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Walnuts (*Juglans regia*) Chemical Composition and Research in Human Health

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Walnuts are among the most widely consumed commercially grown tree nuts in the world. Many health benefits have been claimed for the consumption of these, including reduced risk of cardiovascular disease, coronary heart disease, type II diabetes treatment, and prevention and treatment of certain cancers, and the lessening of symptoms attributed to age-related and other neurological disorders. The health-promoting benefits of walnut consumption are ascribed to its fatty acid profile, which is rich in polyunsaturated fatty acids with a particularly high ω3:ω6 ratio—the highest among all the tree nuts. The content of polyphenols and other phytochemicals in walnuts, with their claimed cytotoxic properties, also make them an attractive candidate for research for the prevention of free radical-induced nucleic acid damage. Research of walnut consumption in humans and animals employing a range of data sets and statistical methods suggest that walnuts may be considered a safe potential nutraceutical or possibly pharmaceutical substance. Nevertheless, few reviews of scientific research on the proposed benefits of these nuts exist, in spite of the numerous claims attributed to them in the lay media. This brief review article attempts to disseminate much of the information surrounding walnut consumption, and human health benefits, to other scientists and the interested general reader.

Keywords Walnuts, fatty acids, phytochemicals, anti-carcinogenic, carcinolysis, cardiovascular disease, diabetes, neurological, cytotoxic

INTRODUCTION

Archaeological evidence suggests gathering and consumption of walnuts by humans occurring c.a. 7300 year BP in proximity to the Mediterranean (Carrion et al., 2007). Although the focus of this review is the species *Juglans regia* L (“English” or “Persian” walnut), some of the available scientific literature also involves data on other species within the genus *Juglans*. Figure 1 illustrates the taxonomic classification of the walnut to the level of genus.

Cultivation of walnuts commenced c.a. 4000 year BP within the ancient Greek empire. It is thought that some of the basic nutritional and medicinal knowledge pertaining to walnut consumption were appreciated during this period and possibly earlier. While the Greeks were aware of the nutritional properties of walnuts, they also found other applications for various parts of the nut such as production of dyes for hair and other fibers such as wool (Casas-Agustench et al., 2011a, 2011b). Modern global supply trends for walnuts, valued for their flavor, health, and nutritional properties, are second only to almonds and pistachios when global rates of supply for tree nuts from 2003 through 2009 are compared (International Nut and Dried Fruit Foundation, 2010). Studies into the effects of walnut consumption on human health have focused for the most part on several risk factors associated in the development of cardiovascular disease (CVD), coronary heart disease (CHD), diabetes (Davis et al., 2006; Ros, 2009; Kendall et al., 2011), and neurological disorders (Mazza et al., 2007; Joseph et al., 2009). In addition, some workers have investigated potential relationships between whole walnut kernel consumption and the prevention and treatment of specific cancers (Spaccarotella et al., 2008; Davis et al., 2012). Other workers have focused on elucidation of health effects of isolated individual compounds present at high concentrations in walnuts and other tree nuts (Simon et al., 2009). Some studies have investigated the implications of kernel oxidation, fungal growth (*Aspergillus* spp.), and resulting development of carcinogenic aflatoxins in relation to commercial storage and transportation of walnuts (Campbell et al., 2003; Singh et al., 2009). The intention of this review is to provide a summary about walnuts (*Juglans regia* L and other genera of the

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Kingdom:	*Plantae*
Order:	*Fagales*
Family:	*Juglandaceae*
Genus:	*Juglans*

Figure 1 Taxonomic classification of the walnut.

Juglandaceae), and research into human health. We have attempted to arrange this review on the basis of how compounds identified in walnuts may individually affect human health by analyzing relevant published research and comparing these with the findings from epidemiological studies involving dietary inclusion of whole walnuts. In taking this approach, we aim to provide the reader with some rationale to accompany the empirical evidence that indicates whether certain health outcomes are attributable to one or several compounds present in walnuts. It is understood that the results of several studies cannot be attributed to the action of a single molecule or group of compounds. Nevertheless, we have attempted to accommodate these research outcomes in an ordered manner where possible.

