

CALIFORNIA WALNUT COMMISSION

ABSTRACTS OF PUBLICATIONS SUPPORTED BY THE CALIFORNIA WALNUT COMMISSION

MAY 2023

The California Walnut Commission (CWC) supports health-related research on walnuts through grants and the provision of walnuts. Research priorities are identified in consultation with our Health Research Advisory Group. Since the inception of the health research program, over 200 CWC-supported, peer-reviewed papers have been published examining the effect of walnut consumption on heart health, cognitive health, cancer, diabetes, metabolic syndrome, gut health, body weight/composition, reproductive health, food modeling, bone health as well as nutrient and bioactive composition and research methodology. The CWC is committed to building a strong foundation of high-quality scientific evidence, from pre-clinical research in the early stages of exploration to human observational, epidemiological, and clinical intervention studies that determine the health effects of walnut consumption across the lifespan.

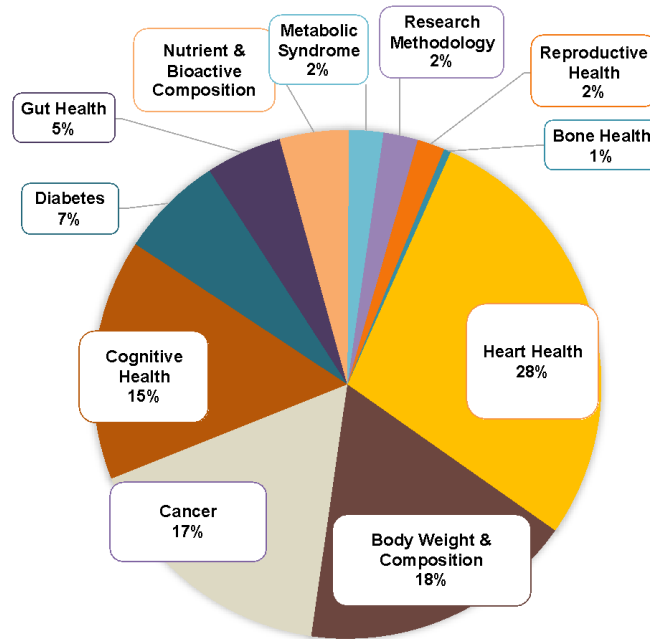


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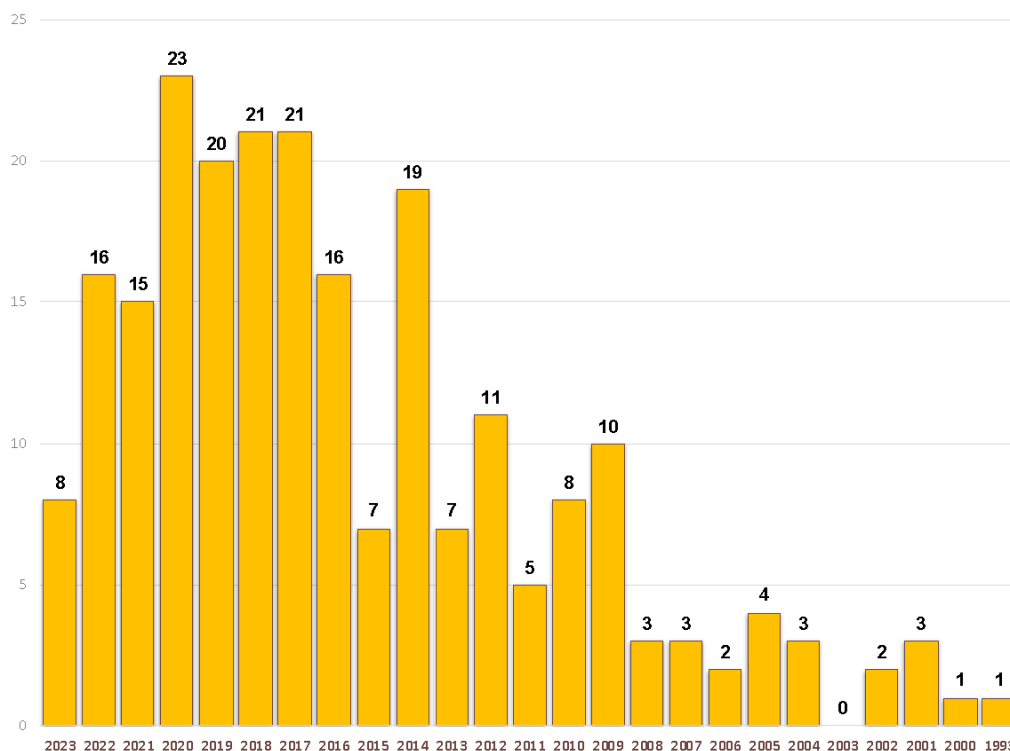
California Walnut Commission Supported Publications At-A-Glance

