Investigación científica publicada: Consumo de nueces y beneficios para la salud

Health Research Publications: Walnut consumption and health outcomes

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Health Research Publications

2013

Berryman CE, Grieger JA, West SG, Chen CY, Blumberg JB, Rothblat GH, Sankaranarayanan S, Kris-Etherton PM. Acute Consumption of Walnuts and Walnut Components Differentially Affect Postprandial Lipemia, Endothelial Function, Oxidative Stress, and Cholesterol Efflux in Humans with Mild Hypercholesterolemia. J Nutr. 2013 Apr 24. [Epub ahead of print]

Abstract: Walnut consumption improves cardiovascular disease risk; however, to our knowledge, the contribution of individual walnut components has not been assessed. This study evaluated the acute consumption of whole walnuts (85 g), separated nut skins (5.6 g), de-fatted nutmeat (34 g), and nut oil (51 g) on postprandial lipemia, endothelial function, and oxidative stress. Cholesterol efflux (ex vivo) was assessed in the whole walnut treatment only. A randomized, 4-period, crossover trial was conducted in healthy overweight and obese adults (n = 15) with moderate hypercholesterolemia. There was a treatment × time point interaction for triglycerides ($P < 0.01$) and increased postprandial concentrations were observed for the oil and whole walnut treatments ($P < 0.01$). Walnut skins decreased the reactive hyperemia index (RHI) compared with baseline ($P = 0.02$) such that a difference persisted between the skin and oil treatments ($P = 0.01$). The Framingham RHI was maintained with the oil treatment compared with the skins and whole nut ($P < 0.05$). There was a treatment effect for the ferric reducing antioxidant potential (FRAP) ($P < 0.01$), and mean FRAP was greater with the oil and skin treatments compared with the nutmeat ($P < 0.01$). Cholesterol efflux increased by 3.3% following whole walnut consumption in J774 cells cultured with postprandial serum compared with fasting baseline ($P = 0.02$). Walnut oil favorably affected endothelial function and whole walnuts increased cholesterol efflux. These 2 novel mechanisms may explain in part the cardiovascular benefits of walnuts.

Pan A, Sun Q, Manson JE, Willett WC, Hu FB. Walnut consumption is associated with lower risk of type 2 diabetes in women. *J. Nutr.* doi: 10.3945/jn.112.172171.

Abstract: Walnuts are rich in polyunsaturated fatty acids and have been shown to improve various cardiometabolic risk factors. We aimed to investigate the association between walnut intake and incident type 2 diabetes in 2 large cohort studies: the Nurses' Health Study (NHS) and NHS II. We prospectively followed 58,063 women aged 52–77 y in NHS (1998–2008) and 79,893 women aged 35–52 y in NHS II (1999–2009) without diabetes, cardiovascular disease, or cancer at baseline. Consumption of walnuts and other nuts was assessed every 4 y using validated food frequency questionnaires. Self reported type 2 diabetes was confirmed by a validated supplemental questionnaire. We documented a total of 5930 incident type 2 diabetes cases during 10 y of follow-up. In the multivariable-adjusted Cox proportional hazards model without body mass index (BMI), walnut consumption was associated with a lower risk of type 2 diabetes, and the HRs (95% CIs) for participants consuming 1–3 servings/mo (1 serving = 28 g), 1 serving/wk, and ≥2 servings/wk of walnuts were 0.93 (0.88–0.99), 0.81 (0.70–0.94), and 0.67 (0.54–0.82) compared with women who never/rarely consumed walnuts (P-trend < 0.001). Further adjustment for updated BMI slightly attenuated the association and the HRs (95% CIs) were 0.96 (0.90–1.02), 0.87 (0.75–1.01), and 0.76 (0.62–0.94), respectively (P-trend = 0.002). The consumption of total nuts (P-trend < 0.001) and other tree nuts (P-trend = 0.03) was also inversely associated with risk of type 2 diabetes, and the associations were largely explained by BMI. Our results suggest that higher walnut consumption is associated with a significantly lower risk of type 2 diabetes in women.

2012

Carey AN, Fisher DR, Joseph JA, Shukitt-Hale B. The ability of walnut extract and fatty acids to protect against the deleterious effects of oxidative stress and inflammation in hippocampal cells. *Nutritional Neuroscience*. Epub December 4, 2012.

Abstract: Previous research from our lab has demonstrated that dietary walnut supplementation protects against agerelated cognitive declines in rats; however, the cellular mechanisms by which walnuts and polyunsaturated fatty acids (PUFAs) may affect neuronal health and functioning in aging are undetermined. Objectives:We assessed if pretreatment of primary hippocampal neurons with walnut extract or PUFAs would protect cells against dopamine- and lipopolysaccharide-mediated cell death and calcium dysregulation. Methods: Rat primary hippocampal neurons were pretreated with varying concentrations of walnut extract, linoleic acid, alpha-linolenic acid, eicosapentaenoic acid, or docosahexaenoic acid prior to exposure to either dopamine or lipopolysaccharide. Viability was assessed using the Live/Dead Cellular Viability/Cytotoxicity Kit. Also, the ability of the cells to return to baseline calcium levels after depolarization was measured with fluorescent imaging. Results: Results indicated that walnut extract, alpha-linolenic acid, and docosahexaenoic acid provided significant protection against cell death and calcium dysregulation; the effects were pretreatment concentration dependent and stressor dependent. Linoleic acid and eicosapentaenoic acid were not as effective at protecting hippocampal cells from these insults. Discussion: Walnut extract and omega-3 fatty acids may protect against age-related cellular dysfunction, but not all PUFAs are equivalent in their beneficial effects.

Chiang YL, Haddad E, Rajaram S, Shavlik D, Sabaté J. The effect of dietary walnuts compared to fatty fish on eicosanoids, cytokines, soluble endothelial adhesion molecules and lymphocyte subsets: a randomized, controlled crossover trial. *Prostaglandins Leukot Essent Fatty Acids.* 2012 Oct;87(4-5):111-7. doi:10.1016/j.plefa.2012.07.007. Epub 2012 Sep 5.

Abstract: We tested the hypothesis that walnut consumption can exert effects on markers of inflammation and endothelial activation similar to those produced by fish consumption. In a crossover dietary intervention trial, 25 normal to mildly hyperlipidemic men and women were randomly assigned to one of three isoenergetic diets: a walnut diet incorporating 42.5g of walnuts per 10.1mJ 6 times per week (1.8% of energy n-3 fat); a fish diet providing 113g of fatty fish per 10.1mJ 2 times per week (0.8% of energy n-3 fat), or a control diet (no nuts or fish, 0.4% of energy n-3 fat) for 4 weeks on each diet. Both the walnut and fish diets inhibited circulating concentrations of prostaglandin E metabolite (PGEM) and 11-dehydro thromboxane B2, but demonstrated no effect on blood interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α¯ (TNF-α¯), and C-reactive protein (CRP) or the number of circulating lymphocyte subsets. On the walnut diet the proportion of plasma phospholipid α¯-linolenic acid (ALA) increased 140% and arachidonic acid (AA) decreased 7% compared to both the control and fish diets. The proportion of plasma phospholipid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased about 200% and 900% respectively on the fish diet relative to either the control or walnut diet. The walnut diet inhibited E-selectin by 12.7% relative to the fish diet, and the fish diet inhibited secretory intercellular adhesion molecule-1 (s-ICAM-1) by 4.5% relative to the control diet. Both walnuts and fish in commonly consumed amounts may have modest albeit distinct effects on circulating adhesion molecules.

Dalamaga M, Diakopoulos KN, Mantzoros CS. The role of adiponectin in cancer: a review of current evidence. *Endocr Rev.* 2012 Aug;33(4):547-94. Epub 2012 Apr 30.

Abstract: Excess body weight is associated not only with an increased risk of type 2 diabetes and cardiovascular disease (CVD) but also with various types of malignancies. Adiponectin, the most abundant protein secreted by adipose tissue, exhibits insulin-sensitizing, antiinflammatory, antiatherogenic, proapoptotic, and antiproliferative properties. Circulating adiponectin levels, which are determined predominantly by genetic factors, diet, physical activity, and abdominal adiposity, are decreased in patients with diabetes, CVD, and several obesity-associated cancers. Also, adiponectin levels are inversely associated with the risk of developing diabetes, CVD, and several malignancies later in life. Many cancer cell lines express adiponectin receptors, and adiponectin in vitro limits cell proliferation and induces apoptosis. Recent in vitro studies demonstrate the antiangiogenic and tumor growth-limiting properties of adiponectin. Studies in both animals and humans have investigated adiponectin and adiponectin receptor regulation and expression in several cancers. Current evidence supports a role of adiponectin as a novel risk factor and potential diagnostic and prognostic biomarker in cancer. In addition, either adiponectin per se or medications that increase adiponectin levels or up-regulate signaling pathways downstream of adiponectin may prove to be useful anticancer agents.This review presents the role of adiponectin in carcinogenesis and cancer progression and examines the pathophysiological mechanisms that underlie the association between adiponectin and malignancy in the context of a dysfunctional adipose tissue in obesity. Understanding of these mechanisms may be important for the development of preventive and therapeutic strategies against obesity-associated malignancies.

Davis PA, Vasu VT, Gohil K, Kim H, Khan IH, Cross CE, Yokoyama W. A high-fat diet containing whole walnuts (Juglans regia) reduces tumour size and growth along with plasma insulin-like growth factor 1 in the

transgenic adenocarcinoma of the mouse prostate model. *Br J Nutr.* 2012 Nov 28;108(10):1764-72. doi: 10.1017/S0007114511007288.

Abstract: Prostate cancer (PCa) has been linked to fat intake, but the effects of both different dietary fat levels and types remain inconsistent and incompletely characterised. The effects on PCa in the transgenic adenocarcinoma of the mouse prostate (TRAMP) cancer model of an elevated fat (20 % of energy as fat) diet containing 155 g of whole walnuts were compared to those of an elevated fat (20 % of energy as soyabean oil) diet with matched macronutrients, tocopherols as well as a low-fat (8 % of energy as soyabean oil) diet. Mice, starting at 8 weeks of age, consumed one of the three different diets ad libitum; and prostates, livers and blood were obtained after 9, 18 or 24 weeks of feeding. No differences were observed in whole animal growth rates in either high-fat (HF) diet group, but prostate tumour weight and growth rate were reduced in the walnut diet group. Walnut diet group prostate weight, plasma insulin-like growth factor 1, resistin and LDL were lower at 18 weeks, while no statistically significant prostate weight differences by diet were seen at 9 or 24 weeks. Multiple metabolites in the livers differed by diet at 9 and 18 weeks. The walnut diet's beneficial effects probably represent the effects of whole walnuts' multiple constituents and not via a specific fatty acid or tocopherols. Moreover, as the two HF diets had dissimilar effects on prostate tumour growth rate and size, and yet had the same total fat and tocopherol composition and content, this suggests that these are not strongly linked to PCa growth.

Heuvel JP, Belda BJ, Hannon DB, Kris-Etherton PM, Grieger JA, Zhang J, Thompson JT. Mechanistic examination of walnuts in prevention of breast cancer. *Nutr Cancer.* 2012 Oct;64(7):1078-86. doi:10.1080/01635581.2012.717679.

Abstract: Walnuts contain bioactive molecules that may contribute to their beneficial effects, including alpha-linolenic acid (ALA) and phytosterols. In these studies, extracts of walnut, purified compounds, or postprandial serum were examined for effects on breast cancer cell proliferation and gene expression. Extracts derived from walnut oil decreased proliferation of MCF-7 cells, as did ALA and β-sitosterol. The gene expression response of ALA in the mouse breast cancer cell line TM2H indicates this molecule has multiple cellular targets with peroxisome proliferator-activated receptor (PPAR) target genes, liver X receptor (LXR), and farnesoid X receptor (FXR) target genes being affected. In transactivation assays, walnut oil extracts increased activity of FXR to a greater extent than the other tested nuclear receptors. When examined separately, walnut components ALA and β-sitosterol were the most efficacious activators of FXR. When serum from individuals fed walnut components were applied to MCF-7 cells, there was a correlation between body mass index and breast cancer cell proliferation in vitro. Taken together, these data support an effect of walnut and its bioactive constituents on mammary epithelial cells and that multiple molecular targets may be involved.

Moon HS, Liu X, Nagel JM, Chamberland JP, Diakopoulos KN, Brinkoetter MT, Hatziapostolou M, Wu Y, Robson SC, Iliopoulos D, Mantzoros CS.Salutary effects of adiponectin on colon cancer: in vivo and in vitro studies in mice. *Gut.* 2012 Jun 26.

