

Health benefits of nuts: potential role of antioxidants

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A diet rich in fruits, vegetables and minimally refined cereals is associated with lower risk for chronic degenerative diseases. Since oxidative stress is common in chronic degenerative disease, it has been assumed that dietary antioxidants may explain this protective effect. Every dietary plant contains numerous types of antioxidants with different properties. Many of these antioxidants cooperate in oxidative stress reduction in plants, and we hypothesize that many different antioxidants may also be needed for the proper protection of animal cells. To test this hypothesis, it is useful to identify dietary plants with high total antioxidant content. Several nuts are among the dietary plants with the highest content of total antioxidants. Of the tree nuts, walnuts, pecans and chestnuts have the highest contents of antioxidants. Walnuts contain more than 20 mmol antioxidants per 100 g, mostly in the walnut pellicles. Peanuts (a legume) also contribute significantly to dietary intake of antioxidants. These data are in accordance with our present extended analysis of an earlier report on nut intake and death attributed to various diseases in the Iowa Women's Health Study. We observed that the hazard ratio for total death rates showed a U-shaped association with nut/peanut butter consumption. Hazard ratio was 0.89 (CI = 0.81–0.97) and 0.81 (CI = 0.75–0.88) for nut/peanut butter intake once per week and 1–4 times per week, respectively. Death attributed to cardiovascular and coronary heart diseases showed strong and consistent reductions with increasing nut/peanut butter consumption. Further studies are needed to clarify whether antioxidants contribute to this apparent beneficial health effect of nuts.

Nuts: Antioxidants: LDL oxidation: Flavonoids: FRAP: Antioxidant defence: Oxidative damage

Nuts are not easily defined in a manner that would be both compatible with popular usage and acceptable to botanists. For example, the groundnut or peanut is a legume, and the chufa nut, which is common in south Europe and Africa, is a tuber. Some languages such as French, even lack an umbrella word equivalent to nuts. 'Noix' in French looks like one, but just means walnuts. In this text we will not only include botanically defined nuts, but will also include data on some foods which have traditionally been defined as nuts.

Nuts are highly nutritious and of prime importance for people in several regions in Asia and Africa. Most nuts contain a great deal of fat (e.g. pecan 70%, macadamia nut 66%, Brazil nut 65%, walnut 60%, almonds 55% and peanut butter 55%). Most have a good protein content (in the 10–30% range), and only a few have a very high starch content (Davidson, 1999). Recently, many nuts have also been identified as especially rich in antioxidants (Halvorsen *et al.* 2002; Wu *et al.* 2004). Nuts therefore constitute one of the most nutritionally concentrated kinds of food available. Most nuts, left in their shell, have a remarkably long shelf-life and can conveniently be stored for winter use.

The aim of this paper is first to present the importance of antioxidants and oxidative stress for human disease, and second to report new data on the antioxidant content of various nuts. We also extend the analysis of Ellsworth *et al.* (2001) concerning the association between nut intake and

death attributed to various diseases in the Iowa Women's Health Study (IWHs).

Non-enzymatic oxidative damage and oxidative stress

Free radicals and other reactive oxygen and nitrogen species (ROS and RNS) are formed as a result of normal cellular oxidative metabolic reactions. Such molecules are also formed as a consequence of diseases (e.g. inflammations) and from tobacco smoke, environmental pollutants, natural food constituents, drugs, ethanol and radiation. If not quenched by antioxidants, these highly reactive compounds will react non-enzymatically with, and potentially alter the structure and function of, several cellular or extracellular components, such as cell membranes, lipoproteins, proteins, carbohydrates, RNA and DNA (Halliwell, 1996; Sies, 1997; Beckman & Ames, 1998; Gutteridge & Halliwell, 2000; McCord, 2000).

When the critical balance between the generation of free radicals and other ROS or RNS and the antioxidant defences is unfavourable, oxidative damage can accumulate. Oxidative stress is defined as 'a condition that is characterized by accumulation on non-enzymatic oxidative damage to molecules that threaten the normal function of the cell or the organism' (Blomhoff, 2005). Compelling evidence has emerged in the last two decades demonstrating that oxidative stress is intimately involved in the pathophysiology of many seemingly unrelated types of disease. Thus, oxidative stress

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is now thought to significantly contribute to all inflammatory diseases (e.g. arthritis, vasculitis, glomerulonephritis, lupus erythematosus, adult respiratory distress syndrome), ischemic diseases (heart disease, stroke, intestinal ischemia), cancer, hemochromatosis, acquired immunodeficiency syndrome (AIDS), emphysema, organ transplantation, gastric ulcers, hypertension and preeclampsia, neurologic diseases (multiple sclerosis, Alzheimer's disease, Parkinson disease, amyotrophic lateral sclerosis, muscular dystrophy), alcoholism, smoking-related diseases and many others (see Halliwell, 1996; Sies, 1997; Beckman & Ames, 1998; Gutteridge & Halliwell, 2000; McCord, 2000; Blomhoff, 2005 for reviews).