Total: 229 publications

Areas of Research Covered



Number of Publications by Year



Abstract List by Year of Publication

Abstracts extracted from <http://www.ncbi.nlm.nih.gov/pubmed> or through open-access journals.

2023

Amen RI, Sirirat R, Oda K, Rajaram S, Nwachukwu I, Cofan M, Ros E, Sabate J, Haddad EH. Effect of Walnut Supplementation on Dietary Polyphenol Intake and Urinary Polyphenol Excretion in the Walnuts and Healthy Aging Study.

Nutrients. 2023 Mar 2;15(5):1253. <https://doi.org/10.3390/nu15051253>

Abstract: Among all tree nuts, walnuts contain the highest total polyphenols by weight. This secondary data analysis examined the effect of daily walnut supplementation on the total dietary polyphenols and subclasses and the urinary excretion of total polyphenols in a free-living elderly population. In this 2-year prospective, randomized intervention trial (ID NCT01634841), the dietary polyphenol intake of participants who added walnuts daily to their diets at 15% of daily energy were compared to those in the control group that consumed a walnut-free diet. Dietary polyphenols and subclasses were estimated from 24 h dietary recalls. Phenolic estimates were derived from Phenol-Explorer database version 3.6. Participants in the walnut group compared to the control group had a higher intake of total polyphenols, flavonoids, flavanols, and phenolic acids in mg/d (IQR): 2480 (1955, 3145) vs. 1897 (1369, 2496); 56 (42,84) vs. 29 (15, 54); 174 (90, 298) vs. 140 (61, 277); and 368 (246, 569) vs. 242 (89, 398), respectively. There was a significant inverse association between dietary flavonoid intake and urine polyphenol excretion; less urinary excretion may imply that some of the polyphenols were eliminated via the gut. Nuts had a significant contribution to the total polyphenols in the diet, suggesting that a single food like walnuts added to habitual diet can increase the polyphenol intake in a Western population.

Key Area: Nutrient & Bioactive Composition

Fan N, Fusco JL, Rosenberg DW. Antioxidant and Anti-Inflammatory Properties of Walnut Constituents: Focus on Personalized Cancer Prevention and the Microbiome.