Background: Obesity and a high-fat diet are associated with the risk and progression of colon cancer. Low adiponectin levels may play an important role in the development of colon and other obesity-related malignancies. No previous studies have directly investigated the mechanistic effects of adiponectin on colon cancer in the settings of obesity, a high-fat diet and/or adiponectin deficiency. Objective: To investigate the effects of adiponectin on the growth of colorectal cancer in adiponectin-deficient or wild-type-C57BL/6 mice fed a low-fat or high-fat diet. Results: Mice fed a high-fat-diet gained more weight and had larger tumours than mice fed a low-fat-diet. Adiponectin administration suppressed implanted tumour growth, causing larger central necrotic areas. Adiponectin treatment also suppressed angiogenesis assessed by CD31 staining and VEGFb and VEGFd mRNA expression in tumours obtained from mice fed a high-fat-diet and from adiponectin-deficient mice. Adiponectin treatment decreased serum insulin levels in mice on a high-fat-diet and increased serum-interleukin (IL)-12 levels in adiponectin-deficient mice. In vitro, it was found that adiponectin directly controls malignant potential (cell proliferation, adhesion, invasion and colony formation) and regulates metabolic (AMPK/S6), inflammatory (STAT3/VEGF) and cell cycle (p21/p27/p53/cyclins) signalling pathways in both mouse MCA38 and human HT29, HCT116 and LoVo colon cancer cell lines in a LKB1-dependent way. Conclusion: These new mechanistic and pathophysiology studies provide evidence for an important role of adiponectin in colon cancer. The data indicate that adiponectin or analogues might be useful agents in the management or chemoprevention of colon cancer.

Pan A, Chen M, Chowdhury R, Wu JH, Sun Q, Campos H, Mozaffarian D, Hu FB. α-Linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. Am J Clin Nutr. 2012 Oct 17.

BACKGROUND: Prior studies of α-linolenic acid (ALA), a plant-derived omega-3 (n-3) fatty acid, and cardiovascular disease (CVD) risk have generated inconsistent results. OBJECTIVE: We conducted a meta-

analysis to summarize the evidence regarding the relation of ALA and CVD risk. DESIGN: We searched multiple electronic databases through January 2012 for studies that reported the association between ALA (assessed as dietary intake or as a biomarker in blood or adipose tissue) and CVD risk in prospective and retrospective studies. We pooled the multivariate-adjusted RRs comparing the top with the bottom tertile of ALA using random-effects meta-analysis, which allowed for between-study heterogeneity. RESULTS: Twenty-seven original studies were identified, including 251,049 individuals and 15,327 CVD events. The overall pooled RR was 0.86 (95% CI: 0.77, 0.97; $I^2 = 71.3\%$). The association was significant in 13 comparisons that used dietary ALA as the exposure (pooled RR: 0.90; 95% CI: 0.81, 0.99; $I^2 = 49.0\%$), with similar but nonsignificant trends in 17 comparisons in which ALA biomarkers were used as the exposure (pooled RR: 0.80; 95% CI: 0.63, 1.03; $I^2 = 79.8\%$). An evaluation of mean participant age, study design (prospective compared with retrospective), exposure assessment (self-reported diet compared with biomarker), and outcome [fatal coronary heart disease (CHD), nonfatal CHD, total CHD, or stroke] showed that none were statistically significant sources of heterogeneity. CONCLUSIONS: In observational studies, higher ALA exposure is associated with a moderately lower risk of CVD. The results were generally consistent for dietary and biomarker studies but were not statistically significant for biomarker studies. However, the high unexplained heterogeneity highlights the need for additional well-designed observational studies and large randomized clinical trials to evaluate the effects of ALA on CVD.

Poulose SM, Bielinski DF, Shukitt-Hale B. Walnut diet reduces accumulation of polyubiquitinated proteins and inflammation in the brain of aged rats. *J Nutr Biochem*. 2012 Aug 20.

Abstract: An increase in the aggregation of misfolded/damaged polyubiquitinated proteins has been the hallmark of many age-related neurodegenerative diseases. The accumulation of these potentially toxic proteins in brain increases with age, in part due to increased oxidative and inflammatory stresses. Walnuts, rich in omega fatty acids, have been shown to improve memory, cognition and neuronal effects related to oxidative stress (OS) and inflammation (INF) in animals and human trials. The current study found that feeding 19-month-old rats with a 6% or 9% walnut diet significantly reduced the aggregation of polyubiquitinated proteins and activated autophagy, a neuronal housekeeping function, in the striatum and hippocampus. Walnut-fed animals exhibited up-regulation of autophagy through inhibiting phosphorylation of mTOR, up-regulating ATG7 and Beclin 1, and turnover of MAP1BLC3 proteins. The clearance of polyubiquitinated protein aggregates such as p62/SQSTM1 was more profound in hippocampus, a critical region in the brain involved in memory and cognitive performance, than striatum. The clearance of ubiquitinated aggregates was in tandem with significant reductions in OS/INF, as indicated by the levels of P38-MAP kinase and phosphorylations of nuclear factor kappa B and cyclic AMP response element binding protein. The results demonstrate the effectiveness of a walnut-supplemented diet in activating the autophagy function in brain beyond its traditionally known antioxidant and anti-inflammatory benefits.

Robbins, WA, Lin X, FitzGerald, LZ, Esguerra S, Henning, SM, Carpenter, CL. Walnuts improve semen quality in men consuming a Western-style diet: randomized control dietary intervention Trial. *Biol Reprod.* 2012 Aug 15 [Epub ahead of print].

Purpose: We tested the hypothesis that 75 gm of whole-shelled walnuts/day added to a Western-style diet of healthy young men would beneficially affect semen quality. Methods: A randomized, parallel two-group, dietary intervention trial with single-blind masking of outcome assessors, was conducted with 117 healthy men, age 21 – 35 years, who routinely consumed a Western-style diet. Primary outcome evaluated was improvement from baseline to 12 weeks in conventional semen parameters and sperm aneuploidy. Secondary endpoints included blood serum and sperm fatty acid (FA) profiles, sex hormones, and serum folate. Conclusions: The group consuming walnuts (n=59) experienced improvement in sperm vitality, motility, and morphology and the group continuing their usual diet but avoiding tree nuts (n=58) saw no change. Comparing differences from baseline between the groups, significance was found for vitality p=0.003, motility p=0.009, and morphology (normal forms) p=0.04. Serum FA profiles improved in the walnut group with increases in omega-6 (p=0.0004) and omega-3 (p=0.0007) but not the control group. Only the plant source of omega-3, alpha-linolenic acid (ALA), increased (p=0.0001). Sperm aneuploidy was inversely correlated with sperm ALA, particularly sex chromosome nullisomy (-0.41, p=0.002). Findings demonstrated that walnuts added to a Western-style diet improved sperm vitality, motility and morphology.

Stendell-Hollis NR, Thompson PA, West JL, Wertheim BC, Thomson CA. A comparison of Mediterranean-Style and MyPyramid Diets on weight loss and inflammatory biomarkers in postpartum breastfeeding women. *J Womens Health (Larchmt)*. 2012 Dec 31. [Epub ahead of print]

Abstract Background: Of postpartum women, 15%-20% retain≥5 kg of their gestational weight gain, increasing risk for adult weight gain. Postpartum women are also in a persistent elevated inflammatory state. Both factors

could increase the risk of obesity-related chronic disease. We hypothesized that breastfeeding women randomized to a Mediterranean-style (MED) diet for 4 months would demonstrate significantly greater reductions in body weight, body fat, and inflammation than women randomized to the U.S. Department of Agriculture's (USDA) MyPyramid diet for Pregnancy and Breastfeeding (comparison diet). Methods: A randomized, controlled dietary intervention trial was conducted in 129 overweight (body mass index [BMI] 27.2±4.9 kg/m(2)), mostly exclusively breastfeeding (73.6%) women who were a mean 17.5 weeks postpartum. Dietary change was assessed using a validated Food Frequency Questionnaire (FFQ) before and after intervention as well as plasma fatty acid measures (gas chromatography/flame ionization detector [GC/FID]). Anthropometric measurements and biomarkers of inflammation, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), also were assessed at baseline and 4 months via enzyme-linked immunosorbent assay (ELISA). Results: Participants in both diet groups demonstrated significant ($p<0.001$) reductions in body weight (-2.3±3.4 kg and -3.1±3.4 kg for the MED and comparison diets, respectively) and significant ($p\leq0.002$) reductions in all other anthropometric measurements; no significant between-group differences were shown as hypothesized. A significant decrease in TNF-α but not IL-6 was also demonstrated in both diet groups, with no significant between-group difference. Conclusions: Both diets support the promotion of postpartum weight loss and reduction in inflammation (TNF-α) in breastfeeding women.

Zhang J, Kris-Etherton PM, Thompson JT, Hannon DB, Gillies PJ, Vanden Heuvel JP. Alpha-linolenic acid increases cholesterol efflux in macrophage-derived foam cells by decreasing stearoyl CoA desaturase 1 expression: evidence for a farnesoid-X-receptor mechanism of action. *J Nutr Biochem.* 2012 Apr;23(4):400-9.

Increased cholesterol efflux from macrophage-derived foam cells (MDFCs) is an important protective mechanism to decrease lipid load in the atherosclerotic plaque. Dietary alpha-linolenic acid (ALA), an omega-3 polyunsaturated fatty acid (PUFA), decreases circulating cholesterol, but its role in cholesterol efflux has not been extensively studied. Stearoyl CoA desaturase 1 (SCD1) is the rate-limiting enzyme in the synthesis of monounsaturated fatty acids (MUFAs). Endogenous MUFAs are preferentially incorporated into triglycerides, phospholipids and cholesteryl ester, which are abundant in atherosclerotic plaque. This study investigated the mechanisms by which ALA regulated SCD1 and subsequent effect on cholesterol storage and transport in MDFCs. Small interfering RNA (siRNA) also was applied to modify SCD1 expression in foam cells. Alpha-linolenic acid treatment and SCD1 siRNA significantly decreased SCD1 expression in MDFCs. The reduction of SCD1 was accompanied with increased cholesterol efflux and decreased intracellular cholesterol storage within these cells. Alpha-linolenic acid activated the nuclear receptor farnesoid-X-receptor, which in turn increased its target gene small heterodimer partner (SHP) expression, and decreased liver-X-receptor dependent sterol regulatory element binding protein 1c transcription, ultimately resulting in repressed SCD1 expression. In conclusion, repression of SCD1 by ALA favorably increased cholesterol efflux and decreased cholesterol accumulation in foam cells. This may be one mechanism by which dietary omega-3 PUFAs promote atherosclerosis regression.

2011

Aronis KN, Vamvini MT, Chamberland JP, Sweeney LL, Brennan AM, Magkos F, Mantzoros CS. Short-term walnut consumption increases circulating total adiponectin and apolipoprotein A concentrations, but does not affect markers of inflammation or vascular injury in obese humans with the metabolic syndrome: data from a double-blinded, randomized, placebo-controlled study. *Metabolism.* 2012 Apr;61(4):577-82.

Long-term consumption of walnuts is associated with lower cardiovascular disease risk in epidemiological studies, possibly through improvements in lipid profile and endothelial function. It remains to be elucidated how soon after initiation of walnut consumption beneficial effects on lipid profile and biomarkers of inflammation or vascular injury can be observed. Fifteen obese subjects (9 men and 6 women; age, 58 ± 2.5 years; body mass index, 36.6 ± 1.7 kg/m(2)) with the metabolic syndrome participated as inpatients in a randomized, double-blinded, placebo-controlled crossover study involving short-term placebo or walnut-enriched diet (48 g/d for 4 days). Apolipoproteins and markers of inflammation and vascular injury were measured before and after consumption of the experimental diets. Consumption of walnuts was associated with a statistically significant increase in serum apolipoprotein A concentrations ($P = .03$), but did not affect circulating levels of fetuin A, resistin, C-reactive protein, serum amyloid A, soluble intercellular adhesion molecules 1 and 3, soluble vascular cell adhesion protein 1, interleukins 6 and 8, tumor necrosis factor α, E-selectin, P-selectin, and thrombomodulin. Four days of walnut consumption (48 g/d) leads to mild increases in apolipoprotein A concentrations, changes that may precede and lead to the beneficial effects of walnuts on lipid profile in obese subjects with the metabolic syndrome.

Hardman WE, Ion G, Akinsete JA, Witte TR. Dietary walnut suppressed mammary gland tumorigenesis in the C(3)1 TAg mouse. *Nutr Cancer.* 2011;63(6):960-70.

Walnuts contain multiple ingredients that, individually, have been shown to slow cancer growth, including omega-3 fatty acids, antioxidants, and phytosterols. In previous research, consumption of walnuts has slowed the growth of implanted breast cancers. We wanted to determine whether regular walnut consumption might reduce the risk for developing cancer. Homozygous male C(3)1 TAg mice were bred with female SV129 mice consuming either the control AIN-76 diet or the walnut-containing diet. At weaning, the female hemizygous pups were randomized to control or walnut-containing diets and followed for tumor development. Compared to a diet without walnuts, consumption of walnuts significantly reduced tumor incidence (fraction of mice with at least one tumor), multiplicity (number of glands with tumor/mouse), and size. Gene expression analyses indicated that consumption of the walnut diet altered expression of multiple genes associated with proliferation and differentiation of mammary epithelial cells. A comparison with another dietary intervention indicated that the omega 3 content alone did not account for the extent of tumor suppression due to the walnut. The results of this study indicate that walnut consumption could contribute to a healthy diet to reduce risk for breast cancer.