Antioxidant defence

The term 'antioxidant' cannot be defined purely chemically; it is always related to the cellular or organismal context, and to oxidative stress. Moreover, every molecule can be both an oxidant and a reductant; this is determined by the reduction potential of the molecule with which it reacts. An antioxidant is therefore defined as 'a redox active compound that limits oxidative stress by reacting non-enzymatically with a reactive oxidant', while an antioxidant enzyme is 'a protein that limits oxidative stress by catalysing a redox reaction with a reactive oxidant' (Blomhoff, 2005).

A complex endogenous antioxidant defence system has been developed to counteract oxidative damage and oxidative stress. Such an antioxidant defence is essential for all aerobic cells. The endogenous antioxidant defence has both enzymatic and non-enzymatic components that prevent radical formation, remove radicals before damage can occur, repair oxidative damage and eliminate damaged molecules (Halliwell, 1996; Gutteridge & Halliwell, 2000; Lindsay & Astley, 2002). The endogenous antioxidant defence, which is produced by cells themselves, consists of components such as glutathione, thioredoxin and various antioxidant enzymes. Mutations in genes coding for these peptides or proteins often lead to increased incidence of oxidative stress-related diseases as well as premature death (Dalton *et al.* 2004; Selverstone *et al.* 2005).

In addition to endogenous antioxidant defence, it has been hypothesized that dietary components may also contribute to antioxidant defence either by providing redox active compounds that can directly scavenge or neutralize free radicals or other ROS and RNS, or by providing compounds that can induce the gene expression of the endogenous antioxidants (Blomhoff, 2005; Moskaug *et al.* 2005).

Dietary compounds with the ability to induce the production of endogenous antioxidants

An important antioxidant defence mechanism involves detoxification enzymes such as catalase, several types of glutathione peroxidase and superoxide dismutase, the glutathione S-transferase family, γ -glutamyl cysteine synthetase and NAD(P)H:quinone reductase (quinone reductase) (Fahey *et al.* 1997; Talalay, 2000; McEligot *et al.* 2005). Many of these enzymes are generally referred to as phase two enzymes because they catalyze the conversion of xenobiotics, mutagenic metabolites or their precursors to compounds that are more readily excreted. It is believed that if benign or non-damaging plant compounds induce the phase two enzymes, cells are more

readily able to neutralize toxic agents such as free radicals and other toxic electrophiles when they appear.

The major plant compounds believed to be able to support the antioxidant defence via this mechanism include the glucosinolates and several other sulphur-containing plant compounds. Glucosinolates are widespread plant constituents, and it is believed that glucosinolate breakdown products (such as the isothiocyanate sulphoraphane) induce phase two enzymes and are therefore responsible for the protective effects shown by Brassica vegetables (Fahey *et al.* 1997; Talalay, 2000; Lindsay & Astley, 2002). Allium vegetables contain a number of other sulphur-containing compounds that may also induce phase two enzymes. These compounds include the cysteine sulphoxides and the dithiolthiones. Like the glucosinolates, the active principles found in allium vegetables result from enzymatic degradation of the plant compounds (Fahey *et al.* 1997; Talalay, 2000; Lindsay & Astley, 2002).

Dietary plants rich in compounds that induce phase two detoxification enzymes include broccoli, Brussels sprouts, cabbage, kale, cauliflower, carrots, onions, tomatoes, spinach and garlic. The evidence for phase two enzyme inductions at ordinary intake levels of plant foods in humans is, however, limited, and the importance of this defence mechanism in the overall protection against oxidative damage is still uncertain.

Dietary compounds with the ability to directly scavenge or neutralize reactive oxidants

In addition to the well-known antioxidants, vitamin C, vitamin E and selenium, there are numerous other antioxidants in dietary plants. Carotenoids are ubiquitous in the plant kingdom, and as many as 1000 naturally occurring variants have been identified. At least sixty carotenoids occur in the fruit and vegetables commonly consumed by humans (Lindsay & Astley, 2002). Besides the provitamin A carotenoids, α - and β -carotene and β -cryptoxanthin, lycopene and the hydroxy-carotenoids (xanthophylls) lutein and zeaxanthin are the main carotenoids present in diet. Their major role in plants is related to light harvesting as auxiliary components and quenching of excited molecules that may be formed during photosynthesis (Halliwell, 1996; Gutteridge & Halliwell, 2000; Lindsay & Astley, 2002).

