Anti-atherosclerotic and cardiovascular protective benefits of Brazilian nuts

Carlos K B Ferrari

1Instituto de Ciencias Biologicas e da Saude, Campus Universitario do Araguaia, Universidade Federal de Mato Grosso, Av. Valdon Varjao, 6390, Setor Industrial, Barra do Garcas, 78.600-000, MT, Brazil

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1. ABSTRACT

Brazil nuts are rich in magnesium, selenium, arginine and other amino acids, dietary fiber, tocopherols (vitamin E), phytosterols, linoleic acid, linolenic acid, sitosterols, monounsaturated and polyunsaturated fatty acids, polyphenols and other amino acids. Due to such a rich mixture of nutrients, Brazil nuts protect LDL from peroxidation, and improve endothelial function, blood pressure, lipid metabolism, and decrease endothelial inflammatory markers, DNA oxidation, and blood lipids (cholesterol, LDL, triglycerides). Here, we review and propose biological mechanisms by which bioactive compounds of Brazil nuts afford protections against atherosclerosis and cardiovascular diseases. Just a few nuts per day provide sufficient cardiovascular benefits, including protection against development and progression of atherosclerosis.

2. INTRODUCTION

Obtained from a South American tree, from Lecythisidae family, the Brazil nut (Bertholletia excelsa) is an edible oleaginous seed found in the Amazon river basin, including Brazil, Peru, Ecuador, Colombia, and Venezuela (1). Brazil nuts are rich in oil (60-70%), protein (17%), fiber, selenium (Se), magnesium (Mg), potassium (K), phosphorus (P), zinc (Zn), calcium (Ca), copper (Cu), iron (Fe), niacin, thiamine, tocopherol, and vitamin B6 (1). Although Brazil nut is considered the richest food source of Se, the value of this microelement is higher in alkaline and neutral soils and lower in acidic lands (2). In this context, Brazil nuts from the Amazon region soils are very rich in Se, whereas Brazil nuts growing in soils...
from the neighborhood states, the so-called Legal Amazon, tend to have a lower concentration of this microelement (2,3). The objective of this review is to update the relationship of dietary intake of Brazil nuts and the mechanistic role of their bioactive compounds against atherosclerosis and cardiovascular disease.

3. EPIDEMIOLOGICAL ASPECTS

The two oldest studies regarding nuts, recorded in the MEDLINE, are descriptions of *Trapa bispinosa* and *Anacardium occidentale*, respectively published in 1927 and 1930 (4,5). Fraser et al. (6) developed the Adventist Health Study and reported that dietary intake of nuts (5 times per week) was associated with a 48% decrease in the risk of mortality from coronary heart disease (6). Six years later, the Nurses’ Health Study found that regular intake of nuts diminished the risk of coronary heart disease (CHD) and non-fatal myocardial infarction (7). The PREDIMED study, a Spanish cohort study covering 7,216 men and women, concluded that frequent consumption of Brazil nut (>3 servings per week) reduced overall, cardiovascular, and cancer mortality (8). In some populations, intake of Brazil nuts is frequent (9). Some studies suggested an association between dietary intake of nuts and decreased risk of other cardiovascular diseases, stroke, and possibly type 2 diabetes mellitus (10-12).

4. ATEROPROTECTIVE EFFECT OF CONSTITUENTS OF BRAZIL NUTS

Brazil nuts are considered very nutritious, since they contain considerable amounts of fiber, macronutrients, micronutrients and bioactive compounds, such as phytosterols, phenolics, and flavonoids (1,13,14).

4.1. Dietary intake of Brazil nuts and protection against atherosclerosis

It has been suggested that frequent nut consumption is associated with decreased levels of total cholesterol (TC) in blood, improvements on blood pressure, vascular function, oxidative stress, inflammation, and also lowering of visceral adiposity and metabolic syndrome (15-20). Since 2006, Brazil nuts have been considered a rich source of bioactive compounds with antioxidant activity whose intake brings benefits to our health (16). Evaluating twenty foods, some of them regularly eaten by the Brazilian population, it was reported that Brazil nuts had the highest total antioxidant capacity *in vitro* (21). The values of the TEAC assay found in that study (21) were similar to those found in another study (22).