COMPOUNDS OF POTENTIAL IMPORTANCE TO HUMAN HEALTH

Walnuts contain a diverse mixture of nutrients and many phytochemical species of possible importance for human health, including the highest known levels of phenolic antioxidants (phenolic acids, flavonoids, tannins, etc.) when compared with other nut species (Espin et al., 2007; Vinson and Cai, 2011). A diagrammatic representation of the phytochemical compounds found in walnuts is provided in Figure 2. Walnut macronutrients include monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Furthermore, concentration of these provide the highest known ratios of oleic acid (OA) and alpha linolenic acid (ALA) to total fatty acid (FA) content of all tree nuts (Maguire et al., 2004). This review discusses the health implications for a range of these compounds and others identified in walnuts. In doing this, however, we reiterate that it may be the combined action of several compounds that produces positive health effects in humans. While there exists much literature related to the health benefits of walnut consumption, the published research articles often infer correlation between a compound or group thereof and the observed health outcomes. Such an approach, while true to the scientific method, is likely to be an oversimplification of the complex events that eventually lead to the ascribed health outcomes. As a result, any conclusions based on the effects of a change in the total dietary intake of a single fatty acid or other compound can be difficult to consider ab initio and caution must be applied when considering the underlying paradigm (Willett, 2012).

FATTY ACIDS (FA)

Fatty acids are found in saturated, monounsaturated, and polyunsaturated forms in all walnut cultivars of the *Juglandacaea* (Table 1, Fig. 3). The fatty acid composition of oil extracted from walnut kernels show the lowest ratios of saturated fatty acid (SFA) to other less SFAs when compared with other consumable tree nuts. The primary SFA (approximately 9% of the total fatty acids) generally found in walnut kernels are palmitic (16:0) and stearic acids (18:0) (Li et al., 2007). MUFA concentration in oil extracted from kernels (*Juglans regia* L) show the percentage ratio of MUFA to total fatty acids to be 21.2% (Maguire et al., 2004), while Li et al. (2007) determined the mean MUFA to total fatty acids content of oil extracted from kernels of two species (*J. regia* L and *J. ailanthifolia* var. *cordiformis*) to be 16.0 ($\pm$1.6)% (see Table 1 for comparison with other common nuts). Given the relatively low concentration ($<$0.2%) of the *trans* isomeric conformation, the latter figure is based on *cis* isomer MUFA concentration only.

While research on proposed relationships between specific fatty acid types and serum lipid profiles can provide information regarding CVD and CHD risk factors and several other health conditions, no experiment can be based on the human consumption of pure substances alone (Bingham and Morris, 1988). Thus, empirical data gathered from epidemiological research, especially prospective cohort studies, based on regular controlled consumption of whole walnut kernels provide at present the foremost resource for discussion regarding human health and causality. There are many such studies that have demonstrated changes in the serum lipid profiles of subjects consuming walnuts under controlled conditions (Sabate, 1993; Ros et al., 2004; Torabian et al., 2010; Din et al., 2011) that strongly correlate such changes with statistically significant ($P < \alpha$, where α is usually designated as 0.05) reductions in the risk development of CVD and CHD.

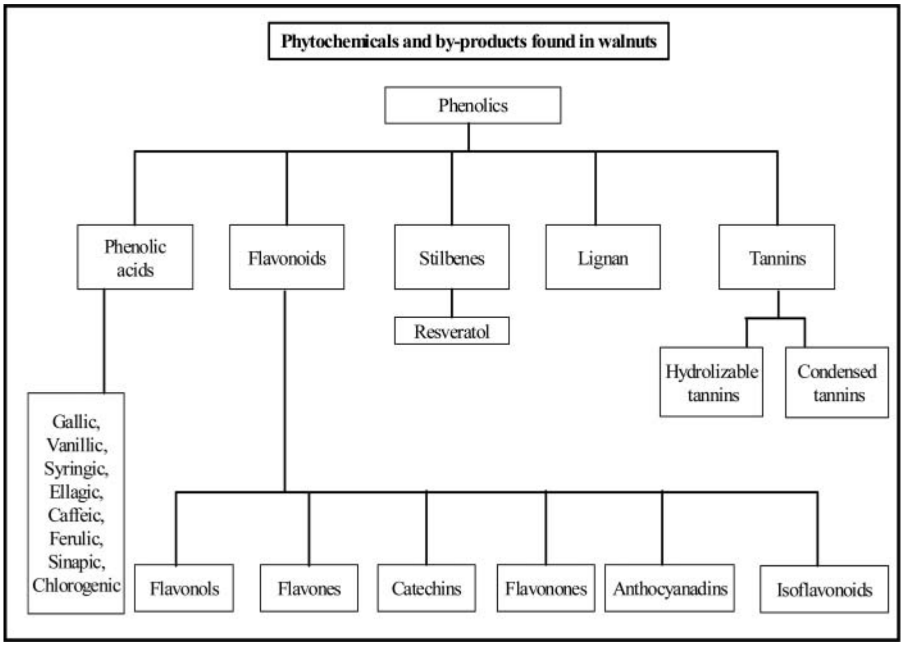

Figure 2 Phytochemicals and their by-products found in walnuts (modified from Alasalvar and Shahidi 2008).