Phenolic compounds are also ubiquitous in dietary plants (Lindsay & Astley, 2002). They are synthesized in large varieties belonging to several molecular families, such as benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignins. Over 8000 plant phenols have been isolated. Plant phenols are antioxidants by virtue of the hydrogen-donating properties of the phenolic hydroxyl groups (Lindsay & Astley, 2002).

Glutathione, thioredoxin and many antioxidant enzymes are present in abundant amounts in the diet. However, they are not absorbed as such from the diet but broken down into their constituent amino acids by digestion. The dietary availability of sulphur amino acids can, however, modulate the cellular glutathione production (Halliwell, 1996; Gutteridge & Halliwell, 2000; Lindsay & Astley, 2002).

It was initially thought that supplementation of a single antioxidant such as ascorbic acid, α -tocopherol or β -carotene

would neutralize free radicals and other ROS or RNS and thereby avoid any oxidative damage. The first strategy for testing this hypothesis was, therefore, to study the ability of these antioxidants to inhibit oxidative damage/stress in cell-free experiments, cell cultures and experimental animals. Such experiments generated many positive results (McCord, 2000; Brigelius-Flohe *et al.* 2002; Cooper *et al.* 2004). Moreover, observational epidemiological studies generally support the hypothesis that the intake of foods rich in these antioxidants is associated with reduced oxidative stress-related diseases (Cooper *et al.* 2004; Stanner *et al.* 2004). However, large randomized intervention trials that have been conducted to further test the protective effect of individual antioxidant compounds have not been supportive (Albanes *et al.* 1996; Omenn *et al.* 1996a, 1996b; Rapola *et al.* 1997). Several reviews and meta-analyses have recently concluded that, for most forms of oxidative-stress-related diseases tested so far, there is no beneficial effect of supplemental α -tocopherol or, probably, of supplemental β -carotene and ascorbic acid (Vivekananthan, 2003; Eidelman *et al.* 2004; Shekelle *et al.* 2004). The results of intervention studies using supplements of selenium, which is not an antioxidant itself but an essential building block of the endogenous antioxidant defence, are more positive, however (Bjelakovic *et al.* 2004; Etminan *et al.* 2005).

The conclusion that antioxidant supplements do not contribute to the beneficial effects of fruits and vegetables is probably premature. One explanation could be that the beneficial health effect arises from the contribution made by other antioxidants in fruits and vegetables. There are numerous antioxidants in dietary plants, and only a small number of the antioxidants in most dietary plants are contributed by α -tocopherol, ascorbic acid and β -carotene. There is a distinct possibility that some of these antioxidants, whose role in living plants is pathogen protection and oxidative stress reduction, can do better in randomized intervention trials than α -tocopherol, ascorbic acid and β -carotene.

Antioxidant network

An alternative antioxidant hypothesis is built on the observation that antioxidants, which cooperate in an integrated manner in plant cells, may also cooperate in animal cells. A network of antioxidants with different chemical properties may, therefore, be needed for proper protection against oxidative stress (Jacobs & Steffen, 2003; Blomhoff, 2005). The concerted action from a number of dietary antioxidants should be expected from the complex physical structure that constitutes a human being. The human body, its tissues and organs, cells and macromolecules, consists of phases with a range of physical variables, anatomical subdivisions, and water- and lipid-soluble phases. Within these phases, and at interfaces between phases, there are numerous chemical variables, such as pH, ionic strength, osmolality, electrical charge and chemical concentration. These variables influence the ability of the phases to act as solvents for lipid- and water-soluble antioxidants. As some water-soluble antioxidants have low partition coefficients into a lipid-soluble phase, their entry or retention in a water-soluble phase will depend on their pKa and the pH gradient across the membrane. In addition, antioxidants with both hydrophobic and hydrophilic characteristics may be distributed between water- and

lipid-soluble phases depending on the relative contribution and stereochemistry of hydrophobic and hydrophilic substitutions. Solubility is further modified when an antioxidant is conjugated or bound into more complex substances such as proteins. Although it would be much simpler to test the protective effect of a single or of a limited number of antioxidants, we may never find such an association if it is actually true that maybe hundreds of dietary antioxidants, such as carotenoids, polyphenolic acids, sulfides, flavonoids and lignans, are bioactive and work synergistically.