Beyond improvement of body antioxidant status, dietary intake of Brazil nuts also increases Se levels increasing Se-dependent GPX as showed by three Brazilian studies in pre-school children, adults, and elderly subjects (23-25). If Brazil nuts enhance body antioxidant status it also decreases peroxidative reactions such as DNA oxidation and lipid peroxidation (23, 26, 27). A Brazilian study evaluated the effects of Brazil nut supplementation (one nut per day) on blood TC and blood glucose of 130 healthy volunteers (28). Authors found that Brazil nut supplementation significantly decreased both TC and blood glucose levels with no effect on malondialdehyde (MDA), HDL fraction, LDL fraction, C-reactive protein, and triglycerides. Lipid peroxidation and an inflammatory microenvironment on subendothelial space are responsible for atherosclerosis pathogenesis, including transformation of vessel macrophages into foam cells, apoptosis and necrosis of foam cells and progression of both atherosclerosis and thrombosis (26,29-31). Dietary intake of Brazil nuts and Brazil nuts flour also decreases TC, triglycerides, apolipoprotein-A1 as well as oxidized LDL particles (32-34). Intake of defatted Brazil nuts improved GPX3 and decreased the oxidation of LDL in hyperlipidemic and hypertensive Brazilian patients (34). Farther those atheroprotective mechanisms, eating Brazil nuts has been also associated with decreased inflammatory biomarkers (IL-1, IL-6, INF-γ, TNF-α) in human subjects (35). Cardoso et al. (36) studied the possible role of Brazil nut supplementation on antioxidant and anti-inflammatory response. Authors observed that intake of Brazil nut augmented the expression of nuclear factor E2-related factor (Nrf2), a mechanism which was associated with increased antioxidant response and decreased lipid peroxidation, and decreased inflammatory response as measured by lowered
Atheroprotective effects of Brazil nuts

Table 1. Health benefits of Brazil nuts

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<thead>
<tr>
<th>Source of micronutrients</th>
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<tr>
<td>Fiber</td>
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<td>Polyphenols</td>
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<td>Fatty acids</td>
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<td>Phytosterols</td>
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<td>Sphingolipids</td>
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<td>Amino acids (arginine)</td>
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<td>Glutathione peroxidase</td>
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<td>Vitamin B1 (thiamine)</td>
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<td>Vitamin B3 (niacin)</td>
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<td>Vitamin E (tocopherol)</td>
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<tr>
<th>Antioxidant activity</th>
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<tr>
<td>Increased expression of nuclear factor E2-related factor (Nrf2)</td>
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<td>Decrease in reactive oxygen species (ROS)</td>
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<td>Decrease in reactive nitrogen species</td>
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<td>Decrease in lipid peroxidation</td>
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<td>Decrease in DNA peroxidation</td>
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<th>Anti-inflammatory activity</th>
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<td>Decrease in the blood levels of IL-1, IL-6, INF-γ, TNF-α</td>
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<td>Decrease in vascular inflammation</td>
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<th>Antidiabetic activity</th>
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<td>Decrease in blood glucose level</td>
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<td>Decrease in pancreatic load</td>
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<th>Anti-atherosclerosis activity</th>
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<td>Decrease in formation of macrophage foam cells</td>
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<td>Decrease in atherogenesis</td>
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<td>Decrease in total amount of cholesterol, triglycerides, and apolipoprotein-A in the blood</td>
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<td>Improves blood viscosity</td>
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Levels of C-reactive protein, and interleukin-6 (IL-6) in hemodialysis patients (36). Evaluating 17 obese female subjects receiving 3 to 5 units of Brazil nuts per day, this small sample randomized controlled trial reported that intake of Brazil nuts decreased TC and LDL cholesterol, triglycerides and oxidized form of LDL, effects that were accompanied by improved red blood cell viscosity, but other microvascular parameters were not significantly affected (37). Mukuddem-Petersen et al. (38), based on results of their systematic review, proposed that regular intake (≥5 times per week) of 50 to 100g of nuts may significantly reduce total cholesterol and LDL cholesterol in normal and hyperlipidemic subjects. Health benefits of eating Brazil nuts are summarized in Table 1.