Muthaiyah B, Essa MM, Chauhan V, Chauhan A. Protective effects of walnut extract against amyloid beta peptide-induced cell death and oxidative stress in PC12 cells. *Neurochem Res.* 2011. 36(11):2096-103.

Amyloid beta-protein (Aβ) is the major component of senile plaques and cerebrovascular amyloid deposits in individuals with Alzheimer's disease. Aβ is known to increase free radical production in neuronal cells, leading to oxidative stress and cell death. Recently, considerable attention has been focused on dietary antioxidants that are able to scavenge reactive oxygen species (ROS), thereby offering protection against oxidative stress. Walnuts are rich in components that have anti-oxidant and anti-inflammatory properties. The inhibition of in vitro fibrillization of synthetic Aβ, and solubilization of preformed fibrillar Aβ by walnut extract was previously reported. The present study was designed to investigate whether walnut extract can protect against Aβ-induced oxidative damage and cytotoxicity. The effect of walnut extract on Aβ-induced cellular damage, ROS generation and apoptosis in PC12 pheochromocytoma cells was studied. Walnut extract reduced Aβ-mediated cell death assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction, and release of lactate dehydrogenase (membrane damage), DNA damage (apoptosis) and generation of ROS in a concentration-dependent manner. These results suggest that walnut extract can counteract Aβ-induced oxidative stress and associated cell death.

Nagel JM, Brinkoetter M, Magkos F, Liu X, Chamberland JP, Shah S, Zhou J, Blackburn G, Mantzoros CS. Dietary walnuts inhibit colorectal cancer growth in mice by suppressing angiogenesis. *Nutrition*. 2012. 28(1):67-75.

OBJECTIVE: Animal studies have demonstrated that dietary supplementation with flaxseed oil inhibits colorectal cancer growth. Recent data indicate that walnuts have strong antiproliferative properties against colon cancer cells in vitro but no previous study has assessed the effects of walnuts in vivo or performed a joint evaluation of flaxseed oil and walnuts. The aim of the present study was to examine the effect of dietary walnuts on colorectal cancer in vivo and to comparatively evaluate their efficacy in relation to flaxseed oil. METHODS: HT-29 human colon cancer cells were injected in 6-wk-old female nude mice. After a 1-wk acclimation period, mice (n = 48) were randomized to diets containing ~19% of total energy from walnuts, flaxseed oil, or corn oil (control) and were subsequently studied for 25 d. RESULTS: Tumor growth rate was significantly slower in walnut-fed and flaxseed-fed mice compared with corn oil-fed animals ($P < 0.05$) by 27% and 43%, respectively. Accordingly, final tumor weight was reduced by 33% and 44%, respectively ($P < 0.05$ versus control); the differences between walnut and flaxseed diets did not reach significance. We found no differences among groups in metabolic and hormonal profile, serum antioxidant capacity, or inflammation ($P > 0.05$). However, walnuts and flaxseed oil significantly reduced serum expression levels of angiogenesis factors, including vascular endothelial growth factor (by 30% and 80%, respectively), and approximately doubled total necrotic areas despite smaller tumor sizes ($P < 0.05$ versus control). Dietary walnuts significantly decreased angiogenesis (CD34 staining; $P = 0.017$ versus control), whereas this effect did not reach significance in the flaxseed oil group ($P = 0.454$ versus control). CONCLUSION: We conclude that walnuts in the diet inhibit colorectal cancer growth by suppressing angiogenesis. Further studies are needed to confirm our findings in humans and explore underlying mechanisms.

Pribis P, Bailey RN, Russell AA, Kilsby MA, Hernandez M, Craig WJ, Grajales T, Shavlik DJ, Sabatè J. Effects of walnut consumption on cognitive performance in young adults. *Br J Nutr*. 2011.19:1-9.

Walnuts contain a number of potentially neuroprotective compounds like vitamin E, folate, melatonin, several antioxidative polyphenols and significant amounts of n-3 α-linolenic fatty acid. The present study sought to determine the effect of walnuts on verbal and non-verbal reasoning, memory and mood. A total of sixty-four

college students were randomly assigned to two treatment sequences in a crossover fashion: walnuts-placebo or placebo-walnuts. Baseline data were collected for non-verbal reasoning, verbal reasoning, memory and mood states. Data were collected again after 8 weeks of intervention. After 6 weeks of washout, the intervention groups followed the diets in reverse order. Data were collected once more at the end of the 8-week intervention period. No significant increases were detected for mood, non-verbal reasoning or memory on the walnut-supplemented diet. However, inferential verbal reasoning increased significantly by 11·2 %, indicating a medium effect size (P = 0·009; d = 0·567). In young, healthy, normal adults, walnuts do not appear to improve memory, mood or non-verbal reasoning abilities. However, walnuts may have the ability to increase inferential reasoning.

Stendell-Hollis NR, Laudermilk MJ, West JL, Thompson PA, Thomson CA. Recruitment of lactating women into a randomized dietary intervention: successful strategies and factors promoting enrollment and retention. *Contemp Clin Trials.* 2011. 32(4):505-11.

INTRODUCTION: Recruitment and retention of lactating women require unique strategies to prevent high attrition. The purpose of this report is to identify successful recruitment strategies and evaluate demographic and lifestyle characteristics associated with study completion. METHODS: A randomized, controlled trial was initiated to test the hypothesis that lactating women adhering to a Mediterranean diet will show a significant reduction in anthropometric measurements as compared to lactating women randomized to the USDA's MyPyramid diet for Pregnancy and Breastfeeding (control diet). Measurements were collected at baseline, 2months, and 4months. Recruitment methods and baseline characteristics of completers and non-completers are described. RESULTS: The largest percentage of women, 24.8%, were recruited from a local parenting magazine, 20.9% from Craig's List, 20.2% from local hospitals, and 34.1% from various other sources. At baseline, women (n=129) were mostly Non-Hispanic (75.2%), average age 29.7years, BMI averaged 27.2kg/m (2), waist:hip ratio 0.84cm (SD: 0.07), and body fat averaged 30.8%. Approximately 72% were exclusively breastfeeding, a mean 17.5weeks postpartum, and 69.0% had a college degree. Non-completers were more likely to have supplemented with formula at baseline as compared to completers (P<0.001). No other characteristics were significantly associated with attrition. CONCLUSION: Researchers conducting studies with lactating women may consider "exclusive breastfeeding" as a study inclusion criterion to prevent high attrition rates or include additional breastfeeding support to study participants.

Zhang J, Grieger JA, Kris-Etherton PM, Thompson JT, Gillies PJ, Fleming JA, Vanden Heuvel JP. Walnut oil increases cholesterol efflux through inhibition of stearoyl CoA desaturase 1 in THP-1 macrophage-derived foam cells. *Nutr Metab* (Lond). 2011. 26;8(1):61.

BACKGROUND: Walnuts significantly decrease total and low-density lipoprotein cholesterol in normo- and hypercholesterolemic individuals. No study to date has evaluated the effects of walnuts on cholesterol efflux, the initial step in reverse cholesterol transport, in macrophage-derived foam cells (MDFC). The present study was conducted to investigate the mechanisms by which walnut oil affects cholesterol efflux. METHODS: The extract of English walnuts (walnut oil) was dissolved in DMSO and applied to cultured THP-1 MDFC cells (0.5 mg/mL). THP-1 MDFC also were treated with human sera (10%, v:v) taken from subjects in a walnut feeding study. Cholesterol efflux was examined by liquid scintillation counting. Changes in gene expression were quantified by real time PCR. RESULTS: Walnut oil treatment significantly increased cholesterol efflux through decreasing the expression of the lipogenic enzyme stearoyl CoA desaturase 1 (SCD1) in MDFC. Alpha-linolenic acid (ALA), the major n-3 polyunsaturated fatty acid found in walnuts, recaptured SCD1 reduction in MDFC, a mechanism mediated through activation of nuclear receptor farnesoid-X-receptor (FXR). Postprandial serum treatment also increased cholesterol efflux in MDFC. When categorized by baseline C-reactive protein (CRP; cut point of 2 mg/L), subjects in the lower CRP sub-group benefited more from dietary intervention, including a more increase in cholesterol efflux, a greater reduction in SCD1, and a blunted postprandial lipemia. CONCLUSION: In conclusion, walnut oil contains bioactive molecules that significantly improve cholesterol efflux in MDFC. However, the beneficial effects of walnut intake may be reduced by the presence of a pro-inflammatory state.

2010

Brennan A, Sweeney LL, Liu X, Mantzoros CS. Walnut consumption increases satiation but has no effect on insulin resistance or the metabolic profile over a 4 day period. *Obesity* (Silver Spring). 2010 Jun;18(6):1176-82.

Obesity and diabetes have been associated with increased consumption of highly processed foods, and reduced consumption of whole grains and nuts. It has been proposed, mainly on the basis of observational studies, that nuts may provide superior satiation, may lead to reduced calorie consumption, and may decrease the risk of type

2 diabetes; but evidence from randomized, interventional studies is lacking. A total of 20 men and women with the metabolic syndrome participated in a randomized, double-blind, crossover study of walnut consumption. Subjects had two 4-day admissions to the clinical research center where they were fed an isocaloric diet. In addition, they consumed shakes for breakfast containing either walnuts or placebo (shakes were standardized for calories, carbohydrate, and fat content). Appetite, insulin resistance, and metabolic parameters were measured. We found an increased level of satiety (overall P value = 0.0079) and sense of fullness (P = 0.05) in prelunch questionnaires following the walnut breakfast as compared to the placebo breakfast, with the walnut effect achieving significance on day 3 and 4 (P = 0.02 and P = 0.03). We did not find any change in resting energy expenditure, hormones known to mediate satiety, or insulin resistance when comparing the walnut vs. placebo diet. Walnut consumption over 4 days increased satiety by day 3. Long-term studies are needed to confirm the physiologic role of walnuts, the duration of time needed for these effects to occur, and to elucidate the underlying mechanisms.

Ma Y, Njike VY, Millet J, Dutt S, Doughty K, Treu JA, Katz D. Effects of walnut consumption on endothelial function in type 2 diabetic subjects: a randomized controlled crossover trial. *Diabetes Care.* 2010 Feb;33(2):227-32.

OBJECTIVE: To determine the effects of daily walnut consumption on endothelial function, cardiovascular biomarkers, and anthropometric measures in type 2 diabetic individuals. RESEARCH DESIGN AND METHODS: This study was a randomized, controlled, single-blind, crossover trial. Twenty-four participants with type 2 diabetes (mean age 58 years; 14 women and 10 men) were randomly assigned to one of the two possible sequence permutations to receive an ad libitum diet enriched with 56 g (366 kcal) walnuts/day and an ad libitum diet without walnuts for 8 weeks. Subjects underwent endothelial function testing (measured as flow-mediated dilatation [FMD]) and assessment of cardiovascular biomarkers before and after each 8-week treatment phase. The primary outcome measure was the change in FMD after 8 weeks. Secondary outcome measures included changes in plasma lipids, A1C, fasting glucose, insulin sensitivity, and anthropometric measures. RESULTS: Endothelial function significantly improved after consumption of a walnut-enriched ad libitum diet compared with that after consumption of an ad libitum diet without walnuts (2.2 +/- 1.7 vs. 1.2 +/- 1.6%; P = 0.04). The walnut-enriched diet increased fasting serum glucose and lowered serum total cholesterol and LDL cholesterol from baseline (10.0 +/- 20.5 mg/dl, P = 0.04; -9.7 +/- 14.5 mg/dl, P < 0.01; and -7.7 +/- 10 mg/dl, P < 0.01, respectively), although these changes were not significant compared with those for an ad libitum diet without walnuts. There were no significant changes in anthropometric measures, plasma A1C, and insulin sensitivity. CONCLUSIONS: A walnut-enriched ad libitum diet improves endothelium-dependent vasodilatation in type 2 diabetic individuals, suggesting a potential reduction in overall cardiac risk.

McKay DL, Chen CY, Yeum KJ, Matthan NR, Lichtenstein AH, Blumberg JB. Chronic and acute effects of walnuts on antioxidant capacity and nutritional status in humans: a randomized cross-over pilot study. *Nutr J.* 2010 May 12;9:21.