Antioxidants. 2023 Apr 22;12(5):982. <https://doi.org/10.3390/antiox12050982>

Abstract: Walnuts have been lauded as a ‘superfood’, containing a remarkable array of natural constituents that may have additive and/or synergistic properties that contribute to reduced cancer risk. Walnuts are a rich source of polyunsaturated fatty acids (PUFAs: alpha-linolenic acid, ALA), tocopherols, antioxidant polyphenols (including ellagitannins), and prebiotics, including fiber (2 g/oz). There is a growing body of evidence that walnuts may contribute in a positive way to the gut microbiome, having a prebiotic potential that promotes the growth of beneficial bacteria. Studies supporting this microbiome-modifying potential include both preclinical cancer models as well as several promising human clinical trials. Mediated both directly and indirectly via its actions on the microbiome, many of the beneficial properties of walnuts are related to a range of anti-inflammatory properties, including powerful effects on the immune system. Among the most potent constituents of walnuts are the ellagitannins, primarily pedunculagin. After ingestion, the ellagitannins are hydrolyzed at low pH to release ellagic acid (EA), a non-flavonoid polyphenolic that is subsequently metabolized by the microbiota to the bioactive urolithins (hydroxydibenzo[b,d]pyran-6-ones). Several urolithins, including urolithin A, reportedly have potent anti-inflammatory properties. These properties of walnuts provide the rationale for including this tree nut as part of a healthy diet for reducing overall disease risk, including colorectal cancer. This review considers the latest information regarding the potential anti-cancer and antioxidant properties of walnuts and how they may be incorporated into the diet to provide additional health benefits.

Key Area: Cancer

Lázaro I, Bobi J, Cofán M, Kapravelou G, Amor AJ, Surra J, Gómez-Guerrero C, Ortega E, Osada J, Dantas AP, Sala-Vila A. Walnut inclusion in a palm oil-based atherogenic diet promotes traits predicting stable atheroma plaque in Apoe-deficient mice.

Frontiers in Nutrition. 2023 Feb 8;10:1079407. <https://doi.org/10.3389/fnut.2023.1079407>

Abstract: INTRODUCTION: The lower rates of cardiovascular disease in Southern Europe could be partially explained by the low prevalence of lipid-rich atheroma plaques. Consumption of certain foods affects the progression and severity of atherosclerosis. We investigated whether the isocaloric inclusion of walnuts within an atherogenic diet prevents phenotypes predicting unstable atheroma plaque in a mouse model of accelerated atherosclerosis. METHODS: Apolipoprotein E-deficient male mice (10-week-old) were randomized to receive a control diet (9.6% of energy as fat, n = 14), a palm oil-based high-fat diet (43% of energy as fat, n = 15), or an isocaloric diet in which part of palm oil was replaced by walnuts in a dose equivalent to 30 g/day in humans (n = 14). All diets contained 0.2% cholesterol. RESULTS: After 15 weeks of intervention, there were no differences in size and extension in aortic atherosclerosis among groups. Compared to control diet, palm oil-diet induced features predicting unstable atheroma plaque (higher lipid content, necrosis, and calcification), and more advanced lesions (Stary score). Walnut inclusion attenuated these features. Palm oil-based diet also boosted inflammatory aortic storm (increased expression of chemokines, cytokines, inflammasome components, and M1 macrophage phenotype markers) and promoted defective efferocytosis. Such response was not observed in the walnut group. The walnut group's differential activation of nuclear factor kappa B (NF-kappaB; downregulated) and Nrf2 (upregulated) in the atherosclerotic lesion could explain these findings. CONCLUSION: The isocaloric inclusion of walnuts in an unhealthy high-fat diet promotes traits predicting stable advanced atheroma plaque in mid-life mice. This contributes novel evidence for the benefits of walnuts, even in an unhealthy dietary environment.

Key Area: Heart Health

Mandalari, G., Gervasi, T., Rosenberg, D.W., Lapsley, K.G. and Baer, D.J., Effect of Nuts on Gastrointestinal Health.