4.2. Dietary fiber and atherosclerosis

Dietary intake of fiber reduced the risk of peripheral artery disease, stroke, and cardiovascular disease (30). Brazil nut is a good source of dietary
fiber (DF) (8g/100g) (14, 39) which has been associated with cardiovascular health. In the Los Angeles Atherosclerosis Study, regular intake of DF has been associated with reduced progression of atherosclerosis as measured by arterial intima-media thickness (IMT) (40). A French study showed that DF intake was negatively associated with decreased apolipoprotein B, total cholesterol (TC), blood glucose, and triglycerides (41). The highest tercile of dietary intake of linoleic acid and DF were inversely associated with blood triglyceride levels in Japanese-Brazilian descendants (42). The PREDIMED, a Spanish population study, showed that high intake of DF (>35g per day) significantly reduced the risk of carotid atherosclerosis (43). DF intake has a beneficial role on prevention of atherosclerosis and CHD, since it decreases TC and inflammation, two important factors that modify the development and progression of the disease (29, 44). Recently, it was shown that in children and adolescents, the risk of increased carotid IMT was decreased by 59% by the intake of nuts (45).

4.3. The atheroprotective effect of micronutrients of Brazil nuts

4.3.1. Magnesium (Mg)

Brazil nut is an important food source of Mg, since it contains 365mg to 393mg/100g of this microelement (39). Mg deficiency has been linked to endothelial dysfunction, arterial stiffening, atherosclerosis, mortality due to coronary heart disease mortality and sudden cardiac death (SCD) (46-51). Dietary intake of Mg reduced arterial calcification and stiffening and improves arterial flow-mediated dilation and decreases carotid IMT (50, 52).

4.3.2. Selenium (Se)

The richest source of Se in the planet is represented by Brazil nut (1-3). It is important to note that selenium content varies according to soil conditions. Brito et al. (53) reported Se values since 1. 4µg/g until 91. 69µg/g. Se is an antioxidant, e. g., a free radical scavenger, active site (selenocysteine) of the glutathione peroxidase (GPX) enzyme complex, thyroid hormone deiodinases, thioredoxin-reductases, and 25 types of selenoproteins (54-60). Se deficiency impairs antioxidant defenses by decreasing glutathione peroxidase synthesis as well as selenoproteins, plasma Se, erythrocyte Se status (54, 61), and has been linked to the pathogenesis of a heart disease, firstly described in China at 1964, the Keshan’s disease (62, 63). However, restoration of Se levels efficiently recovered cardiac, mitochondrial and cytosolic GPX values in laboratory animal studies and human studies (61, 64). In a short-term clinical trial, Se supplementation during 8 weeks were sufficient to promote reduction on fasting blood glucose and TC in human volunteers (65). In the SUBRANUT study, Se supplementation increased selenoprotein p, plasma Se, erythrocyte Se, and the levels of GPX isoforms, GPX-1 and GPX-3 (65). The same study, showed that rare single nucleotide polymorphisms were associated with decreased Se biomarkers even after supplementation, and that Se supplementation increased expression of GPX-1 only in subjects with polymorphism at rs1050450, whereas expression of selenoprotein p were lower only in subjects with rs7579 polymorphism.

4.4. Arginine and other amino acids

Brazil nut is a good source of protein and amino acids. It is rich in essential amino acids like leucine, valine, lysine, methionine, and phenylalanine, but it also contains a considerable amount of non-essential amino acids such as glycine, proline, serine, alanine. It contains also the highest arginine amounts among the oilseeds, and has a higher content of aspartic and glutamic acids (66). Some studies from the 90’s suggested that arginine could decrease formation of atherosclerotic plaques (67-69), but other studies showed no anti-atherogenic effect or even a proatherogenic effect of arginine (70-73). The study of Oomen et al. (71) demonstrated no effect of dietary arginine on CHD mortality, whereas Bahls et al. (73) reported deleterious atherosclerotic effects of dietary arginine. Many studies have been suggested that arginine can improve endothelial dysfunction in animals due to improvement of nitric oxide biosynthesis (68, 69, 74, 75), but a recent meta-analysis found no beneficial effects of arginine on endothelial function (76). However, a study reported that dietary intake of arginine, cysteine, glycine, lysine, tyrosine, and
glutamic acid was associated with diminished arterial stiffness and blood pressure values (77).