BACKGROUND: Compared with other common plant foods, walnuts (Juglans regia) are consistently ranked among the highest in antioxidant capacity. In vitro, walnut polyphenols inhibit plasma and LDL oxidation, while in animal models they lower biomarkers of oxidative stress and raise antioxidant capacity. A limited number of human feeding trials indicate that walnuts improve some measures of antioxidant status, but not others. METHODS: A 19 wk, randomized crossover trial was conducted in 21 generally healthy men and postmenopausal women > or = 50 y to study the dose-response effects of walnut intake on biomarkers of antioxidant activity, oxidative stress, and nutrient status. Subjects were randomized to receive either 21 or 42 g raw walnuts/d during each 6 wk intervention phase with a 6 wk washout between phases. Subjects were instructed to consume their usual diet, but refrain from eating any other tree nuts, seeds, peanuts, or ellagitannin-rich foods during the entire study, and other polyphenol-rich foods for 2 d prior to each study visit. RESULTS: Compared to baseline levels, red blood cell (RBC) linoleic acid and plasma pyridoxal phosphate (PLP) were significantly higher after 6 wk with 42 g/d walnuts (P < 0.05 for both). Overall, changes in plasma total thiols, and other antioxidant biomarkers, were not significant with either walnut dose. However, when compared to fasting levels, plasma total thiols were elevated within 1 h of walnut consumption with both doses during the baseline and end visits for each intervention phase (P < 0.05 for all). Despite the observed increase in RBC linoleic and linolenic acids associated with walnut consumption, this substrate for lipid peroxidation only minimally affected malondialdehyde (MDA) and antioxidant capacity. The proportional changes in MDA and Oxygen Radical Absorbance Capacity (ORAC) were consistent with a dose-response effect, although no significant within- or between-group differences were observed for these measures. CONCLUSIONS: Walnut consumption did not significantly change the plasma antioxidant capacity of healthy, well-nourished older adults in this pilot study. However, improvements in linoleic acid and pyridoxal phosphate were observed with chronic consumption, while total plasma thiols were enhanced acutely. Future studies investigating the

antioxidant effects of walnuts in humans are warranted, but should include either a larger sample size or a controlled feeding intervention.

Ros E, Tapsell LC, Sabaté J. Nuts and berries for heart health. *Curr Atheroscler Rep.* 2010 Nov;12(6):397-406.

Nuts are nutrient-dense foods with complex matrices rich in unsaturated fatty acids and other bioactive compounds, such as L-arginine, fiber, minerals, tocopherols, phytosterols, and polyphenols. By virtue of their unique composition, nuts are likely to beneficially impact heart health. Epidemiologic studies have associated nut consumption with a reduced incidence of coronary heart disease in both genders and diabetes in women. Limited evidence also suggests beneficial effects on hypertension and inflammation. Interventional studies consistently show that nut intake has a cholesterol-lowering effect and there is emerging evidence of beneficial effects on oxidative stress, inflammation, and vascular reactivity. Blood pressure, visceral adiposity, and glycemic control also appear to be positively influenced by frequent nut consumption without evidence of undue weight gain. Berries are another plant food rich in bioactive phytochemicals, particularly flavonoids, for which there is increasing evidence of benefits on cardiometabolic risk that are linked to their potent antioxidant power.

Torabian S, Haddad E, Cordero-Macintyre Z, Tanzman J, Fernandez ML, Sabaté J. Long-term walnut supplementation without dietary advice induces favorable serum lipid changes in free-living individuals. *Eur J Clin Nutr.* 2010 Mar;64(3):274-9.

BACKGROUND/OBJECTIVES: Walnuts have been shown to reduce serum lipids in short-term well-controlled feeding trials. Little information exists on the effect and sustainability of walnut consumption for longer duration in a free-living situation. SUBJECTS/METHODS: A randomized crossover design in which 87 subjects with normal to moderate high plasma total cholesterol were initially assigned to a walnut-supplemented diet or habitual (control) diet for a 6-month period, then switched to the alternate dietary intervention for a second 6-month period. Each subject attended seven clinics 2 months apart. At each clinic, body weight was measured, and in five clinics (months 0, 4, 6, 10 and 12), a blood sample was collected. RESULTS: Our study showed that supplementing a habitual diet with walnuts (12% of total daily energy intake equivalent) improves the plasma lipid profile. This beneficial effect was more significant in subjects with high plasma total cholesterol at baseline. Significant changes in serum concentrations of total cholesterol (P=0.02) and triglycerides (P=0.03) were seen and nearly significant changes in low-density lipoprotein cholesterol (LDL-C) (P=0.06) were found. No significant change was detected in either high-density lipoprotein (HDL) cholesterol LDL to HDL ratio. CONCLUSIONS: Including walnuts as part of a habitual diet favorably altered the plasma lipid profile. The lipid-lowering effects of walnuts were more evident among subjects with higher lipid baseline values, precisely those people with greater need of reducing plasma total and LDL-C.

West SG, Krick AL, Klein LC, Zhao G, Wojtowicz TF, McGuiness M, Bagshaw DM, Wagner P, Ceballos RM, Holub BJ, Kris-Etherton PM. Effects of diets high in walnuts and flax oil on hemodynamic responses to stress and vascular endothelial function. *J Am Coll Nutr.* 2010 Dec;29(6):595-603.

Background: Polyunsaturated fatty acids (PUFA) have beneficial effects on cardiovascular risk, although the mechanisms are incompletely understood. In a previous article, we showed significant reductions in lowdensity lipoprotein cholesterol and several markers of inflammation with increasing intake of alpha-linolenic acid (ALA) from walnuts and flax. Objective: To examine effects of ALA on cardiovascular responses to acute stress, flow-mediated dilation (FMD) of the brachial artery, and blood concentrations of endothelin-1 and arginine-vasopressin (AVP). Design: Using a randomized, crossover study design, cardiovascular responses to acute stress were assessed in 20 hypercholesterolemic subjects, a subset of whom also underwent FMD testing (n 5 12). Participants were fed an average American diet (AAD) and 2 experimental diets that varied in the amount of ALA and linoleic acid (LA) that they contained. The AAD provided 8.7% energy from PUFA (7.7% LA, 0.8% ALA). On the LA diet, saturated fat was reduced, and PUFA from walnuts and walnut oil provided 16.4% of energy (12.6% LA, 3.6% ALA). On the ALA diet, walnuts, walnut oil, and flax oil provided 17% energy from PUFA (10.5% LA, 6.5% ALA). Results: The ALA and LA diets significantly reduced diastolic blood pressure (22 to 23 mm Hg) and total peripheral resistance (24%), and this effect was evident at rest and during stress (main effect of diet, $p < 0.02$). FMD increased (+34%) on the diet containing additional ALA. AVP also increased by 20%, and endothelin-1 was unchanged. Conclusions: These results suggest novel mechanisms for the cardioprotective effects of walnuts and flax, and further work is needed to identify the bioactives responsible for these effects.

Willis LM, Bielinski DF, Fisher DR, Matthan NR, Joseph JA. Walnut extract inhibits LPS-induced activation of BV-2 microglia via internalization of TLR4: possible involvement of phospholipase D2. *Inflammation.* 2010 Oct;33(5):325-33.

Walnuts are a rich source of essential fatty acids, including the polyunsaturated fatty acids alpha-linolenic acid and linoleic acid. Essential fatty acids have been shown to modulate a number of cellular processes in the brain, including the activation state of microglia. Microglial activation can result in the generation of cytotoxic intermediates and is associated with a variety of age-related and neurodegenerative conditions. In vitro, microglial activation can be induced with the bacterial cell wall component lipopolysaccharide (LPS). In the present study, we generated a methanolic extract of English walnuts (Juglans regia) and examined the effects of walnut extract exposure on LPS-induced activation in BV-2 microglial cells. When cells were treated with walnut extract prior to LPS stimulation, production of nitric oxide and expression of inducible nitric oxide synthase were attenuated. Walnut extract also induced a decrease in tumor necrosis-alpha (TNFalpha) production. We further found that walnut extract induced internalization of the LPS receptor, toll-like receptor 4, and that the anti-inflammatory effects of walnut were dependent on functional activation of phospholipase D2. These studies represent the first to describe the anti-inflammatory effects of walnuts in microglia, which could lead to nutritional interventions in the prevention and treatment of neurodegeneration.

Wu H, Pan A, Yu Z, Qi Q, Lu L, Zhang G, Yu D, Zong G, Zhou Y, Chen X, Tang L, Feng Y, Zhou H, Chen X, Li H, Demark-Wahnefried W, Hu FB, Lin X., 2010. Lifestyle counseling and supplementation with flaxseed or walnuts influence the management of metabolic syndrome. *J Nutr.* 140(11):1937-42.

A healthy lifestyle may ameliorate metabolic syndrome (MetS); however, it remains unclear if incorporating nuts or seeds into lifestyle counseling (LC) has additional benefit. A 3-arm, randomized, controlled trial was conducted among 283 participants screened for MetS using the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian Americans. Participants were assigned to a LC on the AHA guidelines, LC + flaxseed (30 g/d) (LCF), or LC + walnuts (30 g/d) (LCW) group. After the 12-wk intervention, the prevalence of MetS decreased significantly in all groups: -16.9% (LC), -20.2% (LCF), and -16.0% (LCW). The reversion rate of MetS, i.e. those no longer meeting the MetS criteria at 12 wk, was not significantly different among groups (LC group, 21.1%; LCF group, 26.6%; and LCW group, 25.5%). However, the reversion rate of central obesity was higher in the LCF (19.2%; P = 0.008) and LCW (16.0%; P = 0.04) groups than in the LC group (6.3%). Most of the metabolic variables (weight, waist circumference, serum glucose, total cholesterol, LDL cholesterol, apolipoprotein (Apo) B, ApoE, and blood pressure) were significantly reduced from baseline in all 3 groups. However, the severity of MetS, presented as the mean count of MetS components, was significantly reduced in the LCW group compared with the LC group among participants with confirmed MetS at baseline (P = 0.045). Our results suggest that a low-intensity lifestyle education program is effective in MetS management. Flaxseed and walnut supplementation may ameliorate central obesity. Further studies with larger sample sizes and of longer duration are needed to examine the role of these foods in the prevention and management of MetS.

2009

Banel DK, Hu FB. Effects of walnut consumption on blood lipids and other cardiovascular risk factors: a meta-analysis and systematic review. *Am J Clin Nutr.* 2009 Jul; 90 (1):56-63.

BACKGROUND: Consumption of nuts has been associated with a decreased risk of cardiovascular disease events and death. Walnuts in particular have a unique profile: they are rich in polyunsaturated fatty acids, which may improve blood lipids and other cardiovascular disease risk factors. OBJECTIVES: We aimed to conduct a literature review and a meta-analysis to combine the results from several trials and to estimate the effect of walnuts on blood lipids. DESIGN: Literature databases were searched for published trials that compared a specifically walnut-enhanced diet with a control diet. We conducted a random-effects meta-analysis of weighted mean differences (WMDs) of lipid outcomes. RESULTS: Thirteen studies representing 365 participants were included in the analysis. Diets lasted 4-24 wk with walnuts providing 10-24% of total calories. When compared with control diets, diets supplemented with walnuts resulted in a significantly greater decrease in total cholesterol and in LDL-cholesterol concentrations (total cholesterol: WMD = -10.3 mg/dL, P < 0.001; LDL cholesterol: WMD = -9.2 mg/dL, P < 0.001). HDL cholesterol and triglycerides were not significantly affected by walnut diets more than with control diets (HDL cholesterol: WMD = -0.2, P = 0.8; triglycerides: WMD = -3.9, P = 0.3). Other results reported in the trials indicated that walnuts provided significant benefits for certain antioxidant capacity and inflammatory markers and had no adverse effects on body weight [body mass index (kg/m(2)): WMD = -0.4, P = 0.5; weight (kg): WMD = -0.05, P = 0.97]. CONCLUSIONS: Overall, high-walnut-enriched diets significantly decreased total and LDL cholesterol for the duration of the short-term trials. Larger and longer-term trials are needed to address the effects of walnut consumption on cardiovascular risk and body weight.

Joseph JA, Shukitt-Hale B, Willis LM. Grape juice, berries, and walnuts affect brain aging and behavior. *J Nutr.* 2009 Sep;139(9):1813S-7S.

Numerous studies have indicated that individuals consuming a diet containing high amounts of fruits and vegetables exhibit fewer age-related diseases such as Alzheimer's disease. Research from our laboratory has suggested that dietary supplementation with fruit or vegetable extracts high in antioxidants (e.g. blueberries, strawberries, walnuts, and Concord grape juice) can decrease the enhanced vulnerability to oxidative stress that occurs in aging and these reductions are expressed as improvements in behavior. Additional mechanisms involved in the beneficial effects of fruits and vegetables include enhancement of neuronal communication via increases in neuronal signaling and decreases in stress signals induced by oxidative/inflammatory stressors (e.g. nuclear factor kappaB). Moreover, collaborative findings indicate that blueberry or Concord grape juice supplementation in humans with mild cognitive impairment increased verbal memory performance, thus translating our animal findings to humans. Taken together, these results suggest that a greater intake of high-antioxidant foods such as berries, Concord grapes, and walnuts may increase "health span" and enhance cognitive and motor function in aging.

Rajaram S, Haddad E, Meija A, Sabaté J. Walnuts and fatty fish influence different serum lipid fractions in normal to mildly hyperlipidemic individuals: a randomized controlled study. *Am J Clin Nutr.* 2009. May; 89(5):1657S-1663S.