One theoretical, but likely, possibility is that antioxidants with different partition coefficients recharge neighbouring antioxidants in an integrated and complementary manner. Such interactions have been proven *in vitro* for α -tocopherol, α -tocotrienol, vitamin C, lipoic acid and thiols by Packer and colleagues (Packer *et al.* 2001). Flavonoids from almonds have also been shown to act in synergy with vitamin C and vitamin E and other bioactive plant compounds to enhance LDL resistance to oxidation (Chen *et al.* 2005).

A total antioxidant food table

A diet containing a high total content of antioxidants and a variety of different antioxidants may therefore yield the best protection against oxidative-stress-related diseases. A ranked table with the total concentration of redox-active antioxidants of various foods was, therefore, considered a useful tool for testing alternative antioxidant hypotheses.

In previous studies, three methods were used to assess total antioxidant capacity in dietary plants: the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay of Miller *et al.* (1996), the ferric reducing ability of plasma (FRAP) assay of Benzie & Strain (1996), and the oxygen radical absorbance capacity (ORAC) assay of Glazer's laboratory (DeLange *et al.* 1989) and others (Cao *et al.* 1993). The TEAC and the ORAC assay are based on the antioxidant's ability to react with or neutralize free radicals generated in the assay systems, while the FRAP assay measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron) in the presence of antioxidants. Since the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half-reaction reduction potentials above that of $\text{Fe}^{3+}/\text{Fe}^{2+}$, the values in the FRAP assay express the corresponding concentration of electron-donating antioxidants.

For several reasons we selected the FRAP analysis, which measures both water- and fat-soluble antioxidants (Halvorsen *et al.* 2002; Dragland *et al.* 2003; Blomhoff, 2005). A major advantage of the FRAP method is its ability to quantify the amounts of total antioxidants or reductants in foods. Values generated by the FRAP method can, therefore, be used to calculate the total intake of antioxidants and the contributions by various food groups to total dietary intake. The FRAP assay is also the only one to directly measure antioxidants or reductants in a sample. The other assays are more indirect because they measure the inhibition of reactive species (free radicals) generated in the reaction mixture. These results also strongly depend on the type of reactive species used. The FRAP assay, in contrast, uses antioxidants as reductants in a redox-linked colorimetric reaction. Moreover, the other assays (but not FRAP) use a lag phase type of measurement. In previous experiments this has been difficult to standardize and has

generated different results between different laboratories. In the FRAP assay, pretreatment is not required, stoichiometric factors are constant and linearity is maintained over a wide range. One possible disadvantage of FRAP is the fact that it does not react with thiols, because the reduction potential for thiols is generally lower than that of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ half-reaction. However, since only limited amounts of plant glutathione are absorbed by humans (Buettner, 1993), and almost no other antioxidant thiols are present in dietary plants (one exception is garlic), the FRAP method may be suitable for assessing total antioxidants in plants. One disadvantage with all of these assays is that they measure the total antioxidant content of foods as eaten, but do not address bioavailability. This problem exists for all nutrients, however, since food tables list quantities of substances as eaten, not post-absorption.

Total amounts of antioxidants in nuts and other dietary plants

We have systematically assessed total antioxidant content (TAC) in a variety of dietary plants (Halvorsen *et al.* 2002; Dragland *et al.* 2003; Blomhoff, 2005). Foods identified by the FRAP assay as containing a high level of total antioxidants include several berries, fruits, nuts, drinks and spices. Table 1 presents TAC values for several nuts while Table 2 contains values for some other antioxidant-rich foods.

Our analyses demonstrate that walnuts contain massive amounts of antioxidants, i.e. more than 20 mmol antioxidants per 100 g. Pecans, chestnuts, peanuts and pistachios are also very rich in total antioxidants with average values of 8.3, 4.7, 2.0 and 1.3 mmol/100 g, respectively. Hazelnuts, almonds, Brazil nuts, macadamias, pine kernels and cashew nuts also contained significant amounts of total antioxidants (i.e. 0.3–0.7 mmol/100 g). Peanut butter, a common form of nut consumption, contains only approximately a quarter of the total

antioxidants of peanuts. Though not in the top ranks of antioxidant containing foods, it is still an important contributor.