4.5. Fatty acids, phytosterols, and sphingolipids

*Bertholletia excelsa* nuts are rich food sources of both monounsaturated (omega-9=oleic acid) and polyunsaturated (n-6 or omega-6, the cis-linoleic acid) fatty acids as well as tocopherols (α and γ), and phytosterols such as β-sitosterol and stigmasterol, with considerable amounts of campesterol, another important phytosterol (78, 79). It should be emphasized that phytosterols can reduce both TC and inflammatory biomarkers decreasing the risk of atherosclerosis and thrombosis (80-82). The composition of monounsaturated, polyunsaturated fatty acids and fiber of those nuts are responsible for the protective effect against cardiovascular diseases (83). Monounsaturated and polyunsaturated fatty acids have antioxidant activity and their regular intake protects brain, heart and other tissues, organs and cells against cell degeneration, cell death and aging (84). Furthermore, both mono- and polyunsaturated fats have anti-inflammatory properties and also acts as lipid-layers that can stabilize mitochondrial membranes, improving cell respiratory function which is associated with increased lifespan and decreased risk of cardiometabolic and neurodegenerative diseases (85, 86). It is important to note that Brazil nuts are very rich in β-tocopherol (88.3%), unsaturated free fatty acids, monounsaturated fatty acids (linoleic acid-39.3%; linolenic acid-36.1%), β-sitosterol (76%) and other phytosterols (87). High dietary intake of linoleic acid was recently associated with decreased levels of blood lipids (total cholesterol and its fractions) in population of more than 340,000 subjects (88). Dietary linolenic acid is inversely associated with waist circumference, blood lipids, inflammatory biomarkers, and positively associated with HDL cholesterol levels (89, 90). Using a structured lipid containing equal proportions of linoleic and linolenic fatty acids, it was reported that this product decreased lipid liver weight, serum TC, LDL cholesterol, and triglycerides, but enhanced serum HDL cholesterol levels (91). One of the oldest studies regarding the cholesterol lowering effects of sitosterol was published in more than 60 years ago. Sitosterol administration to human subjects resulted in lowered levels of TC, phospholipids, and triglycerides (92). In 1956, one of the first studies regarding β-sitosterol supplementation on blood lipids, an experimental rabbit model of atherosclerosis, reported that β-sitosterol added to diet decreased liver cholesterol and blood TC (93). In the same year, a human experimental study observed that β-sitosterol supplementation reduced TC in blood (94). In 1963, it was reported that β-sitosterol decreased blood and arterial TC levels (95).

During many decades, other experimental animal and human studies corroborated the anti-atherosclerotic role of β-sitosterol and other phytosterols (96). Brazil nuts are also rich in stigmasterol (1). However, there is a very rare genetic condition in which phytosterols accumulate in body tissues, including in the arterial walls, the sitosterolemia is associated with high levels of cholesterol and increased risk of cardiac events (97). β-sitosterol administration in a hamster experimental model of atherosclerosis decreased by 20% the plasma cholesterol compared to cholesterol-fed group, and that phytosterol also lowered liver cholesterol (98). β-sitosterol decreased inflammatory biomarkers such as interleukin-6 and tumor necrosis factor-alpha reducing fatty liver and body adiposity (99). Brazil nuts are a rich source of food sphingolipids (100). Food sphingolipids are suggested to decrease serum LDL and to enhance HDL levels (101). Long-term dietary of sphingomyelin did no atherogenic effect, but significantly decreased atherogenesis in mice (102). Brazil nuts also have significant amounts of squalene (1). The role of squalene in atherosclerosis is still controversial. Some studies found squalene in arterial fatty plaques, whereas other studies found that squalene increase cholesterol levels, others found no effect on blood cholesterol, and other papers also reported a cholesterol lowering effect (103). This lipid has been pointed as having an antioxidant role protecting LDL from oxidation due to down-regulation of CD36 receptor expression, decreasing the risk of atherosclerosis (104). In a mice model, dietary intake of squalene was linked to enhanced levels of HDL
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cholesterol (105). A review regarding health benefits of squalene showed that this compound seems to be beneficial against atherosclerosis, since it protects lipoproteins against peroxidation, and improves HDL cholesterol levels (106). It should be assumed that more basic and clinical studies regarding to the potential roles of squalene are necessary.