Nutrients, 2023 Apr 1;15(7):1733. <https://doi.org/10.3390%2Fnu15071733>

Abstract: Nuts are high nutrient-dense foods containing healthy lipids, dietary fiber, and bioactive phytochemicals, including vitamins and minerals. Although the beneficial effect of nut consumption on different chronic diseases has been well documented, especially in relation to their cardiometabolic benefits, less scientific evidence is available on their possible beneficial effects on gastrointestinal health. In this narrative review, we summarize the most important findings and new research perspectives in relation to the importance of nut consumption on gastrointestinal health. The integrity of the cell wall structure, cell size and particle size after mastication are known to play a crucial role in energy, nutrient and bioactive release from nuts during digestion, therefore affecting bioaccessibility. Other mechanisms, such as cell wall composition, thickness and porosity, as well as stability of the membranes surrounding the oil bodies within the cell, are also important for energy extraction. As the undigested nutrients and phytochemicals are delivered to the colon, effects on gut microbiota composition are predicted. Although the overall effect of nut consumption on microbial alpha- and beta-diversity has been inconsistent, some scientific evidence suggests an increase in fecal butyrate after almond consumption, and a beneficial role of walnuts on the prevention of ulcerative colitis and protection against the development of gastric mucosal lesions.

Key Area: Gut Health

Novaj A, Engel MG, Wang R, Mao K, Xue X, Amir Y, Atzmon G, Huffman DM. Dietary Walnuts Preserve Aspects of Health Span and Alter the Hippocampal Lipidome in Aged High-Fat Diet-Fed Mice.

International Journal of Molecular Sciences. 2023 Jan 24;24(3):2314.
<https://doi.org/10.3390/ijms24032314>

Abstract: Evidence continues to accrue that aging and its diseases can be delayed by pharmacologic and dietary strategies that target the underlying hallmarks of the aging process. However, identifying simple, safe, and effective dietary strategies involving the incorporation of whole foods that may confer some protection against the aging process is also needed. Recent observational studies have suggested that nut consumption can reduce mortality risk in humans. Among these, walnuts are particularly intriguing, given their high content of n-3 fatty acids, fiber, and antioxidant and anti-inflammatory compounds. To this end, 12-month-old male CB6F1 mice were provided either a defined control low-fat diet (LFD), a control high-fat diet (HFD), or an isocaloric HFD containing 7.67% walnuts by weight (HFD + W), and measures of healthspan and related biochemical markers (n = 10-19 per group) as well as survival (n = 20 per group) were monitored. Mice provided the HFD or HFD + W demonstrated marked weight gain, but walnuts lowered baseline glucose ($p < 0.05$) and tended to temper the effects of HFD on liver weight gain ($p < 0.05$) and insulin tolerance ($p = 0.1$). Additional assays suggested a beneficial effect on some indicators of health with walnut supplementation, including preservation of exercise capacity and improved short-term working memory, as determined by Y maze ($p = 0.02$). However, no effect was observed via any diet on inflammatory markers, antioxidant capacity, or survival ($p = 0.2$). Ingenuity Pathway Analysis of the hippocampal transcriptome identified two processes predicted to be affected by walnuts and potentially linked to cognitive function, including estrogen signaling and lipid metabolism, with changes in the latter confirmed by lipidomic analysis. In summary, while walnuts did not significantly improve survival on a HFD, they tended to preserve features of healthspan in the context of a metabolic stressor with aging.

Key Area: Cognitive Health

Pinar-Martí A, Gignac F, Fernández-Barrés S, Romaguera D, Sala-Vila A, Lázaro I, Ranzani OT, Persavento C, Delgado A, Carol A, Torrent J. Effect of walnut consumption on neuropsychological development in healthy adolescents: a multi-school randomised controlled trial.
eClinicalMedicine. 2023 May 1;59. <https://doi.org/10.1016/j.tjnnt.2023.01.003>