Remarkably, in walnuts, most of the antioxidants are located in the pellicle and less than 10% is retained in the walnuts when the skin is removed. In most other cases, nuts without the pellicle contained less than 50% of the total antioxidants compared to nuts with the pellicle. Also, Brazil nuts and pistachios purchased without the outer shell contained much fewer antioxidants than nuts purchased with the shell. A significant portion of nut antioxidants is therefore located in the pellicle, and nuts stored with the outer shell tend to contain more antioxidants than nuts stored without the shell. The TAC of most nuts are comparable with the TAC of other antioxidant-rich foods (Table 2) and are much higher than the TAC values of foods of animal origin such as meat, cheese and milk, which contain 0.0–0.1 mmol/100 g (Blomhoff, 2005).

Based on their USDA-specified serving sizes, walnuts, pecans, chestnuts, peanuts, and peanut butter contribute 6.5, 2.4, 1.3, 0.6 and 0.2 mmol antioxidants per serving, respectively. In comparison, other foods that are rich in antioxidants, such as blueberries, cloves, coffee, dark chocolate and red wine, contribute 11.9, 9.8, 6.7, 3.8 and 3.1 mmol per serving, respectively. The contribution of nut antioxidants to total dietary intake of antioxidants may also be significant, since the daily intake of total antioxidants in cereals, fruits and berries, fruit juices, vegetables, tea and wine each contributes between 0.4–1.5 mmol TAC (Svilaas *et al.* 2004).

The results shown in Table 1 are mean results based on the analysis of several samples. The full list of results with all individual items is shown in Table 3. As we can see from this table, there are relatively large variations within each nut product. For example, TAC in walnuts with pellicle ranged from 10.8 to 33.3 mmol per 100 g. Hazelnuts and almonds with pellicle ranged from 0.5 to 1.2 mmol and 0.2 to 0.7 per 100 g, respectively. These values suggest that nut variety, cultivation conditions and/or storage conditions

Table 1. Total antioxidant content (TAC) in nuts and nut products

Products	TAC (mmol/100 g)	Serving size (g)	TAC (mmol/ serving size)
Almonds with pellicle	0.412	28.35	0.117
Almonds without pellicle	0.112	28.35	0.032
Brazil nuts with pellicle	0.249	28.35	0.071
Brazil nuts without pellicle	0.191	28.35	0.054
Brazil nuts with pellicle but purchased without outer shell	0.090	28.35	0.026
Cashews with pellicle	0.388	28.35	0.110
Chestnuts with pellicle	4.668	28.35	1.323
Chestnuts without pellicle	0.755	28.35	0.214
Hazelnuts with pellicle	0.701	28.35	0.199
Hazelnuts without pellicle	0.160	28.35	0.045
Macadamia nuts with pellicle	0.424	28.35	0.120
Peanut butter	0.571	32	0.183
Peanuts with red pellicle	1.967	28.35	0.558
Peanuts without pellicle	0.838	28.35	0.238
Pecans with pellicle	8.330	28.35	2.362
Pine kernels	0.367	28.35	0.104
Pistachios with pellicle	1.274	28.35	0.361
Pistachios with pellicles but purchased without outer shell	0.890	28.35	0.252
Walnuts with pellicle	23.073	28.35	6.541
Walnuts without pellicle	1.131	28.35	0.321

Mean values of items presented in Table 3 are given. Serving size as given by the USDA (U.S. Department of Agriculture, Agricultural Research Service, 2004. USDA National Nutrient Database for Standard Reference, Release 17).

Table 2. Total antioxidant values in other antioxidant-rich foods

Product	TAC (mmol/100 g)	Serving size (g)	TAC (mmol/serving size)
Cloves	465.3	2.1	9.8
Cinnamon	98.4	2.3	2.3
Oregano	45.0	1.0	0.5
Blueberry	8.2	145	11.9
Blackberry	5.1	144	7.3
Raspberry	3.1	123	3.8
Strawberry	2.2	166	3.6
Dark chocolate (70 % cocoa)	13.4	28.35	3.8
Red grapes	2.4	160	3.8
Orange	1.1	140 (1 fruit)	1.5
Red Wine	2.9	103 (1 glass)	3.1
Grape juice (blue)	1.6	249 (240 ml)	4.0
Orange juice	0.8	249 (240 ml)	2.0
Black coffee, filtered	2.8	240 (240 ml)	6.7
Green tea	2.5	240 (240 ml)	6.0
Black tea	0.8	240 (240 ml)	1.9

The serving size of each food is in accordance with the USDA National Nutrient Database for Standard Reference (USDA, Agricultural Research Service, 2004. USDA National Nutrient Database for Standard Reference. Release 17.)

markedly modify the content of total antioxidants. Nuts with much higher antioxidant contents could reach the market and be available for human consumption if these parameters were systematically optimized.