4.6. Tocopherols

Although some studies did not report the occurrence of β-tocopherol in Brazil nuts, in fact both major forms of tocopherol (α, β, γ) are found in those seeds (87, 100). Vitamin E should also be considered a mitochondrial stabilizer agent. It has been observed that vitamin E deficiency was associated with increased lipid peroxidation and partially impaired mitochondrial respiration, since NADH-CoQ10 reductase and cytochrome oxidase activities were diminished in skeletal muscle cells (107). However, the same authors reported increased mitochondrial activities and lipid peroxidation in the liver. Other authors have found mitochondrial failure during liver aging in vitamin E-deficient rats (107). Far beyond its general protective effect on biological membranes (108), tocopherol blocks the oxidative decay of respiratory complex III (109). β-tocopherol is an antioxidant, membrane stabilizer, and nutrient which knowledge has been well established (110).

Tocopherols can stabilize mitochondrial and plasma membranes and protect LDL cholesterol from oxidative modification fostered by oxygen, nitrogen, and chlorine reactive species (111, 112). Beyond those activities, tocopherols can also trigger anti-inflammatory effects on liver, connective tissues, brain and other organs (113).

4.7. Polyphenols

Brazil nuts are rich in phenolic compounds (1, 78, 113). John and Shahidi (22) revealed a substantial antioxidant activity of Brazil nuts, measured by trolox equivalent antioxidant capacity test (TEAC assay), DPPH radical scavenging test, Hydroxyl radical scavenging capacity test, oxygen radical absorbance capacity (ORAC), and reducing power test. Authors also describe the following polyphenolics found in Brazil nuts: citric acid, galocatechin, catechin, vanillic acid, taxifolin, myrecetin-3-o-rhamnoside, elagic acid, and quercetin among others. Catechin can protect endothelial function reducing the expression of vascular adhesion molecules and other molecules associated with inflammatory endothelial activation (114). Elagic acid can contribute to improve HDL cholesterol and to decrease TC and LDL cholesterol (115). Quercetin feeding can modulate serum lipids, raising HDL levels, but decreasing serum triglycerides (116). Furthermore, quercetin can reduce oxidized LDL and blood pressure in human subjects (117). Polyphenols also display antioxidant, anti-inflammatory and anti-apoptotic activities which can rescue cell life, decreasing cell degeneration and death. Their ingestion displays positive impacts on reduction of both morbidity and mortality as well as improvement on life span (30, 45). Polyphenols can scavenge free radicals and reactive oxygen and nitrogen species, as well as they can reduce platelet activity, decreasing the risk of thrombosis. Furthermore, polyphenols display anti-inflammatory properties (118). Both antioxidant and anti-inflammatory activities can reduce atherogenesis, decreasing the development of fatty plaques (29, 30). Polyphenols can specifically reduce oxidation of cholesterol oxidation which is also related to decreased risk of atherosclerosis (Cory et al., 2018, Williamson, 2017). Since atherosclerosis is used to be associated with thrombosis, it is relevant to mention that polyphenols can foster the production of nitric oxide, scavenge free radicals, and inhibit platelet aggregation by multiple pathways (15, 118, 119). It is important to note that when Brazil nut is sold peeled off it is expected that the samples can have some reduction of polyphenol content (118).

Based on the studies above referred, the major anti-atherosclerotic mechanisms associated with eating Brazil nuts are represented in Figure 1.

5. SUMMARY AND PERSPECTIVES

Eating Brazil nuts can afford protection against atherosclerosis. However, this dietary intake should be accompanied by healthy eating habits which comprise a difficult issue in public health
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Figure 1. Biological mechanisms of bioactive compounds from Brazil nuts against atherosclerosis.

More research is still important to determine the possible effects of Brazil nut intake in other endothelial regulators and its effects on atherosclerosis and thrombosis risk.

6. ACKNOWLEDGEMENTS

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**Abbreviation**: CHD: coronary heart disease, DF: dietary fiber, K: potassium, Mg: magnesium, TC: total cholesterol, Se: selenium, TEAC assay = trolox equivalent antioxidant capacity

**Key Words**: Atherosclerosis, beta-sitosterol, Linolenic acid, Magnesium, Tocopherol, Selenium, Arginine, Review

**Send correspondence to**: Carlos K B Ferrari, ICBS, Campus do Araguaia, Universidade Federal de Mato Grosso (UFMT), Av. Valdon Varjao, 6390, Setor Industrial, Barra do Garças, 78. 600-000, MT, Brazil, Tel: 55663402-0717, Fax: 55663402-0718, E-mail: drcarlosferrari-ufmt@gmail.com