41.18%, +41.36%, respectively; $P<.05$) was observed after strawberry consumption. Moreover, the spontaneous and oxidative hemolysis were significant reduced (−31.7% and −39.03%, respectively; $P<.05$), compared to the baseline point, which remained stable after the washout period. Finally, strawberry intake significant decrease ($P<.05$) the number of activated platelets, compared to both baseline and washout values. Strawberries consumption improves plasma lipids profile, biomarkers of antioxidant status, antihemolytic defenses and platelet function in healthy subjects, encouraging further evaluation on a population with higher cardiovascular disease risk.

Keywords: Strawberry consumption; CVD risk; Platelet activation; LDL-C; Cholesterol; Triglycerides

1. Introduction

Cardiovascular diseases (CVD) are the world's "biggest killer" and major cause of death among non-transmittable diseases [1]. Substantial evidence indicates that CVD is a life course disease that begins with the development of subclinical atherosclerosis and the latent increase of risk factors prior to culminating in the diagnosed pathological state. Consequently, primary and secondary prevention of CVD and early monitoring of CVD-related risk factors are dramatically urgent public health priorities.

Diet plays a crucial role in the prevention of CVD [2], and dietary patterns based on a high consumption of fruits and vegetables, such as the Mediterranean diet [2], have been particularly associated with a

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longer life expectancy and a significative decrease of incidence and prevalence of CVD [3]. Since an imbalance between oxidative stress and endogenous/exogenous body antioxidant defenses is involved in its pathogenesis, antioxidant components of fruit and vegetables such as polyphenols have been described to possess antiatherosclerotic properties and play a role in protecting cellular macromolecules from reactive oxygen species (ROS)/reactive nitrogen species (RNS)-induced damage, and improving antioxidant status and endothelial function [3].

Among fruits, soft berries are of particular interest because of their high antioxidant phytochemical content. Even if it is difficult to prove categorically that certain foods can affect the decreased risk of CVD, several studies carried out on berry supplementation are encouraging [4–7]. A possible mechanism to explain the decreased risk is related to the high content of antioxidants, i.e., polyphenols and vitamin C, in the fruit, but growing evidence suggests that additional direct and indirect mechanisms of action appear to be involved in the protective effects provided by the fruit intake [3]. Among the mechanisms already proposed, a regular consumption of berries/strawberries may positively affect risk factors for CVD by improving the plasma lipid profile, increasing plasma antioxidant activity [8] low-density lipoprotein (LDL) resistance to oxidation [3] and improving endothelial function [9]. Significant increases in LDL peroxidation lag time [9], as well as significant decreases of total and LDL cholesterol, small LDL particles [10,11], and favourable changes in high-density lipoprotein (HDL) cholesterol and blood pressure [12,13] were reported after relatively protracted periods of strawberry consumption.

Platelets deserve special attention: in part, activated blood platelets are important in the development of CVD as they are major components of thrombi that occlude arteries [14]; therefore, favouring an optimal platelet function, *via* the reduction of diet-based platelet hyperactivity, can be considered a feasible approach for the maintenance of cardiovascular health. Impaired platelet reactivity can be found in smokers and people under stress. Hence, favouring an optimal platelet function can be considered a feasible approach for the maintenance of cardiovascular health. However, current evidence on the effects of the consumption of specific food items, such as strawberries, on platelets activation is still scarce.

In the last few years, our group has been conducted several acute and protracted strawberry consumption studies by selecting strawberry varieties particularly rich in phytochemical compounds [15–19]. Although our findings already suggested several health promoting effects of strawberries, particularly improving plasma antioxidant status and erythrocyte resistance to oxidative haemolysis in humans [16], more information is clearly needed about the effects of strawberry intake as a preventive factor of CVD. Moreover, at present there are few studies to verify the possible maintenance of the healthy effects following a nutritional intervention with strawberries (washout period).

Since the increases of markers as cholesterol, LDL cholesterol (LDL-C) and triglycerides are an alarm bell and represent risk factors for the predisposition of CVD, a more detailed study of the effect of strawberry on these markers could be an important target to elucidate how the consumption of these fruits may be beneficial in the prevention of such pathologies. Until now, our group has shown a significant improvement of oxidative status in volunteers after the consumption of strawberries, but more deep information is necessary about the effect of their consumption on specific markers of CVD, paying particular attention on lipid profile and platelet activity, markers that have never been evaluated in depth in our studies. Therefore, the aim of the present study was to assess in vivo the possible beneficial effect of a protracted consumption of “Alba” strawberry cultivar fruits on serum lipid profile, biomarkers of antioxidant status and erythrocyte resistance to oxidative hemolysis, as well as on platelet function in healthy subjects.