Evidence of the unfavorable human health effects resulting from changes to serum lipid profiles following a diet high in SFA content has been generally accepted for over 50 years (Keys et al., 1957; Kuo et al., 1961; Mente et al., 2009). Nevertheless, it has been suggested that direct correlation between total quantitative SFA intake and changes to serum lipid profiles may be dependent on the type of SFA, what is consumed as the alternative to SFA (Mensink et al., 2003), and the qualitative profile of all fatty acids present in a particular food (Astrup et al., 2011; Willett, 2012). In a follow up of a longitudinal cohort study of the dietary fatty acid intake of 83, 818 women aged 34 to 59 years with no previous diagnosis of CHD, CVD, stroke, or cancer at the baseline year 1980 (Nurses Health Study, 1980), Hu et al. (1999) concluded that the type of SFA did not appear important in determining the risk of CHD.Furthermore, in a review of relationships between SFA intake and CVD risk factors, SFA molecular characteristics were found of negligible significance in reducing CVD risk factors (Micha and Mozaffarian, 2010). In addition, when replacement of total dietary SFA with carbohydrates (CHO) was assessed, no significant benefit, as measured by the ratio of total cholesterol (TC) to high-density lipoprotein (HDL), was evident (Mensink et al., 2003). Rather, a slight increase in fasting triacylglycerol (TG) concentrations was found. Nevertheless, experimental data regarding CHO replacement for SFA remains uncertain (Siri-Tarino et al., 2010).

Walnuts are a rich source of n-3 and n-6 PUFA (ω-3 and ω-6 PUFA) (Table 1). While some workers have suggested that n-6 PUFAs may be associated with an increased pro-inflammatory vascular response (Hamazaki and Okuyama, 2003; Ailhaud et al., 2008), most researchers concur that consumption of these has no adverse effects on cardiovascular health in humans (Czernichow et al., 2010). Several studies have concluded that inclusion of n-3 and n-6 PUFAs through regular consumption of walnuts (30–100 g/day) lowers CVD risk factors in non-hyperlipidaemic individuals (total cholesterol levels <5.2 mmol/L or 200 mg/dL) (Manninen et al., 1988; Feldman, 2002). Din et al. (2011) found that a moderate dietary supplementation of walnuts (15 g/day – approximately four whole kernels) (Anderson et al., 2001) produced no effect on cardiac health. While, in an animal model in vivo study designed to further understand the several mechanisms

Table 1 Comparison of fatty acid content of nuts (USDA, 2012)

Nut Type	TF (%)	SFA (%)	MUFA (%)	Total PUFA*	n-3 PUFA (%)	n-6 PUFA (%)
Walnut	65.2	9.4	13.6	72.4	14.0	58.4
Almond	76.0	19	63	24.5	1.2	23.3
Pistachio	44.4	12.1	52.5	30.4	0.7	29.7
Peanut	49.2	13.8	49.5	31.7	0.09	31.6(1)
Chestnut	2.3	17.0	34.0	39.1	4.4	34.7

*TF (%) based on 100-g sample.

involved in CVD risk factor reduction and whole walnut intake, Davis et al. (2006) monitored concentrations of aortic endothelin 1 (ET-1) and other CVD risk markers in hamsters fed with high fatty acids diets with the inclusion of walnuts. The authors concluded that ET-1 regulator levels decreased as walnut consumption increased, thereby producing "beneficial effects on CVD risk in part via the ET-1-related effects on endothelial processes" (Davis et al., 2006). Overall, the current state of play relating fatty acid intake to risk of CVD in adults (Elmadfa and Kornsteiner, 2009) concludes that a proportionally high intake of SFA over MUFA and PUFA increases CVD risk, although the authors do note the limited presence of studies that have produced conflicting outcomes.