BACKGROUND: Increased consumption of n-3 (omega-3) fatty acids decreases the incidence of coronary heart disease (CHD). OBJECTIVE: The objective was to determine whether walnuts (plant n-3 fatty acid) and fatty fish (marine n-3 fatty acid) have similar effects on serum lipid markers at intakes recommended for primary prevention of CHD.DESIGN: In a randomized crossover feeding trial, 25 normal to mildly hyperlipidemic adults consumed 3 isoenergetic diets (approximately 30% total fat and <10% saturated fat) for 4 wk each: a control diet (no nuts or fish), a walnut diet (42.5 g walnuts/10.1 mJ), or a fish diet (113 g salmon, twice/wk). Fasting blood was drawn at baseline and at the end of each diet period and analyzed for serum lipids. RESULTS: Serum total cholesterol and LDL cholesterol concentrations in adults who followed the walnut diet (4.87 +/- 0.18 and 2.77 +/- 0.15 mmol/L, respectively) were lower than in those who followed the control diet (5.14 +/- 0.18 and 3.06 +/- 0.15 mmol/L, respectively) and those who followed the fish diet (5.33 +/- 0.18 and 3.2 +/- 0.15 mmol/L, respectively; $P < 0.0001$). The fish diet resulted in decreased serum triglyceride and increased HDL-cholesterol concentrations (1.0 +/- 0.11 and 1.23 +/- 0.05 mmol/L, respectively) compared with the control diet (1.12 +/- 0.11 and 1.19 +/- 0.05 mmol/L, respectively) and the walnut diet (1.11 +/- 0.11 mmol/L, $P < 0.05$, and 1.18 +/- 0.05 mmol/L, $P < 0.001$, respectively). The ratios of total cholesterol:HDL cholesterol, LDL cholesterol:HDL cholesterol, and apolipoprotein B:apolipoprotein A-I were lower ($P < 0.05$) in those who followed the walnut diet compared with those who followed the control and fish diets. CONCLUSION: Including walnuts and fatty fish in a healthy diet lowered serum cholesterol and triglyceride concentrations, respectively, which affects CHD risk favorably.

Tapsell L, Batterham M, Tan SY, Warensjö E. The effect of a calorie controlled diet containing walnuts on substrate oxidation during 8 hours in a room calorimeter. *J Am Coll Nutr.* 2009 Oct;28(5):611-7.

OBJECTIVE: Dietary macronutrient proportions affect substrate utilization, but in practice people consume foods. We hypothesized that in overweight adults, a calorie controlled diet based on core foods and including walnuts may be advantageous in promoting greater use of fat stores. METHODS: This crossover study tested the effects of diet-related energy expenditure and fat oxidation in 16 overweight individuals over an 8-hour period. The 2 diets included breakfast and lunch meals during the measurement period and an evening meal the night before. They comprised core foods of bread/cereals, fruit, vegetables, milk/yogurt, and meat, and either walnuts (walnut diet) or olive oil (control diet). There was no difference in the energy and macronutrient composition of the diets in the measurement period. Energy expenditure, respiratory quotient (RQ), and macronutrient oxidation were assessed during two 8-hour stays in a room calorimeter facility. RESULTS: During the 8-hour measurement period, no difference in energy expenditure was noted between the diets, but a significant difference in RQ was observed between diets (control 0.908 +/- 0.046 vs. walnut 0.855 +/- 0.036, $p = 0.029$). Carbohydrate oxidation was lower and fat oxidation was higher during the walnut period than during the control period. CONCLUSIONS: A calorie controlled diet of core foods including walnuts may be advantageous in promoting the use of body fat stores, at least under acute conditions.

Tapsell LC, Teuss G, Tan S-Y, Dalton S, Quick CJ, Gillen LJ, Charlton KE. Long-term effects of increased dietary polyunsaturated fat from walnuts on metabolic parameters in type II diabetes. *Eur J Clin Nutr.* 2009 Aug;63(8):1008-15.

BACKGROUND/OBJECTIVES: Most dietary interventions have metabolic effects in the short term, but long-term effects may require dietary fat changes to influence body composition and insulin action. This study

assessed the effect of sustained high polyunsaturated fatty acids (PUFA) intake through walnut consumption on metabolic outcomes in type II diabetes. SUBJECTS/METHODS: Fifty overweight adults with non-insulin-treated diabetes (mean age 54+/-8.7 years) were randomized to receive low-fat dietary advice +/-30 g per day walnuts targeting weight maintenance (around 2000 kcal, 30% fat) for 1 year. Differences between groups were assessed by changes in anthropometric values (body weight, body fat, visceral adipose tissue) and clinical indicators of diabetes over treatment time using the general linear model. RESULTS: The walnut group consumed significantly more PUFA than the control (P=0.035), an outcome attributed to walnut consumption (contributing 67% dietary PUFA at 12 months). Most of the effects were seen in the first 3 months. Despite being on weight maintenance diets, both groups sustained a 1-2 kg weight loss, with no difference between groups (P=0.680). Both groups showed improvements in all clinical parameters with significant time effects (P<0.004), bar triacylglycerol levels, but these were just above normal to begin with. The walnut group produced significantly greater reductions in fasting insulin levels (P=0.046), an effect seen largely in the first 3 months. CONCLUSIONS: Dietary fat can be manipulated with whole foods such as walnuts, producing reductions in fasting insulin levels. Long-term effects are also apparent but subject to fluctuations in dietary intake if not of the disease process.

Torabian S, Haddad E, Rajaram S, Banta J, Sabaté J. Acute effect of nut consumption on plasma total polyphenols, antioxidant capacity and lipid peroxidation. *J Hum Nutr Diet,* 22, pp. 64–71.

BACKGROUND: Nuts have been shown to have beneficial effects on human health due to the healthy fat content; however, the effect of antioxidants (i.e. polyphenols) in nuts have not been fully investigated. The present study aimed to assess the immediate effect of a polyphenol-rich meal (75% of energy from nuts: walnuts or almonds) and a polyphenol-free meal on plasma polyphenol content, antioxidant capacity and lipid peroxidation in healthy volunteers. METHODS: Thirteen subjects participated in a randomized, crossover, intervention study. After an overnight fast, walnuts, almonds or a control meal in the form of smoothies were consumed by study subjects. Each subject participated on three occasions, 1 week apart, consuming one of the smoothies each time. Blood samples were obtained at fasting and then at intervals up to 3.5 h after consumption of the smoothies. RESULTS: There was a significant increase in plasma polyphenol concentration following both nut meals, with peak concentrations being achieved at 90 min, and with a walnut meal having a more sustained higher concentration than an almond meal. The plasma total antioxidant capacity reached its highest point at 150 min postconsumption of the nut meals, and was higher after the almond compared to walnut meal. A gradual significant ($P < 0.05$) reduction in the susceptibility of plasma to lipid peroxidation was observed 90 min after ingestion of the nut meals. No changes were observed following consumption of control meal. CONCLUSIONS: Consumption of both nuts increased plasma polyphenol concentrations, increased the total antioxidant capacity and reduced plasma lipid peroxidation.

Velliquette RA, Gillies PJ, Kris-Etherton PM, Green JW, Zhao G, Vanden Heuvel JP. Regulation of human stearoyl-CoA desaturase by omega-3 and omega-6 fatty acids: implications for the dietary management of elevated serum triglycerides. *J Clin Lipidol.* 2009 Aug;3(4):281-8.

BACKGROUND: Polyunsaturated fatty acids lower serum triglycerides by a mechanism that may involve the inhibition of stearoyl-CoA desaturase (SCD).OBJECTIVE: We sought to evaluate the effects of serum fatty acids on 1) the SCD index in a controlled clinical setting, and 2) SCD regulation in Hep G2 cells. METHODS: The SCD index was determined in 23 subjects randomly sequenced through 3 diets for 6 weeks in a crossover study. Diets were variably enriched with n-3 and n-6 polyunsaturated fatty acids; notably, monounsaturated fatty acids were held constant. Effects of linoleic acid (LA), α-linolenic acid (ALA), and eicosapentaenoic acid (EPA) on mRNA levels of SCD, fatty acid elongases 5 and 6 (Elovl5 and Elovl6), fatty acid synthase, carnitine palmitoyltransferase-1, and sterol response element binding protein-1c were investigated in Hep G2 cells after 24-hour incubations. RESULTS: The SCD indexes C18:1/18:0 and C16:1/C16:0 were significantly ($P < .0001$) correlated with serum TG with R(2) values of 0.71 and 0.58. The correlation was negatively associated with LA and positively associated with ALA. LA and EPA decreased SCD mRNA (EC(50) of 0.50 and 1.67μM), whereas ALA did not. Likewise, LA and EPA decreased sterol response element binding protein-1c mRNA (EC(50) of 0.78 and 1.78μM), but ALA did not. Similar results were observed for Elovl6. GW9662, a peroxisome proliferation activator receptor antagonist, did not obviate the effects of LA and EPA on SCD mRNA. CONCLUSIONS: Diets enriched in LA, ALA, and by metabolic inference EPA, can regulate SCD activity at the level of transcription, a nutritional intervention that may be useful in the management of increased levels of serum triglycerides in cardiometabolic disorders

Willis L, Shukitt-Hale B, Cheng V, Joseph J. Dose-dependent effects of walnuts on motor and cognitive function in aged rats. *Br J Nutr.* 2009; 101:1140-1164.

Aged rats show decrements in performance on motor and cognitive tasks that require the use of spatial learning and memory. Previously we have shown that these deficits can be reversed by the polyphenolics in fruits and vegetables. Walnuts, which contain the n-3 fatty acids a-linolenic acid and linoleic acid, are a dietary source of polyphenols, antioxidants and lipids. Thus, the present study examined the effects of walnut supplementation on motor and cognitive ability in aged rats. Fischer 344 rats, aged 19 months, were fed a control, or a 2, 6 or 9% walnut diet for 8 weeks before motor and cognitive testing. Results for the motor testing showed that the 2% walnut diet improved performance on rod walking, while the 6% walnut diet improved performance on the medium plank walk; the higher dose of the 9% walnut diet did not improve psychomotor performance and on the large plank actually impaired performance. All of the walnut diets improved working memory in the Morris water maze, although the 9% diet showed impaired reference memory. These findings show for the first time that moderate dietary walnut supplementation can improve cognitive and motor performance in aged rats.

Willis LM, Shukitt-Hale B, Joseph JA. Modulation of cognition and behavior in aged animals: role for antioxidant- and essential fatty acid–rich plant foods. *Am J Clin Nutr.* 2009 May;89(5):1602S-1606S.

Aging results in the development of cognitive and motor deficits in humans and animals that are evident by midlife. These deficits are thought to stem from neuronal damage and dysfunction as a result of a variety of stressors, including increased oxidative stress and modifications in brain lipid composition. Recent clinical and animal studies have identified nutritional intervention as a viable method to curtail the cognitive aging process. Human studies have been primarily observational and have indicated that inclusion of antioxidant-rich foods in the diet can slow the progression of cognitive decline. Basic science studies investigating nutritional modulation of age-related cognitive decline have focused on foods rich in antioxidants or essential fatty acids. The purpose of this review is to discuss recent advancements in animal research showing that age-related cognitive and behavioral decline can be ameliorated with nutritional supplementation with polyphenol- or polyunsaturated fatty acid-rich plant foods.

Willis, L, Shukitt-Hale, B, Joseph, JA. Dietary polyunsaturated fatty acids improve cholinergic transmission in the aged brain. *Genes Nutr.* 2009 December; 4(4): 309–314.

The cholinergic theory of aging states that dysfunction of cholinergic neurons arising from the basal forebrain and terminating in the cortex and hippocampus may be involved in the cognitive decline that occurs during aging and Alzheimer's disease. Despite years of research, pharmacological interventions to treat or forestall the development of Alzheimer's disease have primarily focused on enhancing cholinergic transmission, either through increasing acetylcholine (ACh) synthesis or inhibition of the acetylcholinesterase enzyme responsible for ACh hydrolysis. However, recent studies have indicated that dietary supplementation can impact the cholinergic system, particularly during aging. The purpose of the present review is to examine the relevant research suggesting that cholinergic functioning may be maintained during aging via consuming a diet containing polyunsaturated fatty acids (PUFAs). The data reviewed herein indicate that, at least in animal studies, inclusion of PUFAs in the diet can improve cholinergic transmission in the brain, possibly leading to improvements in cognitive functioning.

2008

Hardman WE, Ion G. Suppression of implanted MDA-MB 231 human breast cancer growth in nude mice by dietary walnut. *Nutr Cancer.* 2008;60(5):666-74.

Walnuts contain components that may slow cancer growth including omega 3 fatty acids, phytosterols, polyphenols, carotenoids, and melatonin. A pilot study was performed to determine whether consumption of walnuts could affect growth of MDA-MB 231 human breast cancers implanted into nude mice. Tumor cells were injected into nude mice that were consuming an AIN-76A diet slightly modified to contain 10% corn oil. After the tumors reached 3 to 5 mm diameter, the diet of one group of mice was changed to include ground walnuts, equivalent to 56 g (2 oz) per day in humans. The tumor growth rate from Day 10, when tumor sizes began to diverge, until the end of the study of the group that consumed walnuts (2.9 +/- 1.1 mm(3)/day; mean +/- standard error of the mean) was significantly less ($P > 0.05$, t-test of the growth rates) than that of the group that did not consume walnuts (14.6 +/- 1.3 mm(3)/day). The eicosapentaenoic and docosahexaenoic acid fractions of the livers of the group that consumed walnuts were significantly higher than that of the group that did not consume walnuts. Tumor cell proliferation was decreased, but apoptosis was not altered due to walnut consumption. Further work is merited to investigate applications to cancer in humans.