Types of antioxidants found in nuts

Nuts contain numerous types of antioxidants. In almonds, for example, there is a range of flavonoids, including catechins, flavonols and flavonones in their aglycone and glycoside forms (Sang *et al.* 2002; Frison-Norrie *et al.* 2002). Peanuts and pistachios contain several flavonoids and are rich in resveratrol (Lou *et al.* 2001), while walnuts contain a variety of polyphenols and tocopherols. Polyphenols in walnuts are typically non-flavonoid ellagitannins (Anderson *et al.* 2001). Alkyl phenols are found in abundance in cashews (Trevisan *et al.* 2005).

Protective effects of nut antioxidants on LDL oxidation in *in vitro* experiments and animal models

Nuts probably have favourable effects on cardiovascular diseases through several mechanisms. These effects may be mediated by their fatty acid profiles, fibre or antioxidant contents, or by a combination of these mechanism. Several recent observations suggest that nut antioxidants have interesting biological effects that may be related to a favourable effect on cardiovascular diseases (Mukuddem-Petersen *et al.* 2005). Anderson *et al.* (2001) used polyphenol-rich extracts from English walnuts and ellagic acid and studied both their ability to inhibit LDL oxidation *in vitro* and their effects on LDL alpha-tocopherol during oxidative stress: 2,2'-Azobis(2-amidino propane) hydrochloride-induced LDL oxidation was significantly inhibited by 87 and 38% with the highest concentration (1.0 micromol/l) of ellagic acid and walnut

Table 3. Variations in antioxidant content of nuts (from different countries and geographic sources)

Product	n	FRAP mmol/100 g		
		Mean \pm SD	Range	Origin
Almonds with pellicle	9	0.41 \pm 0.16	0.18–0.67	USA, Spain, Norway, Australia,
Almonds without pellicle	5	0.11 \pm 0.02	0.09–0.13	USA, Unknown, Norway
Brazil nuts with pellicle	5	0.15 \pm 0.09	0.01–0.26	USA, Norway, Unknown
Brazil nuts without pellicle	2	0.19 \pm 0.05	0.09–0.24	USA, Unknown
Cashews	3	0.39 \pm 0.18	0.23–0.64	USA
Chestnuts with pellicle	1	4.67	–	Italy
Chestnuts without pellicle	1	0.75	–	Italy
Hazelnuts with pellicle	5	0.70 \pm 0.28	0.49–1.24	USA, Norway
Hazelnuts without pellicle	1	0.16	–	USA
Macadamia nuts	4	0.42 \pm 0.12	0.25–0.59	USA, Norway, Australia
Peanut butter	6	0.57 \pm 0.16	0.31–0.83	USA, Norway
Peanuts with red pellicle	1	1.97	–	USA
Peanuts without pellicle	5	0.84 \pm 0.43	0.32–1.40	USA, Mali, China
Pecans	6	8.33 \pm 1.50	6.32–11.05	USA, Norway, Mexico
Pine kernels	5	0.37 \pm 0.29	0.08–0.77	USA, Netherlands, Norway
Pistachios	4	1.27 \pm 0.16	1.00–1.47	USA, Iran
Pistachios bought without outer shell	2	0.89 \pm 0.11	0.78–1.00	Norway, Unknown
Walnuts with pellicle	17	23.07 \pm 7.19	10.75–33.29	USA, Norway, France, Italy, Unknown
Walnuts without pellicle	7	1.13 \pm 0.64	0.46–2.58	Italy and Unknown

extract, respectively. In addition, copper-mediated LDL oxidation was inhibited by 14 and 84% in the presence of ellagic acid and walnut extract, respectively. LC-ELSD/MS analysis of the walnut extracts identified ellagic acid monomers, polymeric ellagitannins and other phenolics, mainly non-flavonoid compounds. These results demonstrate that walnut polyphenolics are effective inhibitors of *in vitro* LDL oxidation.

More recently, Chen *et al.* (2005) demonstrated that almond-pellicle flavonoids have a relatively high bioavailability in hamsters and increase the resistance of copper-mediated LDL *in vitro* and *ex vivo*. They also demonstrated that almond pellicle flavonoids act in synergy with vitamins C and E.

Hatipoglu *et al.* (2004) investigated the effect of hazelnut oil on prooxidant-antioxidant status in rabbits fed a high-cholesterol diet. As expected, a high-cholesterol diet significantly increased lipid and lipid peroxide levels in the plasma, liver and aorta and led to histopathological atherosclerotic changes in the aorta. Hazelnut oil supplementation reduced plasma, liver and aorta lipid peroxide levels and aorta cholesterol levels and ameliorated atherosclerotic lesions in the aortas of rabbits fed the high-cholesterol diet.