Abstract: Background Omega-3 fatty acids are critical for neuropsychological functioning. Adolescence is increasingly believed to entail brain vulnerability to dietary intake. The potential benefit on adolescent neurodevelopment of consuming walnuts, a source of omega-3 alpha-linolenic acid (ALA), remains unclear. Methods We conducted a 6-month multi-school-based randomised controlled nutrition intervention trial to assess whether walnut consumption has beneficial effects on the neuropsychological and behavioural development of adolescents. The study took place between 04/01/2016 and 06/30/2017 in twelve different high schools in Barcelona, Spain (ClinicalTrials.gov Identifier: NCT02590848). A total of 771 healthy teenagers aged 11–16 years were randomised into two equal groups (intervention or control). The intervention group received 30 g/day of raw walnut kernels to be incorporated into their diet for 6 months. Multiple primary endpoints concerning neuropsychological (working memory, attention, fluid intelligence, and executive function) and behavioural (socioemotional and attention deficit hyperactivity disorder [ADHD] symptoms) development were assessed at baseline and after intervention. Red blood cell (RBC) ALA status was determined at baseline and 6 months as a measure of compliance. Main analyses were based on intention-to-treat using a linear mixed-effects model. A per-protocol effect of the intervention was analysed using inverse-probability weighting to account for post-randomisation prognostic factors (including adherence) using generalised estimating equations. Findings In intention-to-treat analyses, at 6 months there were no statistically significant changes between the intervention and control groups for all primary endpoints. RBC ALA (%) significantly increased only in the intervention group, coefficient = 0.04 (95% Confidence Interval (CI) = 0.03, 0.06; $p < 0.0001$). The per-protocol (adherence-adjusted) effect on improvement in attention score (hit reaction time variability) was -11.26 ms (95% CI = -19.92 , -2.60 ; $p = 0.011$) for the intervention group as compared to the control group, improvement in fluid intelligence score was 1.78 (95% CI = 0.90 , 2.67 ; $p < 0.0001$), and reduction of ADHD symptom score was -2.18 (95% CI = -3.70 , -0.67 ; $p = 0.0050$). Interpretation Our study suggested that being prescribed eating walnuts for 6 months did not improve the neuropsychological function of healthy adolescents. However, improved sustained attention, fluid intelligence, and ADHD symptoms were observed in participants who better complied with the walnut intervention. This study provides a foundation for further clinical and epidemiological research on the effect of walnuts and ALA on neurodevelopment in adolescents.

Key Area: Cognitive Health

Spence, L.A.; Henschel, B.; Li, R.; Tekwe, C.D.; Thiagarajah, K. Adding Walnuts to the Usual Diet Can Improve Diet Quality in the United States: Diet Modeling Study Based on NHANES 2015–2018.

Nutrients 2023 Jan;15(2):258. <https://doi.org/10.3390/nu15020258>

Abstract: Background: The under-consumption of calcium, potassium, fiber, and vitamin D is considered a U.S. public health concern. Shifts in eating patterns that increase the consumption of vegetables, fruits, whole grains, nuts/seeds, and dairy products can help achieve the recommended intakes of these nutrients, leading to healthier diets. Objective: We assessed the impact of adding 1 ounce (28.35 g) of walnuts to usual diets on diet quality and nutrients of concern, including magnesium, fiber, and potassium. Methods: We utilized 24 h dietary recalls obtained from the What We Eat in America, National Health and Nutrition Examination Survey (NHANES) and modeled the addition of 1 ounce (28.35 g) of walnuts to the usual diets of no-nut consumers. No-nut consumers aged ≥ 4 years ($n =$

7757) from the 2015–2018 NHANES study were included. Population percentages with intakes below the estimated average requirement (EAR) values for calcium, magnesium, folate, and vitamin E and above the adequate intake (AI) values for potassium and fiber were examined. Diet quality was assessed using the Healthy Eating Index-2015 (HEI-2015). The National Cancer Institute method was used to estimate the usual and modeled intakes. Significant differences between usual (current) and modeled intakes were determined using non-overlapping 95% confidence intervals. All analyses included sample weights to account for the NHANES survey design. Results: Adding 1 ounce (28.35 g) of walnuts to the usual diet resulted in significant reductions in the percentages of adults with intakes below the EAR for magnesium and folate (69.6% vs. 52.0%; 49.2% vs. 40.6%, respectively), and increased the percentage of adults above the AI for potassium (22.8% vs. 26.5%). A similar trend was observed among children (4–18 years). HEI scores improved significantly from 49.1 (95% CI: 48.0–50.4) to 58.5 (95% CI: 57.5–59.6) in children and from 52.4 (95% CI: 51.0–53.8) to 59.2 (95% CI: 58.0–60.5) in adults. Conclusions: Adding 1 ounce (28.35 g) of walnuts to the usual diet of no-nut consumers improved the diet quality and adequacy of some under-consumed nutrients.