Segovia-Siapco G, Singh P, Haddad E, Sabaté J. Relative validity of a food frequency questionnaire used to assess food intake during a dietary intervention study. *Nutr Cancer.* 2008;60(5):603-11.

To develop a cost-effective alternative for evaluating dietary intake in large-scale intervention trials of cancer and cardiovascular disease outcomes, we designed and validated a semiquantitative food frequency questionnaire (FFQ). We collected 6 to 8 of the 24-hr dietary recalls from 87 adults (ages 30-72 yr) who were randomly assigned to a walnut-supplemented diet or a control diet in a 6-mo dietary intervention trial. Relative validity of a 171-item FFQ in assessing intake of selected foods and the prescribed intervention (intake > or =25 g/day or intake < 2 g of walnuts) was determined using 24-h dietary recalls as the reference. De-attenuated correlations between FFQ and dietary recalls were .82 for walnuts, .80 for fruits, .79 for grains, .77 for vegetables, .63 for water, .44 for sweets, and .36 for dairy/eggs. High within-person variation did not allow de-attenuation for the remaining foods, but uncorrected correlations were high (> .7) for the beverage variables. The FFQ correctly classified 86 out of 87 subjects in the 2 prescribed intervention groups. The FFQ can provide an accurate measure of a food-based intervention (i.e., walnut supplementation) in a trial setting and can also accurately estimate a number of other food groups consumed during the trial.

Spaccarotella KJ, Kris-Etherton PM, Stone, WL, Bagshaw, DM, Fishell VK, West SG, Lawrence FR, and Hartman TJ. The effect of walnut intake on factors related to prostate and vascular health in older men. *Nutr J.* 2008 May 2;7:13.

BACKGROUND: Tocopherols may protect against prostate cancer and cardiovascular disease (CVD). METHODS: We assessed the effect of walnuts, which are rich in tocopherols, on markers of prostate and vascular health in men at risk for prostate cancer. We conducted an 8-week walnut supplement study to examine effects of walnuts on serum tocopherols and prostate specific antigen (PSA). Subjects (n = 21) consumed (in random order) their usual diet +/- a walnut supplement (75 g/d) that was isocalorically incorporated in their habitual diets. Prior to the supplement study, 5 fasted subjects participated in an acute time course experiment and had blood taken at baseline and 1, 2, 4, and 8 h after consuming walnuts (75 g). RESULTS: During the time course experiment, triglycerides peaked at 4 h, and gamma-tocopherol (gamma-T) increased from 4 to 8 h. Triglyceride - normalized gamma-T was two-fold higher (P = 0.01) after 8 versus 4 h. In the supplement study, change from baseline was +0.83 +/- 0.52 micromol/L for gamma-T, -2.65 +/- 1.30 micromol/L for alpha-tocopherol (alpha-T) and -3.49 +/- 1.99 for the tocopherol ratio (alpha-T: gamma-T). A linear mixed model showed that, although PSA did not change, the ratio of free PSA:total PSA increased and approached significance (P = 0.07). The alpha-T: gamma-T ratio decreased significantly (P = 0.01), partly reflecting an increase in serum gamma-T, which approached significance (P = 0.08). CONCLUSION: The significant decrease in the alpha-T: gamma-T ratio with an increase in serum gamma-T and a trend towards an increase in the ratio of free PSA:total PSA following the 8-week supplement study suggest that walnuts may improve biomarkers of prostate and vascular status.

2007

Griel AE, Kris-Etherton PM, Hilpert KF, Zhao G, West SG, Corwin RL. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. *Nutr J.* 2007 Jan 16;6:2.

Human, animal, and in vitro research indicates a beneficial effect of appropriate amounts of omega-3 (n-3) polyunsaturated fatty acids (PUFA) on bone health. This is the first controlled feeding study in humans to evaluate the effect of dietary plant-derived n-3 PUFA on bone turnover, assessed by serum concentrations of N-telopeptides (NTx) and bone-specific alkaline phosphatase (BSAP). Subjects (n = 23) consumed each diet for 6 weeks in a randomized, 3-period crossover design: 1) Average American Diet (AAD; [34% total fat, 13% saturated fatty acids (SFA), 13% monounsaturated fatty acids (MUFA), 9% PUFA (7.7% LA, 0.8% ALA)]), 2) Linoleic Acid Diet (LA; [37% total fat, 9% SFA, 12% MUFA, 16% PUFA (12.6% LA, 3.6% ALA)]), and 3) alpha-Linolenic Acid Diet (ALA; [38% total fat, 8% SFA, 12% MUFA, 17% PUFA (10.5% LA, 6.5% ALA)]). Walnuts and flaxseed oil were the predominant sources of ALA. NTx levels were significantly lower following the ALA diet (13.20 +/- 1.21 nM BCE), relative to the AAD (15.59 +/- 1.21 nM BCE) ($p < 0.05$). Mean NTx level following the LA diet was 13.80 +/- 1.21 nM BCE. There was no change in levels of BSAP across the three diets. Concentrations of NTx were positively correlated with the pro-inflammatory cytokine TNFalpha for all three diets. The results indicate that plant sources of dietary n-3 PUFA may have a protective effect on bone metabolism via a decrease in bone resorption in the presence of consistent levels of bone formation.

Simon J, Sabaté J, Tanzman J. Lack of effect of walnuts on serum levels of prostate specific antigen: a brief report. *J Am Coll Nutr.* 2007; 26; 4; 317-320.

OBJECTIVE: To examine whether the short-term consumption of walnuts, a food rich in alpha-linolenic acid, affects levels of serum prostate-specific antigen (PSA), a marker of prostate enlargement, inflammation, and cancer. METHODS: Using data from a 12-month randomized crossover study examining the effect of walnut

consumption on body composition, we examined whether increased walnut consumption (mean 35 grams daily, 12% total energy) affected serum PSA levels among 40 middle-aged men. RESULTS: There was no significant difference between mean PSA level at the conclusion of the 6-month walnut-supplemented diet (1.05 mug/L, 95% CI [0.81, 1.37]) and the conclusion of the 6-month control diet (1.06 mug/L, 95% CI [0.81, 1.38]) (P = 0.86) (or a mean proportional decrease in PSA of -1%). CONCLUSIONS: Our results suggest that short-term consumption of walnuts is unlikely to affect PSA levels adversely among otherwise normal men.

Zhao G, Etherton TD, Martin KR, Gillies PJ, West SG, Kris-Etherton PM. Dietary alpha-linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. *Am J Clin Nutr.* 2007; 85:385-91.

BACKGROUND: Atherosclerosis is a chronic inflammatory disease. We previously reported that a diet high in alpha-linolenic acid (ALA) reduces lipid and inflammatory cardiovascular disease risk factors in hypercholesterolemic subjects. OBJECTIVE: The objective was to evaluate the effects of a diet high in ALA on serum proinflammatory cytokine concentrations and cytokine production by cultured peripheral blood mononuclear cells (PBMCs) from subjects fed the experimental diets. DESIGN: A randomized, controlled, 3-diet, 3-period crossover study design was used. Hypercholesterolemic subjects (n = 23) were assigned to 3 experimental diets: a diet high in ALA (ALA diet; 6.5% of energy), a diet high in linoleic acid (LA diet; 12.6% of energy), and an average American diet (AAD) for 6 wk. Serum interleukin (IL)-6, IL-1beta, and tumor necrosis factor-alpha (TNF-alpha) concentrations and the production of IL-6, IL-1beta, and TNF-alpha by PBMCs were measured. RESULTS: IL-6, IL-1beta, and TNF-alpha production by PBMCs and serum TNF-alpha concentrations were lower ($P < 0.05$ and $P < 0.08$, respectively) with the ALA diet than with the LA diet or AAD. PBMC production of TNF-alpha was inversely correlated with ALA ($r = -0.402$, $P = 0.07$) and with eicosapentaenoic acid ($r = -0.476$, $P = 0.03$) concentrations in PBMC lipids with the ALA diet. Changes in serum ALA were inversely correlated with changes in TNF-alpha produced by PBMCs ($r = -0.423$, $P < 0.05$). CONCLUSIONS: Increased intakes of dietary ALA elicit antiinflammatory effects by inhibiting IL-6, IL-1beta, and TNF-alpha production in cultured PBMCs. Changes in PBMC ALA and eicosapentaenoic acid (derived from dietary ALA) are associated with beneficial changes in TNF-alpha release. Thus, the cardioprotective effects of ALA are mediated in part by a reduction in the production of inflammatory cytokines.

2006

Cortés B, Núñez I, Cofán M, Gilabert R, Pérez-Heras A, Casals E, Deulofeu R, Ros E. Acute effects of high-fat meals enriched with walnuts or olive oil on postprandial endothelial function. *J Am Coll Cardiol*. 2006; 48:1666-1671.

OBJECTIVES: We sought to investigate whether the addition of walnuts or olive oil to a fatty meal have differential effects on postprandial vasoactivity, lipoproteins, markers of oxidation and endothelial activation, and plasma asymmetric dimethylarginine (ADMA).BACKGROUND: Compared with a Mediterranean diet, a walnut diet has been shown to improve endothelial function in hypercholesterolemic patients. We hypothesized that walnuts would reverse postprandial endothelial dysfunction associated with consumption of a fatty meal. METHODS: We randomized in a crossover design 12 healthy subjects and 12 patients with hypercholesterolemia to 2 high-fat meal sequences to which 25 g olive oil or 40 g walnuts had been added. Both test meals contained 80 g fat and 35% saturated fatty acids, and consumption of each meal was separated by 1 week. Venipunctures and ultrasound measurements of brachial artery endothelial function were performed after fasting and 4 h after test meals. RESULTS: In both study groups, flow-mediated dilation (FMD) was worse after the olive oil meal than after the walnut meal ($p = 0.006$, time-period interaction). Fasting, but not postprandial, triglyceride concentrations correlated inversely with FMD ($r = -0.324$; $p = 0.024$). Flow-independent dilation and plasma ADMA concentrations were unchanged, and the concentration of oxidized low-density lipoproteins decreased ($p = 0.051$) after either meal. The plasma concentrations of soluble inflammatory cytokines and adhesion molecules decreased ($p < 0.01$) independently of meal type, except for E-selectin, which decreased more ($p = 0.033$) after the walnut meal. CONCLUSIONS: Adding walnuts to a high-fat meal acutely improves FMD independently of changes in oxidation, inflammation, or ADMA. Both walnuts and olive oil preserve the protective phenotype of endothelial cells.

Davis P, Valacchi G, Pagnin E, Shao, Gross HB, Calo L, Yokoyama W. Walnuts reduce aortic ET-1 mRNA levels in hamsters fed a high-fat, atherogenic diet. *J of Nutr.* 2006; 136: 428-32.

Walnut consumption is associated with reduced coronary vascular disease (CVD) risk; however, the mechanisms responsible remain incompletely understood. Recent clinical studies suggested that these mechanisms involve non-plasma lipid-related effects on endothelial function. Male Golden Syrian hamsters (12

groups, n=10-15) were fed for 26 wk atherosclerotic, high-fat, hyperlipidemic diets with increasing concentrations of whole walnuts (61-150 g/kg diet), or alpha-tocopherol (alpha-T, 8.1-81 mg/kg diet) and single diets with either walnut oil (32 g/kg diet) or pure gamma-tocopherol (gamma-T; 81 mg/kg diet) added. Aortic endothelin 1 (ET-1), an important endothelial regulator, was assayed as mRNA. Aortic cholesterol ester (CE) concentration along with other vascular stress markers (Cu/Zn and Mn superoxide dismutase, biliverdin reductase) and plasma lipid concentrations were determined. Hyperlipidemia (plasma LDL cholesterol approximately 6 times normal) occurred in all groups. Aortic CE concentration, a measure of atherosclerotic plaque, was highest in the lowest alpha-T only group and declined significantly with increasing alpha-T. The aortic CE of all walnut groups was decreased significantly relative to the lowest alpha-T only group but showed no dose response. The diets did not produce changes in the other vascular stress markers, whereas aortic ET-1 mRNA levels declined dramatically with increasing dietary walnuts (to a 75% reduction in the highest walnut content group compared with the lowest alpha-T group) but were unaltered in the alpha-T groups or gamma-T group. The study results are consistent with those of human walnut feeding studies and suggest that the mechanisms underlying those results are mediated in part by ET-1-dependent mechanisms. The contrasting results between the alpha-tocopherol or gamma-tocopherol diets and the walnut diets also make it unlikely that the non-plasma lipid-related CVD effects of walnuts are due to their alpha-tocopherol or gamma-tocopherol content. Finally, the results indicate that the walnut fat compartment is a likely location for the components responsible for the reduced aortic CE concentration.