Nut antioxidants and human intervention studies

In a crossover trial with hypercholesterolemic patients, Zambon *et al.* (2000) showed that the mean total cholesterol level and the mean LDL cholesterol level decreased by 9.0 and 11.2%, respectively, when patients were submitted to walnut dietary interventions. Moreover, the LDL particles were enriched with polyunsaturated fatty acids from walnuts but preserved their resistance to oxidation. Ros *et al.* (2004) showed that, in moderately hypercholesterolemic patients, walnuts significantly improved oxidative stress-related vascular endothelial function. In a study with a crossover design by Iwamoto *et al.* (2002), forty healthy individuals were randomly assigned to consume a walnut diet (44–58 g/d) or a control diet for 4 weeks. Although plasma lipid levels improved significantly when subjects were fed the walnut diet (i.e. total cholesterol and serum apolipoprotein B concentrations and the ratio of LDL cholesterol to HDL cholesterol decreased significantly), LDL oxidizability was not influenced by the diets in this study.

Hyson *et al.* (2002) compared the effects of whole-almond versus almond-oil consumption on LDL oxidation in healthy men and women. Using a randomized crossover trial design, twenty-two normolipemic men and women replaced half of their fat intake with either whole almonds or almond oil for 6-week periods. Both treatments improved lipid parameters but neither treatment affected *in vitro* LDL oxidizability.

In a study by Durak *et al.* (1999), thirty healthy individuals consumed hazelnuts (1 g/d per kg body weight) in addition to their normal daily diet for 30 days. These authors observed that hazelnut supplementation improved both lipid biomarkers and oxidative stress markers (i.e. malondialdehyde levels decreased and antioxidant capacity increased).

Inflammation is often a cause or effect of oxidative stress. A recent cross-sectional epidemiologic study of the consumption of nuts and seeds (based on the sum of self-reported frequency of 'almonds, walnuts, pecans, other nuts', 'sunflower, pinyon, other seeds', and 'peanuts, peanut butter') found lower levels of the circulating inflammatory markers

C-reactive protein, interleukin-6, and fibrinogen with a higher nut consumption (Jiang *et al.* 2006). Therefore, the results of this study support the hypothesis that nut antioxidants may reduce oxidative stress and, therefore, oxidative-stress-related disease such as inflammation.

Nut/peanut butter consumption is associated with a decreased incidence of cardiovascular diseases

We have extended the analysis of Ellsworth *et al.* (2001) on the association between nut intake and death attributed to various diseases in the Iowa Women's Health Study (IWHS). As previously described (Ellsworth *et al.* 2001), this is a prospective cohort comprising 41 836 women aged 55–69 years recruited via a baseline questionnaire (including a 127-item food frequency questionnaire) mailed in 1986 and followed through to 31 December 2001. Ellsworth *et al.* reported on nut consumption (based on self-reported frequency of a single item, 'nuts') in 34 111 postmenopausal women in the first 12 years of follow-up, during which 3726 women died, 657 from coronary heart disease (CHD). After multiple adjustment, they compared two or more 28.5 g servings per week to less than one serving per month and found a 19% lower risk of death from CHD and a 12% lower risk of all-cause mortality. In the present study, we excluded women who were not postmenopausal and those with self-reported baseline heart disease or diabetes. Our study consisted of a 15-year follow-up of 31 778 women, comprising a total of 472 354 person years, with 5451 total deaths, including 1675 from cardiovascular disease (CVD) and 948 from coronary heart disease. We studied whether the risk of death due to coronary heart disease (CHD) or cardiovascular disease (CVD) is associated with the sum of self-reported 'nuts' and 'peanut butter' consumption in these women, who consume nuts as part of their normal diet.

The mean (SD) consumption of nuts/peanut butter in the IWHS was 2.37 servings/week (3.44), of which 0.75 (1.75) and 1.62 (2.81) servings per week were nuts and peanut butter, respectively. Median intakes of nuts plus peanut butter and of each item separately are given in Table 4 for four intake levels.