The prevalence of individuals with diabetes in the world is increasing. Estimates based on the 216-member nations of the United Nations suggest that 285 million adults were diabetic in 2010 (Shaw et al., 2010). This total is expected to increase to approximately 440 million adults by 2030. Percentage increase by 2030 is estimated to be 69% in developing nations and 20% in developed countries (Shaw et al., 2010). This rate of change is too rapid to be considered genetic in origin (Kolb and Mandrup-Poulsen, 2010). Numerous studies have shown a direct causative link between obesity (Ford et al., 1997; Steppan et al., 2001; Barnett, 2009), low physical activity (Hu et al., 1999, 2001), psychological factors (Anderson et al., 2001; Knol et al., 2006), and the general effects of excessive SFA consumption levels on several contributing CVD risk factors as discussed above. All of these causative factors are considered associated with a pro-inflammatory response in several organs leading, in some cases, to insulin resistance (Kolb and Mandrup-Poulson, 2005; Kolb and Mandrup-Poulsen, 2010). The results of an innovative study (Jiang et al., 2002) designed to assess the possible effects of nut consumption on the development of type II diabetes in women selected from the Nurses Cohort Study, after adjustment for confounders, demonstrated that regular dietary inclusion of peanut butter reduced the overall risk of developing type II diabetes. Following this, Tapsell et al. (2004) designed a controlled trial to establish whether walnut consumption by type II non-insulin-dependent diabetic subjects produced an effect on blood lipid profiles. The group whose diet included walnut consumption of 30 g/day was found to have a statistically significant increase in HDL-cholesterol to total cholesterol and consequently a reduced risk of developing CHD (Arsenault et al., 2010). Following these interesting findings, Ma et al. (2010) carried out a randomized controlled crossover trial with non-insulin-dependant type II diabetics using a walnut-enriched ad libitum diet over an eight-week period to assess endothelial function/dysfunction (measured as flow mediated dilatation [FMD]) and risk factors for CVD. To the author's knowledge, this remains the only study designed to observe in vivo effects on vasodilation-mediated endothelium flow in humans affected by walnut consumption in type II diabetic subjects. Results demonstrated a significant ($P < 0.05$) improvement 2.2% (±1.7) in endothelial function as measured by FMD in subjects receiving a walnut-enriched diet. Interestingly, no significant changes were observed in total cholesterol and low-density lipoprotein (LDL)—cholesterol, blood serum A1C, or fasting serum insulin. The authors suggested that while walnuts posses a fatty acid profile that has been shown to be beneficial in reducing risk factors associated with CVD, results of such studies may be difficult to interpret due to confounding factors. The authors further note these limitations to the study are likely an outcome of a constrained sample size ($n = 22$), unknown patient genetic factors, duration of the disease, and what other medications patients may have received prior to the study. Interestingly, results from a randomized cross-sectional study conducted by Casas-Agustench et al. (2011a, 2011b) found patients with metabolic syndrome (MetS) placed on a supplementation diet of raw walnuts, almonds, and hazelnuts (15, 7.5, and 7.5 g/day respectively) over 12 weeks demonstrated a significant ($P < 0.05$) reduction in levels of insulin resistance as quantified by the homeostatic assessment model, HOMA (Matthews et al., 1985) along with "borderline" anti-inflammatory results.

Similarly, workers using a subset from the Prevencion con Dieta Mediterranea (PREDIMED) study, Salas-Salvado et al. (2011) conducted a randomized trial using approximately 400 non-diabetic subjects at high risk of developing CVD. The results of this study when pooled indicated that individuals given a calorie-restricted diet enriched with either virgin olive oil or nuts comprising a mix of almonds, walnuts, and hazelnuts showed a significant 52% (95% CI: 27–86) risk reduction for developing type II diabetes compared with the control group who consumed a low-fat diet. The authors similarly suggested that the high unsaturated fat dietary profile, through the provision of virgin olive oil (rich in MUFA) and mixed nuts (rich in both MUFA and PUFA), was most likely, "instrumental in achieving diabetes risk reduction" (Salas-Salvado et al., 2011).

The dietary inclusion of fatty acids suitable for prevention of cardio-related health problems associated with type II diabetes as recommended (American Diabetes Association, 2004) must take into consideration difficulties often faced by patients in achieving recommended fatty acid intake while maintaining energy intake and avoiding weight gain. In a controlled study by Gillen and Tapsell (2005) to assess the efficacy of walnut consumption (30 g/day) in achieving suitable fatty acid (PUFA) intake in type II non-insulin-dependent diabetic individuals, a statistically significant improvement in total PUFA intake was evident for the group consuming walnuts without affecting change in glycemic control or body mass index (BMI). Sabate et al. (2005) proposed that the lack of weight gain for subjects consuming 35 g/day of walnuts in a supplemented diet was likely a reflection of increased satiety levels. Coupled with this, a controlled randomized double-blind study (Brennan et al., 2010), addressing the hypothesized existence of correlation between energy expenditure, satiety, and body mass in regular consumers of walnuts and risk of type II diabetes development, found that while total dietary intake of fatty acids is higher in habitual consumers of walnuts, no correlation was found linking increased consumption of fatty acids to an increase in body mass. Nevertheless, the authors conclude that similar studies of longer duration (more than four days) are required to assess the role of walnut consumption in further elucidating its effects on insulin resistance and body mass (Brennan et al., 2010).