2005

Reiter RJ, Manchester LC, Tan DX. Melatonin in walnuts: Influence in levels of melatonin and total antioxidant capacity of blood. *Nutrition.* 2005 Sep; 21(9):920-4.

OBJECTIVE: We investigated whether melatonin is present in walnuts (Juglans regia L.) and, if so, tested whether eating walnuts influences melatonin levels and the total antioxidant status of the blood. METHODS: Melatonin was extracted from walnuts and quantified by high-performance liquid chromatography. After feeding walnuts to rats, serum melatonin concentrations were measured using a radioimmunoassay and the "total antioxidant power" of the serum was estimated by using the trolox equivalent antioxidant capacity and ferric-reducing ability of serum methods. RESULTS: Mean +/- standard error melatonin concentrations were 3.5 +/- 1.0 ng/g of walnut. After food restriction of rats and then feeding them regular chow or walnuts, blood melatonin concentrations in the animals that ate walnuts were increased over those in the rats fed the control diet. Increases in blood melatonin were also accompanied by increases in trolox equivalent antioxidant capacity and ferric-reducing ability of serum values. CONCLUSIONS: Melatonin is present in walnuts and, when eaten, increase blood melatonin concentrations. The increase in blood melatonin levels correlates with an increased antioxidative capacity of this fluid as reflected by augmentation of trolox equivalent antioxidant capacity and ferric-reducing ability of serum values.

Sabaté J, Cordero-Macintyre Z, Siapco G, Torabian S, Haddad E. Does regular walnut consumption lead to weight gain? *Br J Nutr.* 2005; 94:859-64.

Studies consistently show the beneficial effects of eating nuts, but as high-energy foods, their regular consumption may lead to weight gain. We tested if daily consumption of walnuts (approximately 12 % energy intake) for 6 months would modify body weight and body composition in free-living subjects. Ninety participants in a 12-month randomized cross-over trial were instructed to eat an allotted amount of walnuts (28-56 g) during the walnut-supplemented diet and not to eat them during the control diet, with no further instruction. Subjects were unaware that body weight was the main outcome. Dietary compliance was about 95 % and mean daily walnut consumption was 35 g during the walnut-supplemented diet. The walnut-supplemented diet resulted in greater daily energy intake (557 kJ (133 kcal)), which should theoretically have led to a weight gain of 3.1 kg over the 6-month period. For all participants, walnut supplementation increased weight (0.4 (se 0.1) kg), BMI (0.2 (se 0.1) kg/m(2)), fat mass (0.2 (se 0.1) kg) and lean mass (0.2 (se 0.1) kg). But, after adjusting for energy differences between the control and walnut-supplemented diets, no significant differences were observed in body weight or body composition parameters, except for BMI (0.1 (se 0.1) kg/m(2)). The weight gain from incorporating walnuts into the diet (control-->walnut sequence) was less than the weight loss from withdrawing walnuts from the diet (walnut-->control sequence). Our findings show that regular walnut intake resulted in weight gain much lower than expected and which became non-significant after controlling for differences in energy intake.

Tapsell LC, Gillen LJ, Patch CS, Owen A, Baré M, Kennedy M. Structured dietary advice incorporating walnuts achieves optimal fat and energy balance in patients with type 2 diabetes mellitus. *J Amer Diet Assoc.* 2005;105:1087-96.

OBJECTIVE: A cardioprotective dietary fat profile is recommended for the treatment of type 2 diabetes. The clinical feasibility of advice strategies targeting specific fatty acid intakes and the extent to which they can be achieved by free-living populations needs to be tested. Walnuts, with high n-3 polyunsaturated fatty acid (PUFA) content, may help optimize fatty acid intakes, but regular consumption might increase total fat and energy intakes. This study examined whether advice that refers to a total dietary pattern inclusive of walnuts would result in low-fat energy-controlled diets with optimal dietary fat proportions for patients with type 2 diabetes mellitus. RESEARCH DESIGN AND METHODS: A parallel-design, controlled trial was completed by 55 free-living men and women with established type 2 diabetes mellitus. Participants were randomly assigned to one of three groups: low-fat (general advice), modified low-fat (total diet advice using exchange lists to differentiate PUFA-rich foods), walnut-specific (modified low fat including 30 g walnuts/day). Dietary intakes and clinical outcomes were measured at baseline, and at 3 and 6 months. Dietary goals were: less than 10% of energy from saturated fat, 7% to 10% of energy from PUFA, adequate n-3 PUFA (>or=2.22 g alpha-linolenic acid, >or=0.65 g eicosapentaenoic acid [EPA]+docosahexaenoic acid [DHA]) and n-6 to n-3 ratio less than 10. The proportion of subjects achieving dietary goals and major food sources of fat were determined. RESULTS: At baseline, dietary intakes were not significantly different between groups. No group and few individuals (10%) were consuming adequate PUFA, with meat the main source of dietary fat (22% total dietary fat). At 3 and 6 months, energy and macronutrient intakes were similar among groups. The walnut group, however, was the only group to achieve all fatty acid intake targets ($P < .01$), and had the greatest proportion of subjects achieving targets ($P < .05$). Walnuts were the main source of dietary fat (31%) and n-3 PUFA (50%), while 350 g oily fish/day provided a further 17% n-3 PUFA consumed by this group. CONCLUSIONS: Specific advice for the regular inclusion of walnuts in the context of the total diet helps achieve optimal fat intake proportions without adverse effects on total fat or energy intakes in patients with type 2 diabetes mellitus.

Zhao G, Etherton TD, Martin KR, Vanden Heuval JP, Gillies PJ, West, SG, Kris-Etherton PM. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. *Biochem Biophys Res Commun.* 2005 Oct 28;336(3):909-17.

The effects of linoleic acid (LA), alpha-linolenic acid (ALA), and docosahexaenoic acid (DHA) were compared to that of palmitic acid (PA), on inflammatory responses in human monocytic THP-1 cells. When cells were pre-incubated with fatty acids for 2-h and then stimulated with lipopolysaccharide for 24-h in the presence of fatty acids, secretion of interleukin (IL)-6, IL-1beta, and tumor necrosis factor-alpha (TNFalpha) was significantly decreased after treatment with LA, ALA, and DHA versus PA ($P < 0.01$ for all); ALA and DHA elicited more favorable effects. These effects were comparable to those for 15-deoxy-delta12,14-prostaglandin J2 (15d-PGJ2) and were dose-dependent. In addition, LA, ALA, and DHA decreased IL-6, IL-1beta, and TNFalpha gene expression ($P < 0.05$ for all) and nuclear factor (NF)-kappaB DNA-binding activity, whereas peroxisome proliferator-activated receptor-gamma (PPARgamma) DNA-binding activity was increased. The results indicate that the anti-inflammatory effects of polyunsaturated fatty acids may be, in part, due to the inhibition of NF-kappaB activation via activation of PPARgamma.

2004

Ros E, Núñez I, Pérez-Heras A, Serra M, Gilabert R, Casals E, Deulofeu R. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. *Circulation.* 2004; 109:1609-14.

BACKGROUND: Epidemiological studies suggest that nut intake decreases coronary artery disease (CAD) risk. Nuts have a cholesterol-lowering effect that partly explains this benefit. Endothelial dysfunction is associated with CAD and its risk factors and is reversed by antioxidants and marine n-3 fatty acids. Walnuts are a rich source of both antioxidants and alpha-linolenic acid, a plant n-3 fatty acid. METHODS AND RESULTS: To test the hypothesis that walnut intake will reverse endothelial dysfunction, we randomized in a crossover design 21 hypercholesterolemic men and women to a cholesterol-lowering Mediterranean diet and a diet of similar energy and fat content in which walnuts replaced approximately 32% of the energy from monounsaturated fat. Participants followed each diet for 4 weeks. After each intervention, we obtained fasting blood and performed ultrasound measurements of brachial artery vasomotor function. Eighteen subjects completing the protocol had suitable ultrasound studies. Compared with the Mediterranean diet, the walnut diet improved endothelium-dependent vasodilation and reduced levels of vascular cell adhesion molecule-1 ($P<0.05$ for both). Endothelium-independent vasodilation and levels of intercellular adhesion molecule-1, C-reactive protein, homocysteine, and oxidation biomarkers were similar after each diet. The walnut diet significantly reduced total cholesterol (-4.4+/-7.4%) and LDL cholesterol (-6.4+/-10.0%) ($P<0.05$ for both). Cholesterol reductions correlated with increases of both dietary alpha-linolenic acid and LDL gamma-tocopherol content, and changes of endothelium-dependent vasodilation correlated with those of cholesterol-to-HDL ratios ($P<0.05$ for all). CONCLUSIONS: Substituting walnuts for monounsaturated fat in a Mediterranean diet improves endothelium-dependent

vasodilation in hypercholesterolemic subjects. This finding might explain the cardioprotective effect of nut intake beyond cholesterol lowering.

Tapsell LC, Gillen LJ, Patch CS, Batterham M, Owen A, Baré M, Kennedy M. Including walnuts in a low-fat/modified-fat diet improves HDL cholesterol-to-total cholesterol ratios in patients with type 2 diabetes. *Diabetes Care*. 2004; 27:2777-83.

OBJECTIVE: The aim of this study was to examine the effect of a moderate-fat diet inclusive of walnuts on blood lipid profiles in patients with type 2 diabetes. RESEARCH DESIGN AND METHODS: This was a parallel randomized controlled trial comparing three dietary advice groups each with 30% energy as fat: low fat, modified low fat, and modified low fat inclusive of 30 g of walnuts per day. Fifty-eight men and women, mean age 59.3 +/- 8.1 years, started the trial. Dietary advice was given at baseline with monthly follow-up and fortnightly phone calls for support. Body weight, percent body fat, blood lipids, HbA1c, total antioxidant capacity, and erythrocyte fatty acid levels were measured at 0, 3, and 6 months. Data were assessed by repeated-measures ANOVA with an intention-to-treat model. RESULTS: The walnut group achieved a significantly greater increase in HDL cholesterol-to-total cholesterol ratio (P=0.049) and HDL (P=0.046) than the two other treatment groups. A 10% reduction in LDL cholesterol was also achieved in the walnut group, reflecting a significant effect by group (P=0.032) and time (P=0.036). There were no significant differences between groups for changes in body weight, percent body fat, total antioxidant capacity, or HbA1c levels. The higher dietary polyunsaturated fat-to-saturated fat ratio and intakes of omega-3 fatty acids in the walnut group were confirmed by erythrocyte biomarkers of dietary intake. CONCLUSIONS: Structured "whole of diet" advice that included 30 g of walnuts/day delivering substantial amounts of polyunsaturated fatty acid improved the lipid profile of patients with type 2 diabetes.

Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *J Nutr.* 2004; 134:2991-7.

Alpha-linolenic acid (ALA) reduces cardiovascular disease (CVD) risk, possibly by favorably changing vascular inflammation and endothelial dysfunction. Inflammatory markers and lipids and lipoproteins were assessed in hypercholesterolemic subjects (n = 23) fed 2 diets low in saturated fat and cholesterol, and high in PUFA varying in ALA (ALA Diet) and linoleic acid (LA Diet) compared with an average American diet (AAD). The ALA Diet provided 17% energy from PUFA (10.5% LA; 6.5% ALA); the LA Diet provided 16.4% energy from PUFA (12.6% LA; 3.6% ALA); and the AAD provided 8.7% energy from PUFA (7.7% LA; 0.8% ALA). The ALA Diet decreased C-reactive protein (CRP, $P < 0.01$), whereas the LA Diet tended to decrease CRP ($P = 0.08$). Although the 2 high-PUFA diets similarly decreased intercellular cell adhesion molecule-1 vs. AAD (-19.1% by the ALA Diet, $P < 0.01$; -11.0% by the LA Diet, $P < 0.01$), the ALA Diet decreased vascular cell adhesion molecule-1 (VCAM-1, -15.6% vs. -3.1%, $P < 0.01$) and E-selectin (-14.6% vs. -8.1%, $P < 0.01$) more than the LA Diet. Changes in CRP and VCAM-1 were inversely associated with changes in serum eicosapentaenoic acid (EPA) ($r = -0.496$, $P = 0.016$; $r = -0.418$, $P = 0.047$), or EPA plus docosapentaenoic acid ($r = -0.409$, $P = 0.053$; $r = -0.357$, $P = 0.091$) after subjects consumed the ALA Diet. The 2 high-PUFA diets decreased serum total cholesterol, LDL cholesterol and triglycerides similarly ($P < 0.05$); the ALA Diet decreased HDL cholesterol and apolipoprotein AI compared with the AAD ($P < 0.05$). ALA appears to decrease CVD risk by inhibiting vascular inflammation and endothelial activation beyond its lipid-lowering effects.

2002

Feldman EB. The scientific evidence for a beneficial health relationship between walnuts and coronary heart disease. *J Nutr.* 2002; 132:1062S-1101S.

