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Cranberry juice improved antioxidant status without affecting bone quality in orchidectomized male rats

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Abstract

Background: We reported that drinking citrus juice improves bone quality in orchidectomized senescent male rats. Because cranberry juice, like citrus, is rich in nutrients and phenolic compounds, beneficial effects of citrus juice might also be seen with cranberry juice. An experiment evaluated effect of drinking cranberry juice on bone quality in orchidectomized rats.

Methods: Thirty-two 1-year-old male rats were randomized to two groups: a sham-control group ($n = 8$) and an orchidectomized group ($n = 24$). The treatments for the 4 months duration of the study were SHAM, orchidectomy (ORX), ORX + drinking either 27% or 45% cranberry juice concentrate added to drinking water. At the termination of the study, the rats were euthanized, blood was collected for plasma antioxidant status and IGF-I. The femur, tibia and the 4th lumbar were evaluated for bone quality. Total calcium and magnesium concentration in the femurs were also evaluated.

Results: ORX did not affect red blood cell (RBC)-induced hemolysis despite lowering ($p < 0.05$) plasma antioxidant capacity; reduced ($p < 0.05$) plasma IGF-I, femoral density, femoral strength, time-induced femoral fracture, bone mineral content, bone mineral area; numerically ($p = 0.07$) lowered 4th lumbar density; decreased ($p < 0.05$) trabecular connectivity, trabecular number, femoral ash; increased ($p < 0.05$) trabecular separation in comparison to the SHAM group. Drinking cranberry juice increased ($p < 0.05$) plasma antioxidant status, protected RBC against hemolysis, but had no positive effect on bone quality or bone mineral status.

Conclusions: Cranberry juice increases plasma antioxidant status without affecting bone quality.

Keywords: Cranberry juice; *Vaccinium macrocarpon*; Bone quality; Rats; Antioxidant status

Introduction

Osteoporosis is a chronic disease of aging that is characterized by micro-architectural deterioration of bone that leads to bone fragility and increase the risk of fractures (National Institute of Health, 2006). Although osteoporosis mostly occurs in women, men also experience

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osteoporosis. Previously, we reported a decrease in bone quality in male senescent rats following orchidectomy (ORX) (Deyhim et al., 2006). In the same study, we observed that the decrease in bone quality in orchidectomized rats coincided with reduced plasma antioxidant status and that increased plasma antioxidant capacity resulted in improvement of bone quality (Deyhim et al., 2006). Reactive oxidative species (ROS) have been implicated in the development of osteoporosis. Oxidative stress plays a significant role in the pathophysiology of primary male osteoporosis by increasing bone resorption (Garrett et al., 1990; Yalin et al., 2005).

Osteoclasts have been reported to generate high levels of superoxide anion and hydrogen peroxide, which modulate intra- and intercellular signaling responsible for bone loss (Lean et al., 2005). In one animal study, vitamin C injection normalized castration-induced increase in the number of osteoclasts and prevented bone loss (Lean et al., 2003).

Fruits and vegetables are rich in vitamins, minerals, and bioactive compounds with antioxidant properties that can protect against development of chronic diseases (Negri et al., 1991; Steinmetz and Potter, 1991; Keli et al., 1996; Kris-Etherton et al., 2002; Ariga, 2004). The health-promoting effects of bioactive compounds have been the subjects of numerous studies (Poulose et al., 2005; Soobrattee et al., 2005; Yu et al., 2005; Lin et al., 2006).

Feeding hesperidin, has been shown to prevent bone loss (Chiba et al., 2003). In other studies, feeding onion and Italian parsley, which are rich in flavonoid have decreased bone resorption in rats (Muhlbauer and Li, 1999; Horcajada-Molteni et al., 2000) Cranberries contain flavonoids including proanthocyanidins, anthocyanins, and flavonols, which may protect bone against resorption. The antioxidant capacity of proanthocyanidin has been reported to be stronger than vitamin C, vitamin E, and catechins (Ariga, 2004). Previously, we reported that drinking citrus juice improves bone quality in orchidectomized senescent male rats (Deyhim et al., 2006). Because cranberry juice, like citrus, is rich in nutrients and phenolic compounds, beneficial effects of citrus juice might also be seen with cranberry juice. If the benefit of cranberry juice is related to its antioxidant capacity, drinking the juice might dose-dependently improve the micro-architecture of bone in orchidectomized rats. The objective of the present study was to evaluate the efficacy of drinking cranberry juice on improving bone quality in orchidectomized senescent male rats.

Materials and methods

Animals and diet

In this experiment, 32 retired male breeder rats upon arrival at Texas A&M University-Kingsville were housed individually in an environmentally controlled laboratory for 3 days of acclimation prior to either surgical ORX or sham surgery. The animals were weighed and divided in two groups: sham (control) group ($n = 8$) and orchidectomized group ($n = 24$). Three days post-surgery when feed intake was normalized, initial body weight was taken and orchidectomized rats were assigned to one of the following three treatments to be compared with the sham (control) group; ORX group drinking 27% cranberry juice concentrate diluted in deionized water, and ORX group drinking 45% cranberry juice concentrate diluted in deionized water. Commercial cranberry drink contains 27% cranberry juice concentrate. Total phenolic and anthocyanin content of cranberry juice is reported to be 1136 ± 3.5 (mg/l gallic acid equivalents) and 2.80 (mg/l malvidin-3-glycoside equivalents), respectively (Duthie et al., 2006). Guidelines for the ethical care and treatment of animals from the Animal Care and Use Committee of Texas A&M University-Kingsville were strictly followed. The animals ate semi-purified, powdered casein-based diet, AIN-93M, for the duration of 4 months study. Food consumption was monitored every 3 days and all ORX rats were pair-fed to the mean food intake of the sham group. Table sugar (10 ± 0.5 g, 42 kcal/100 ml) and baking soda (13 g/l) were added to cranberry juice to adjust for tartness and pH. Sham (control) and ORX groups received deionized water (DI) containing table sugar adjusted for calorie (10 ± 0.5 g; 42 kcal/100 ml) and baking soda (13 g/l) was added for neutralizing pH to 7.2 ± 0.25. All rats were pair-fed to the mean food intake of the SHAM group.

Blood parameter

Four months after drinking cranberry juice, rats were euthanized and bled from the abdominal aorta. Blood samples were collected in heparinized tubes and centrifuged (4 °C) at 1500*g* for 15 min. Plasma was separated and an aliquot of plasma was refrigerated for oxidative stress-induced RBC hemolysis, an index for RBC resistance to hemolysis. The kinetics of RBC resistance to hemolysis was determined at 27 °C by continuous monitoring of changes in 450-nm absorbance. The time to reach 50% of total hemolysis ($T_{50\%\ \text{hemolysis}}$) was used for group comparisons (Blache and Prost, 1992). Plasma antioxidant capacity was evaluated using a commercially available kit (Calbiochem, San Diego, CA) as a quantitative measure of circulating antioxidant status. Plasma IGF-I was evaluated using a commercially available kit (R&D Systems, Minneapolis, MN) as a quantitative measure of bone formation.

Bone quality assessment

The femur was cleaned of soft tissue and stored in a glass vial at −20 °C. Femoral length was measured using