Several studies have attempted to determine a relationship between walnut consumption and serum levels of the hormone adiponectin and the amphipathic protein apolipoprotein A (Spranger et al., 2004; Mantzoros et al., 2006; Aronis et al., 2011).These molecules are known for their anti-inflammatory (Ouchi et al., 2004; Ohashia et al., 2012) and insulin resistance properties (Yamauchi et al., 2001; Ziemke et al., 2010). Aronis et al. (2011) in a crossover study of obese subjects with the metabolic syndrome found daily consumption of walnuts (48 g/day) over a four-day period correlated with a statistically significant increase of approximately 15% in total circulating concentration of apolipoprotein A. However, when adiponectin levels were monitored, the duration of the study (four days) was considered insufficient to allow accurate determination of any changes in total concentration of this. Nonetheless, based on changes in the apolipoprotein A concentrations, the study suggests the possibility of beneficial health outcomes for obese subjects through regular consumption of walnuts. In an animal model study Oshaghi et al. (2012) also investigated empirically the role of walnuts in expression of genes considered essential for production of proteins involved in the synthesis and metabolism of lipids. They found that levels of sterol regulatory element binding protein-1c (SREBP-1c) were significantly reduced ($P < 0.005$) in rats fed with walnuts, leading to a reduction in the synthesis of fatty acids. While levels of the protein Peroxisome Proliferator Activated Receptor α (PPARα), found to be important in reducing triacylglyceride levels (Huang et al., 2010), were shown to rise ($P < 0.05$) in the same study. In addition, the benefits of increased hepatic levels of PPARα lead to lower accumulation of lipids there, reducing further the risk of type II diabetes (Shu et al., 2000).

While the majority of research relating to walnuts, fatty acids, and human health has focused on lipid profile changes in individuals, and how these affect change in CVD, CHD, atherosclerosis, inflammation, and diabetes, workers have also investigated potential relationships between dietary consumption of walnut fatty acids, carcinogenesis, carcinolysis, and suppression of malignancy (Hardman et al., 2011; Davis et al., 2012; Nagel et al., 2012). While research into fatty acids and cancer has provided workers with numerous interesting and novel outcomes over the last 30 years, it should be recognized that many contradictions are evident in the literature (Gerber, 2009). Such contradictions most readily arise when comparing results from cohort and prospective studies and are seen when analyzing and comparing data gained from animal and human studies (Gerber, 2009). Be that as it may, when viewed with appropriate caution research involving consumption of fatty acids, such as those derived from walnuts, and their effects on cancer have provided invaluable data and illustrate the need for continuing research in this area.

Doll and Peto (1981) in an assessment of the causes of cancer in humans concluded that approximately one-third were correlated to dietary factors. Early research into potential relationships between PUFA, such as those found in high concentration in walnuts, e.g., α-linolenic acid (ALA), and various human cancer cells suggested that malignant cells were damaged in vivo by these fatty acids, while non-cancerous cells appeared unaffected (Bégin et al., 1985). The mechanism responsible for carcinolysis, while thought related to the action of PUFA desaturase enzymes, was not empirically established. In a review article commenting on evidence for correlation between dietary intake of PUFA and occurrence of certain cancers, Bartsch et al. (1999) suggest that available evidence demonstrates that high dietary intake of ω-6 PUFAs stimulates carcinogenesis, while ω-3 PUFAs produce the opposite effect. These findings are supported by several studies that have reported that high ratios of ω-6 to ω-3 increase risk of carcinogenesis (Larsson et al., 2004; Simopoulos, 2008; Williams et al., 2011; Jiang et al., 2012). It is estimated that our pre-historic ancestors' PUFA dietary intake (ω-3:ω-6), PUFA ratio, was in proportion (approximately 1) compared with the current western dietary intake ratio of approximately 0.075 ± 0.025 (Molendi-Coste et al., 2011). Of the plant-based sources of PUFAs, walnuts contain an ω-3 to ω-6 ratio of 0.19, which is approximately one order of magnitude higher compared with that of almonds at 0.02(6) and peanuts at 0.03(8) (Russo, 2009), and provide a suitable alternative source for cooking oils when compared with safflower oil (0.006) and sunflower oil (0.008).

In spite of doubts about meta-analysis techniques in accounting for heterogeneity (study-to-study variation in observed data over that expected due to deficiencies in precision present in each individual study) present when data from numerous studies investigating relationships between fatty acids and cancer are compared (Colditz et al., 1995; Thompson and Sharp, 1999; Gerber, 2009), much can still be ascertained from examination of individual studies. Larsson's et al. (2004) review article on proposed mechanisms involving the consumption of ω-3 fatty acids and cancer prevention emphasizes the need for further studies to determine which, or perhaps which combination of several proposed carcinogenic modifying avenues, are responsible for the positive results relating to cancer treatment seen in humans. Many such studies (Berquin et al., 2008; Nagel et al., 2011; Davis et al., 2012) continue to produce results of statistical significance, demonstrating that dietary consumption of walnuts may aid in the treatment and prevention of cancer – particularly those of the colon and stomach.