The author and four independent experts evaluated the intent and quality of scientific evidence for a potential beneficial health relationship between the intake of walnuts and the reduction and prevention of coronary heart disease. The report also addresses the supporting evidence for the health benefit of other tree nuts and selected legumes. Compared to most other nuts, which contain monounsaturated fatty acids, walnuts are unique because they are rich in n-6 (linoleate) and n-3 (linolenate) polyunsaturated fatty acids. Walnuts contain multiple health-beneficial components, such as having a low lysine:arginine ratio and high levels of arginine, folate, fiber, tannins, and polyphenols. Though walnuts are energy rich, clinical dietary intervention studies show that walnut consumption does not cause a net gain in body weight when eaten as a replacement food. Five controlled, peer-reviewed, human clinical walnut intervention trials, involving approximately 200 subjects representative of the 51% of the adult population in the United States at risk of coronary heart disease were reviewed. The intervention trials consistently demonstrated walnuts as part of a heart-healthy diet, lower blood cholesterol

concentrations. None of these studies were of extended duration that would be essential for evaluation of the sustainability of the observed outcomes. These results were supported by several large prospective observational studies in humans, all demonstrating a dose response-related inverse association of the relative risk of coronary heart disease with the frequent daily consumption of small amounts of nuts, including walnuts

Iwamoto M, Imaizumi K, Sato M, Hirooka Y, Sakai K, Takeshita A, Kono, M. Serum lipid profiles in Japanese women and men during consumption of walnuts. *Eur J Clin Nutr.* 2002 Jul;56(7):629-37.

OBJECTIVE: To determine the serum cholesterol, apolipoproteins and LDL oxidizability in young Japanese women and men during walnut consumption and to evaluate its active principle. DESIGN: Experimental study with a randomized design. SUBJECTS: Twenty healthy women and 20 healthy men. INTERVENTIONS: Subjects were randomly assigned to consume each of two mixed natural diets for 4 weeks in a cross-over design. Reference and walnut diets were designed and the walnut diet had 12.5% of the energy derived from walnuts (44-58 g/day). RESULTS: The total cholesterol and serum apolipoprotein B concentrations, and the ratio of LDL cholesterol to HDL cholesterol was significantly lowered in women and men when fed on the walnut diet, than when on the reference diet (P<or=0.05). The LDL cholesterol concentration was significantly lowered in women on the walnut diet (0.22 mmol/l, P=0.0008), whereas this decrease was not significant in men (0.18 mmol/l, P=0.078). The most prominent change in the fatty acid composition of the cholesteryl esters from serum after the walnut diet was an elevation of alpha-linolenic acid in women (76%, P<0.001) and men (107%, P<0.001). This elevation was negatively correlated to the change in LDL cholesterol in women (r=0.496, P=0.019) and men (r=0.326, P=0.138). The LDL oxidizability in women was not influenced by the diets (P=0.19). CONCLUSIONS: alpha-Linolenic acid in the walnut diet appears to be responsible for the lowering of LDL cholesterol in women.

2001

Almario RU, Vonghavaravat V, Wong R, Kasim-Karakas SE. Effects of walnut consumption on plasma fatty acids and lipoproteins in combined hyperlipidemia. *Am J Clin Nutr.* 2001; 74:72-9.

BACKGROUND: Epidemiologic studies show an inverse relation between nut consumption and coronary heart disease. OBJECTIVE: We determined the effects of walnut intake on plasma fatty acids, lipoproteins, and lipoprotein subclasses in patients with combined hyperlipidemia. DESIGN: Participants sequentially adhered to the following diets: 1) a habitual diet (HD), 2) a habitual diet plus walnuts (HD+W), 3) a low-fat diet (LFD), and 4) a low-fat diet plus walnuts (LFD+W). RESULTS: In 13 postmenopausal women and 5 men (+/- SD age 60 +/- 8 y), walnut supplementation did not increase body weight despite increased energy intake and the LFD caused weight loss (1.3 +/- 0.5 kg; P < 0.01). When comparing the HD with the HD+W, linoleic acid concentrations increased from 29.94 +/- 1.14% to 36.85 +/- 1.13% and alpha-linolenic acid concentrations increased from 0.78 +/- 0.04% to 1.56 +/- 0.11%. During the LFD+W, plasma total cholesterol concentrations decreased by 0.58 +/- 0.16 mmol/L when compared with the HD and by 0.46 +/- 0.14 mmol/L when compared with the LFD. LDL-cholesterol concentrations decreased by 0.46 +/- 0.15 mmol/L when compared with the LFD. Measurements of lipoprotein subclasses and particle size suggested that walnut supplementation lowered cholesterol preferentially in small LDL (46.1 +/- 1.9% compared with 33.4 +/- 4.3%, HD compared with HD+W, respectively; P < 0.01). HDL-cholesterol concentrations decreased from 1.27 +/- 0.07 mmol/L during the HD to 1.14 +/- 0.07 mmol/L during the HD+W and to 1.11 +/- 0.08 mmol/L during the LFD. The decrease was seen primarily in the large HDL particles. CONCLUSIONS: Walnut supplementation may beneficially alter lipid distribution among various lipoprotein subclasses even when total plasma lipids do not change. This may be an additional mechanism underlying the antiatherogenic properties of nut intake.

Anderson K, Teuber S, Gobeille A, Cremin P, Waterhouse A, Steinberg F. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. *J Nutr.* 2001; 131:2837-2842.

Recent epidemiologic studies have associated nut consumption with a reduced incidence of cardiovascular mortality. However, little is known about the contribution of nut polyphenols to antioxidant and cardiovascular protection. In this investigation, polyphenol-rich extracts from English walnuts (Juglans regia) were studied and compared with ellagic acid for their ability to inhibit in vitro plasma and LDL oxidation, as well as their effects on LDL alpha-tocopherol during oxidative stress. In addition, the Trolox equivalent antioxidant activity (TEAC) was determined and liquid chromatography electrospray detection mass spectrometry (LC-ELSD/MS) analyses of the walnut extracts were performed. 2,2'-Azobis'(2-amidino propane) hydrochloride (AAPH)-induced LDL oxidation was significantly inhibited by 87 and 38% with the highest concentration (1.0 micromol/L) of ellagic acid and walnut extract, respectively. In addition, copper-mediated LDL oxidation was inhibited by 14 and 84% in the presence of ellagic acid and walnut extract, respectively, with a modest, significant LDL alpha-tocopherol

sparing effect observed. Plasma thiobarbituric acid reacting substance (TBARS) formation was significantly inhibited by walnut extracts and ellagic acid in a dose-dependent manner, and the extracts exhibited a TEAC value greater than that of alpha-tocopherol. LC-ELSD/MS analysis of the walnut extracts identified ellagic acid monomers, polymeric ellagitannins and other phenolics, principally nonflavonoid compounds. These results demonstrate that walnut polyphenolics are effective inhibitors of in vitro plasma and LDL oxidation. The polyphenolic content of walnuts should be considered when evaluating their antiatherogenic potential.

Muñoz S, Merlos M, Zambón D, Rodríguez C, Sabaté J, Ros E, Laguna JC. Walnut-enriched diet increases the association of LDL from hypercholesterolemic men with human HepG2 cells. *J. Lipid Res.* 2001; 42:2069 - 2076.

In a randomized, cross-over feeding trial involving 10 men with polygenic hypercholesterolemia, a control, Mediterranean-type cholesterol-lowering diet, and a diet of similar composition in which walnuts replaced approximately 35% of energy from unsaturated fat, were given for 6 weeks each. Compared with the control diet, the walnut diet reduced serum total and LDL cholesterol by 4.2% (P = 0.176), and 6.0% (P = 0.087), respectively. No changes were observed in HDL cholesterol, triglycerides, and apolipoprotein A-I levels or in the relative proportion of protein, triglycerides, phospholipids, and cholesteryl esters in LDL particles. The apolipoprotein B level declined in parallel with LDL cholesterol (6.0% reduction). Whole LDL, particularly the triglyceride fraction, was enriched in polyunsaturated fatty acids from walnuts (linoleic and alpha-linolenic acids). In comparison with LDL obtained during the control diet, LDL obtained during the walnut diet showed a 50% increase in association rates to the LDL receptor in human hepatoma HepG2 cells. LDL uptake by HepG2 cells was correlated with alpha-linolenic acid content of the triglyceride plus cholesteryl ester fractions of LDL particles ($r(2) = 0.42$, $P < 0.05$). Changes in the quantity and quality of LDL lipid fatty acids after a walnut-enriched diet facilitate receptor-mediated LDL clearance and may contribute to the cholesterol-lowering effect of walnut consumption.

2000

Zambón D, Sabaté J, Muñoz S, Campero B, Casals E, Merlos M, Laguna JC, Ros E. Substituting walnuts for monounsaturated fat improves the serum lipid profile of hypercholesterolemic men and women. A randomized crossover trial. *Ann Intern Med*. 2000; 132:538-46.

BACKGROUND: It has been reported that walnuts reduce serum cholesterol levels in normal young men. OBJECTIVE: To assess the acceptability of walnuts and their effects on serum lipid levels and low-density lipoprotein (LDL) oxidizability in free-living hypercholesterolemic persons. DESIGN: Randomized, crossover feeding trial. SETTING: Lipid clinic at a university hospital. PATIENTS: 55 men and women (mean age, 56 years) with polygenic hypercholesterolemia. INTERVENTION: A cholesterol-lowering Mediterranean diet and a diet of similar energy and fat content in which walnuts replaced approximately 35% of the energy obtained from monounsaturated fat. Patients followed each diet for 6 weeks. MEASUREMENTS: Low-density lipoprotein fatty acids (to assess compliance), serum lipid levels, lipoprotein(a) levels, and LDL resistance to in vitro oxidative stress. RESULTS: 49 persons completed the trial. The walnut diet was well tolerated. Planned and observed diets were closely matched. Compared with the Mediterranean diet, the walnut diet produced mean changes of -4.1% in total cholesterol level, -5.9% in LDL cholesterol level, and -6.2% in lipoprotein(a) level. The mean differences in the changes in serum lipid levels were -0.28 mmol/L (95% CI, -0.43 to -0.12 mmol/L) (-10.8 mg/dL [-16.8 to -4.8 mg/dL]) ($P<0.001$) for total cholesterol level, -0.29 mmol/L (CI, -0.41 to -0.15 mmol/L) (-11.2 mg/dL [-16.3 to -6.1 mg/dL]) ($P<0.001$) for LDL cholesterol level, and -0.021 g/L (CI, -0.042 to -0.001 g/L) ($P = 0.042$) for lipoprotein(a) level. Lipid changes were similar in men and women except for lipoprotein(a) levels, which decreased only in men. Low-density lipoprotein particles were enriched with polyunsaturated fatty acids from walnuts, but their resistance to oxidation was preserved. CONCLUSION: Substituting walnuts for part of the mono-unsaturated fat in a cholesterol-lowering Mediterranean diet further reduced total and LDL cholesterol levels in men and women with hypercholesterolemia.

1993

Sabaté J, Fraser GE, Burke K, Knutson SF, Bennett H, Lindsey KD. Effects of walnuts on serum lipid levels and blood pressure in normal men. *N Engl J Med.* 1993 Mar 4;328(9):603-7.

In a recent six-year follow-up study, we found that frequent consumption of nuts was associated with a reduced risk of ischemic heart disease. To explore possible explanations for this finding, we studied the effects of nut consumption on serum lipids and blood pressure. We randomly placed 18 healthy men on two mixed natural diets, each diet to be followed for four weeks. Both diets conformed to the National Cholesterol Education

Program Step 1 diet and contained identical foods and macronutrients, except that 20 percent of the calories of one diet (the walnut diet) were derived from walnuts (offset by lesser amounts of fatty foods, meat, and visible fat [oils, margarine, and butter]).With the reference diet, the mean (+/- SD) serum values for total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol were, respectively, 182 +/- 23, 112 +/- 16, and 47 +/- 11 mg per deciliter (4.71 +/- 0.59, 2.90 +/- 0.41, and 1.22 +/- 0.28 mmol per liter). With the walnut diet, the mean total cholesterol level was 22.4 mg per deciliter (0.58 mmol per liter) lower than the mean level with the reference diet (95 percent confidence interval, 28 to 17 mg per deciliter [0.72 to 0.44 mmol per liter]); the LDL and HDL cholesterol levels were, respectively, 18.2 mg per deciliter (0.47 mmol per liter) ($P < 0.001$) and 2.3 mg per deciliter (0.06 mmol per liter) ($P = 0.01$) lower. These lower values represented reductions of 12.4, 16.3, and 4.9 percent in the levels of total, LDL, and HDL cholesterol, respectively. The ratio of LDL cholesterol to HDL cholesterol was also lowered ($P < 0.001$) by the walnut diet. Mean blood-pressure values did not change during either dietary period. Incorporating moderate quantities of walnuts into the recommended cholesterol-lowering diet while maintaining the intake of total dietary fat and calories decreases serum levels of total cholesterol and favorably modifies the lipoprotein profile in normal men. The long-term effects of walnut consumption and the extension of this finding to other population groups deserve further study.

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