2. Methods

2.1. Strawberry fruit analysis

The commercial variety of "Alba" strawberry, from the strawberry breeding program of the Marche Polytechnic University, Ancona, Italy, was selected for this study. Compound extraction was carried out depending on the analysis to be performed [19]. The total phenolic content (TPC) was determined by Folin-Ciocalteu method [20], total flavonoid content (TFC) by the aluminium chloride spectrophotometric method [19] while vitamin C was analyzed by reversed-phase high-performance liquid chromatography (HPLC) [19]. Anthocyanins (ACYs) extraction and HPLC-MS/MS analysis were performed as previously described [21]. Total antioxidant capacity (TAC) was determined using Trolox Equivalent Antioxidant Capacity [22] and Oxygen Radical Absorbance Capacity (ORAC) assays [23].

All results were expressed per total fresh weight of strawberries consumed daily (500 g fresh weight [FW]) by each subject during the test as grams of gallic acid (TPC), catechin (TFC), vitamin C (Vit C) and milligrams of pelargonidin-3-glucoside (Pg-3-glc) or cyanidin-3-glucoside equivalents, while TAC values were expressed as mM of Trolox equivalents.

2.2. Subjects and study design

Twenty-three healthy volunteers (11 men and 12 women, age 27±3.2, weight 63.5 ±12.7 kg and body mass index 21.74±2.5 kg/m^2) were included in the study. The study was performed in accordance with the principles of the Declaration of Helsinki as revised in 2000; the protocol was approved by the Medicine School of Marche Polytechnic University Ethical Committee and informed written consent was obtained from each participant. Smokers and subjects declaring vitamin or other dietary supplementation, case history of allergy to strawberries or other berry fruits, history of any chronic disease possibly linked to oxidative stress and current symptoms of any acute disease were excluded from the study.

Fig. 1 shows a scheme of the study design. To standardize baseline data and to prevent dietary changes during the study, two weeks before the experiment (pre-study period), subjects began a strawberry-free and low-in-polyphenolics diet. A list of daily recommended foods, limited foods and beverages and forbidden consumption (strawberries) was provided to the subjects. At baseline, subjects started a 30-day period of consumption of 500 g of fresh strawberries per day[1] (in-study period), preferably at mid-morning and mid-afternoon between meals. Participants were advised not to change their habitual diet until the end of the study (washout) and filled out a detailed daily diet diary from the pre-study period until the end of the 30 days of strawberry supplementation. During the washout period 15 days, subjects were invited to avoid the consumption of strawberries. Energy, macronutrient and micronutrient intake of the subjects before and during the study was calculated using Funiber Nutriber software (version 1.1.1.R4). Table 1 shows the details of the energy, macro and micronutrient intake of the subjects through the baseline diet and contribution of strawberries daily dose.

2.3. Samples

Blood samples (10 mL) were collected by antecubital venipuncture into a sodium citrate vacutainer (BD Vacutainer CPTTM) from overnight fasting subjects at baseline, after a 30-day supplementation period (time 30d), and 15 days after the end of the study (washout). Plasma was isolated and stored at −80°C for biochemical studies. Morning urine samples were also collected at each time points and frozen at −80°C until analysis.

2.4. Biochemical analysis and biomarker determination of antioxidant status and oxidative stress

The enzymatic test kits were used for the determination of plasma glucose (GOD-PAP), total cholesterol (CHOD-PAD), triglycerides (GPO-PAP), high-density lipoprotein cholesterol (HDL-C) and LDL-C (HDL-C plus 3rd generation and LDL-C plus 2nd generation, respectively), while albumin was determined by colorimetric test kit (BCG). All commercial kits were purchased from Roche Diagnostics GmbH, Mannheim, Germany, and the analyses were conducted using automated clinical chemistry analyzers Roche/Hitachi. Plasma TAC was estimated by evaluating the plasma antioxidant capacity by ORAC [20] and by ferric reducing ability of plasma (FRAP) assays [24]. The serum concentrations of ascorbic and uric acids were measured by HPLC coupled to electrochemical detection [15] while the extent of plasma lipid peroxidation was estimated by HPLC-UV quantification of malondialdehyde (MDA) [25].

Evaluation of urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) was determined using a competitive in vitro enzyme-linked immunosorbent assay (ELISA) kit (JaICA, Japan). Determination of urinary isoprostanes level was carried out using an enzyme

[1] The daily dose of strawberries corresponded to the amount given to a hypothetical 63 kg individual. A few extra strawberries were respectively given to subjects with a body weight above the mean value (63 kg), in order to standardize the dose/body weight ratio.