Another area of research that has received much attention in the lay media, especially on websites proffering "expert" health advice, is based on the neurological effects provided by walnut consumption. In a review of ω-3 fatty acids and antioxidants and their potential use in neurological and psychiatric diseases, Mazza et al. (2007) concluded that there was evidence to suggest that diets high in ω-3 fatty acids could be beneficial in the treatment of some psychiatric and neurological conditions, and specifically listed walnuts as a primary dietary source of ω-3 fatty acids. In addition, Joseph et al. (2009) also commented that walnuts potentially provide a source of compounds that improved cognitive function in aging patients, which could be useful in the treatment of diseases such as Alzheimer's. In this case, it was suggested that the polyphenolic compounds were important in this role, although no evidence was provided to justify this inference. Improvement in cognitive functioning in rats on walnut supplemented diets was observed in a laboratory investigation by Willis et al. (2009). This research demonstrated that moderate dietary walnut supplementation could significantly improve cognitive and motor performance in aged rats. The observed improvements were attributed to high levels of PUFA and polyphenolic compounds present in the supplemented walnut diets of these animals. Up until this time the only published human cross-sectional study designed to assess possible benefits in age-related cognitive function gained from a diet rich in polyphenols, antioxidants, and PUFA was conducted by Valls-Pedret et al. (2012) with Spanish subjects selected from the PREDIMED study (Martinez-Gonzalez et al., 2010). Subjects selected ($n = 447$) were aged between 55 and 80 years, were asymptomatic with no prior history of CVD, and diagnosed as possessing risk markers for development of CVD such as diabetes. The authors concluded that a diet rich in polyphenols and antioxidants was quantitatively associated with improvement in cognitive performance in elderly subjects. Interestingly, the results demonstrated positive correlation of walnut consumption with improved working memory (temporary retention and manipulation of information) and an association of increased total urinary polyphenols measured by modification of the Folin–Ciocalteu method (Medina-Remon et al., 2009) with an improved ability to recall words using an auditory verbal learning test (Rey, 1964). These findings were novel and should be followed with further research. Another similar study was performed on young adults aged between 18 and 25 years (Pribis et al., 2012). In this crossover study, walnuts were provided to the non-placebo group at a rate of 60 g/day over eight weeks. Unlike the previously mentioned study of Valls-Pedrit et al. (2012), where dietary intake and urinary concentration of polyphenols were measured, the mean blood plasma concentrations of ALA and LA were considered to be independent variables tested against several measures for memory, non-verbal reasoning, and mood. The authors concluded that no significant effect on cognitive function was produced in this study, and further suggested that benefits from a high dietary intake of walnuts may be confined to age-related cognitive decline.

An animal model study by Asadi-Shekaari et al. (2012), attempting to establish whether walnuts were of benefit in the prevention of experimental epilepsy, showed that male Wistar rats fed with a diet containing 6% walnut kernels were ($P < 0.05$) significantly likely to have an increased seizure threshold resistance. The authors tentatively ascribe these results to the presence of fatty acids and their effects in reducing the production of nitric oxide, which is thought to be involved in the induction of epileptic seizures (Bashkatova et al., 2003).

Phytochemicals

The diversity of phytochemicals found in walnuts is numerous (Fukuda et al., 2003; Li et al., 2007; Bolling et al., 2011) (Figure 2). While some workers have placed much emphasis on the many complex and poorly understood issues that remain regarding consumption of polyphenols and human health outcomes, e.g., food content, bioavailability, role of microorganisms in the body, and conflicting data between in vivo and in vitro studies (Visioli et al., 2011), these issues are outside the scope of this review. Many of the chemical compounds detected in walnuts have been shown to affect beneficial health outcomes for humans (Anderson et al., 2001; Han et al., 2006; Hardman and Ion 2008; Papoutsi et al., 2008; Carvalho et al., 2010). The majority of phytochemical-based health studies have focused on the walnut's phenolic acid (tannin derived), stillbene, tocopherol, and flavinoid types and content (Carvalho et al., 2010; Bolling et al., 2011). Some of these naturally occurring compounds display elevated levels of antioxidant activity in vitro (Fukuda et al., 2003), providing protection against carcinogenesis and atherogenic diseases (Stoner and Mukhtar, 1995; Ros et al., 2004). The chemical compounds and descriptions of relevant studies that follow are those on which research outcomes have provided unequivocal evidence of the efficacy of walnuts as a health promoting food. Although these data allude strongly to health benefits available from human consumption of walnuts, it is important to understand that the results from such studies are based on a quantitative assessment of all phenolic and other reducing compounds present in a given sample (Singleton et al., 1999) of any food