After adjustment for age and energy intake, the hazard ratio for total death rates showed a U-shaped association with nut/peanut butter consumption (Table 4). The hazard ratio was 0.89 (CI = 0.81–0.97) and 0.81 (CI = 0.75–0.88) for nut/peanut butter intake once per week and 1–4 times per week, respectively, but slightly higher for five or more times per week ($P = 0.003$ for quadratic trend). Death attributed to CVD and CHD showed strong and consistent reductions with increasing nut/peanut butter consumption, with hazard ratios of 0.67 (CI = 0.56–0.81) and 0.67 (CI = 0.52–0.86), respectively, for those reporting nut/peanut butter intake five or more times per week. These findings were slightly attenuated by additional adjustment. Most of the association with deaths from cardiovascular and coronary heart disease was attributable to peanut butter intake, which constituted more than two-thirds of the total nut/peanut butter intake in these data. Separate analyses confirmed that the findings for the single 'nuts' item were similar to those of Ellsworth *et al.* (2001) (data not shown). In particular, fewer than 1000 women reported consumption of nuts five

Table 4. Hazard ratios for total and CVD mortality according to category of nuts + peanut butter intake, $n = 31\ 778$, IWHS 1986–2001

	0	<1 /week	1–4 times/week	≥5 times/week	P value for trend‡	P value for quadratic trend
Median intake						
Nuts plus peanut butter, servings/week	0	0.5	1.5	7.0		
Nuts, servings/week (28.5g serving)	0	0	0.5	1.0		
Peanut butter, servings/week (1 tablespoon serving)	0	0.5	1.0	5.5		
Total death						
No of cases	895	1037	2748	771		
Person-years	67 362	88 481	250 985	65 526		
Minimal adjustment*	1	0.89 (0.81–0.97)	0.81 (0.75–0.88)	0.85 (0.77–0.94)	0.23	0.003
Multivariate adjustment†	1	0.93 (0.85–1.02)	0.88 (0.81–0.95)	0.89 (0.81–0.99)	0.26	0.09
CVD death						
No of cases	291	355	834	195		
Minimal adjustment*	1	0.95 (0.81–1.10)	0.76 (0.67–0.88)	0.67 (0.56–0.81)	0.0002	
Multivariate adjustment†	1	1.00 (0.86–1.17)	0.84 (0.73–0.96)	0.72 (0.60–0.88)	0.0008	
CHD death						
No of cases	164	207	466	111		
Minimal adjustment*	1	0.97 (0.79–1.20)	0.75 (0.63–0.90)	0.67 (0.52–0.86)	0.008	
Multivariate adjustment†	1	1.03 (0.84–1.26)	0.82 (0.68–0.98)	0.71 (0.55–0.91)	0.02	

* Adjusted for age, energy intake.

† Additionally adjusted for BMI, waist/hip ratio, education, physical activity, use of estrogens, use of multivitamin supplements, alcohol, intakes of whole grain and refined grain, red meat, fish and seafood, and total fruit and vegetable.

‡ Linear trend was tested in a separate model from the test of quadratic trend.

or more times per week, so there was very little power to study daily consumption of nuts. No significant associations were observed between nut consumption and death from cancer or other diseases (data not shown).

The inverse association between nut intake and cardiovascular and coronary heart diseases in epidemiological studies may, or may not, be associated with antioxidants. Epidemiologic studies are not ideally suited for studying the role of specific nuts or biological mechanism. These studies follow disease rates according to broad consumption categories, such as ‘nuts’ and ‘peanut butter’ in the case of the Iowa Women’s Health Study. Nevertheless, the findings presented here support the theory subscribed to in this paper, namely that the complex and rich mix of nut constituents protects against cardiovascular and perhaps other chronic diseases. Whether this protection is due to the antioxidant content of nuts cannot be ascertained from our data.

In conclusion, here we have identified several nuts as being among the dietary plants with highest contents of total antioxidants. Of the tree nuts, walnuts, pecans and chestnuts have the highest contents of antioxidants. In walnuts most of the antioxidants are located in the walnut pellicle. These data are in accordance with our present extended analysis of an earlier report on the association between nut intake and death attributed to various diseases in the Iowa Women’s Health Study (Ellsworth *et al.* 2001). Death attributed to cardiovascular and coronary heart diseases showed strong and consistent reductions with increasing nut/peanut butter consumption. Interestingly, most of the association with deaths due to cardiovascular and coronary heart disease was attributable to peanut butter intake, which constituted more than two-thirds of the total nut intake in these data. While these findings support the theory subscribed to in this paper, namely that the complex and rich mix of nut constituents are protective against cardiovascular and perhaps other chronic diseases, they are not sufficiently specific to ascertain whether such protection is specific to the antioxidant content of nuts. The area is ripe for intensive studies, including controlled intervention studies, to clarify the extent to which the antioxidant content and other biochemical features of nuts may contribute to long-term health.

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