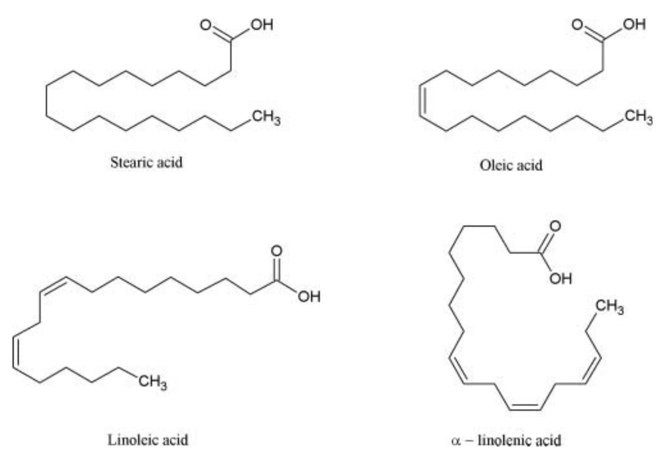

Figure 3 Saturated, monounsaturated, and polyunsaturated fatty acids in walnuts.

product. Qualitative data about these bioactive compounds must also be known to ensure scientifically safe and sensible procedures involving human dietary consumption of walnuts are established. In one regrettable case involving packaging of walnuts for human consumption, it was even suggested by the US Food and Drug Administration (USFDA that the package labeling claiming certain health benefits, although based on scientifically accurate and correct data, provided legal means to declare walnuts as a drug (News Review, 2010).

Phenolic Acids

In a study by Labuckas et al. (2008), in part designed to measure the total concentration of phenols and phenolic acids in walnuts, including the tannin pedunculagin, see Figure 4, which can be chemically degraded during digestion to yield ellagic acid and metabolites (Bate-Smith, 1972; Cerda et al., 2005; Espin et al., 2007)) in dried and ground walnut kernels, they obtained (19.1 ± 9.6) mg of GAE[†]/g ([†]Gallic Acid Equivalent) for the walnut kernel extract (value is the mean ± standard deviation) obtained from three different *Juglans regia* cultivars using methanolic solvent extraction. Interestingly, the study also found much higher GAE concentrations in the hulls (outer skin surrounding kernel) of the same cultivars at (370 ± 140) mg of GAE/g. These results suggest that the walnut seeds, whether roasted or raw, posses differential health properties, thereby implying that the processes involved in storage, transport, and processing are of importance (Sze-Tao et al., 2001). In addition, in a research by Vinson and Cai (2011), the antioxidant efficacy of several nuts was measured, where it was found that almost all varieties evaluated had greater antioxidant efficacy in the roasted form. When the total phenol content (as catechin equivalent) and the dimensionless phenol antioxidant index (PAOXI) of Vinson and Honz (1995) for walnuts are compared with foods such as fruits, vegetables, and others, walnuts posses approximately double the concentration of phenols (7.05 ± 1.00) mg catechin equivalent/g of sample with the next closest being violet carrot (*Daucus carota* ssp.) at (3.05 ± 6.00) mg catechin equivalent/g of sample and a PAOXI index of 9447 compared with the next closest, green pepper (*Capsicum annuum*) at 4355 (Gunduc and El 2003).

Proposed health benefits from human consumption of walnut-derived phenolic acids often focus on ellagic acid (Stoner and Mukhtar, 1995), as quantitative analyses show that walnuts in general contain high levels of the source ellagitannin compared with other tree nuts and peanuts (Daniel et al., 1989), while research over several decades has shown this compound to be an inhibitor of some cancer types in vitro and in animal models (Bhargava and Westfall, 1968; Wood et al., 1982; Boukharta et al., 1992; Han et al., 2006; Papoutsi et al., 2008; Umesalma and Sudhandiran, 2011). To the authors' knowledge, at the time of publication, no human in vivo studies on cancer treatment or prevention have been conducted using either pure ellagic acid or natural dietary sources such as walnuts.

Walnuts show lower levels of auto-oxidation of unsaturated fatty acids than several other nuts (Kagawa, 2001) in spite of presenting a lower concentration of α-tocopherol. It is generally thought that this is because the tannins, flavonoids, and phenolic acid content of walnuts must be sufficient to inhibit

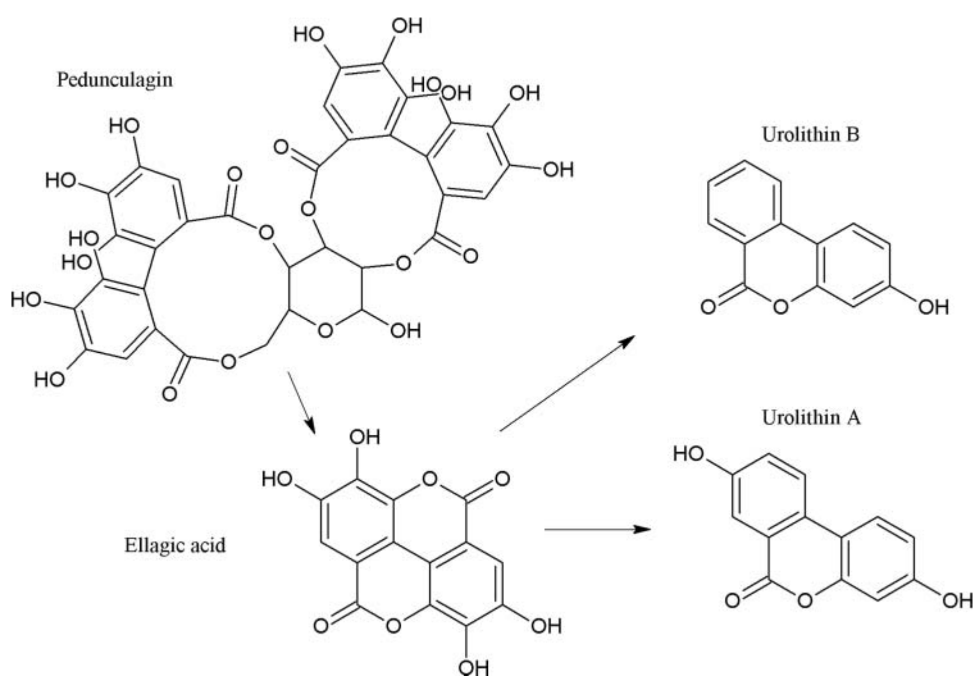

Figure 4 Chemical degradation of pedunculagin (an ellagitannin) to give ellagic acid and urolithins.

auto-oxidation (Anderson et al., 2001). Other tannins that may be responsible for this property and have been isolated from walnuts are glansrin A, glansrin B, glansrin C (Fukuda et al., 2003), and glansrin D (Ito et al., 2007). Interestingly, the content of flavinoids, present primarily as anthocyanins in walnuts is lower than that of pecans, almonds, hazelnuts, and pistachios (Chen and Blumberg, 2008). This suggests that inhibition of auto-oxidation of fatty acids in walnuts is probably due to the concentration of tannins, especially the ellagotannins.

Stilbenes

Stilbenes (1, 2-diarylethene) are present in walnuts predominantly as trans-resveratrol (trans-3,4′,-5-trihydroxystilebene) and are thought to act as a phytoalexin (*de novo* antimicrobial) protecting the plant against infection from fungi (Shakibaei et al., 2009). In laboratory trials and in vitro studies, resveratrol exhibits anti-carcinogenic, anti-inflammatory, and antioxidant properties (Smoliga et al., 2011). In vivo research on human consumption of reseveratrol has raised several questions about the complexity surrounding the numerous mechanisms by which this compound acts in providing its claimed benefits (Baur and Sinclair, 2006). Nevertheless, the concentration of resveratrol in nuts is low (∼100 μg/100 g) when compared with other naturally derived plant-based sources such as red wine (100–2000 μg/100 mL) (Bolling et al., 2010), and further clinical trials are needed to establish the efficacy and safety of this compound in humans.

Flavonoids

The flavonoid content of walnuts is comparable with the concentrations found in most fruit sources, although when compared with other tree nuts and peanuts, its concentration in walnut kernels is approximately 50% lower (Harnly et al., 2006). The flavonoids comprise many sub-groupings such as the flavones, flavonones, and others. Consequently, in excess of 5000 flavonoids have been identified in natural plant-derived sources (Harnly et al., 2006). It is currently thought that the human consumption of flavonoids may reduce the risk of developing several chronic health disorders (Gao et al., 2012). Similarly, as found with the stilbenes, while much of the laboratory-based in vitro studies indicate these compounds to be active in the prevention of carcinogenesis and other age-related neurological disorders, the in vivo data, although promising, are less clear and require further research to establish the mechanisms responsible for the human health benefits of these compounds (Park and Pezzuto, 2012).

CONCLUSIONS

Walnuts are amongst the most highly consumed tree nuts by humans worldwide. Much research focusing on their unique fatty acid profile, polyphenols, and benefits to human health provides evidence that protection against the development of many CVD-related diseases, age-related neurological disorders, and even some cancer types may be afforded through regular consumption of walnuts as part of a healthy diet. While many of the individual mechanisms related to these benefits are yet to be fully elucidated and require much further well-planned and collaborative research, most of the meta-studies based on the results gathered from human dietary consumption trials appear conclusive. That is, the consumption of walnuts as part of regular healthy diet is beneficial for humans.

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