Key Area: Nutrient & Bioactive Composition

Wang R, Hannan MT, Wang M, Schwartz AW, Lopez-Garcia E, Grodstein F. Long-Term Consumption of Nuts (Including Peanuts, Peanut Butter, Walnuts, and Other Nuts) in Relation to Risk of Frailty in Older Women: Evidence from a Cohort Study.

The Journal of Nutrition. 2023 Jan 7;153(3):820-7. <https://doi.org/10.1016/j.tjnut.2023.01.003>

Abstract: Background: Adherence to a healthy diet is inversely associated with frailty. However, the relationship between nuts, a key food group of Mediterranean diet, and frailty is unclear. Objectives: This study aimed to evaluate the association between nut consumption and frailty in an aging female population. Methods: This population-based observational study included nonfrail women (60 y old) in the NHS from 11 states of the United States.

Outcome was incident frailty, defined as having 3 of the FRAIL components (fatigue, lower strength, reduced aerobic capacity, multiple chronic conditions, and significant weight loss) and assessed every 4 y from 1992 to 2016. From 1990 to 2014, FFQs were used to assess the intakes of peanuts, peanut butter, walnuts (added in 1998), and other nuts at 4-y intervals. Exposure was total nut consumption, calculated as the sum of intakes of peanuts, peanut butter, walnuts, and other nuts and categorized into <1 serving/mo, 1–3 servings/mo, 1 serving/wk, 2–4 servings/wk, and ≥5 servings/wk. The relations of intakes of peanuts, peanut butter, and walnuts with frailty were also investigated separately. Cox proportional hazards models were used to assess the associations between nut consumption and frailty after adjusting for age, smoking, BMI, EI, diet quality, and medication use. Results: Among 71,704 participants, 14,195 incident frailty cases occurred over 1,165,290 person-years. The adjusted HR (95% CI) for consuming ≥5 servings/wk of nuts was 0.80 (0.73, 0.87), as compared with <1 serving/mo. Higher intakes of peanuts and walnuts, but not peanut butter, were also inversely associated with frailty.

Conclusions: This large prospective cohort study showed a strong and consistent inverse association between regular nut consumption and incident frailty. This suggests that nut consumption should be further tested as a convenient public health intervention for the preservation of health and well-being in older adults.

Key Area: Body Weight and Composition

Byerley LO, Chang HM, Lorenzen B, Guidry J, Hardman WE. Impact of dietary walnuts, a nutraceutical option, on circulating markers of metabolic dysregulation in a rodent cachectic tumor model.

Biomed Pharmacother. 2022 Nov;155:113728. doi: 10.1016/j.biopha.2022.113728. Epub 2022 Sep 21. PMID: 36152410; PMCID: PMC9618292.

Abstract: Background: Nutraceutical foods, like walnuts which are rich in immunonutrients, can have medicinal benefits. Dietary walnuts have been shown to slow or prevent tumor growth in mice genetically programmed to grow breast or prostate tumors. This study investigated whether walnuts could exert the same preventable effect in a transplantable carcinoma rat model. Methods: Eighteen rats were randomly fed a diet containing walnuts (10% of food by weight), and 36 were fed a diet without walnuts (control) for 21 days. On day 22, 18 control diet rats were switched to the walnut diet. All other animals remained on their same diet. Within each diet group, 6 rats were implanted with the Ward colon carcinoma (TB), and 12 were sham-operated. Five days later, 6 sham-operated animals were weight-matched to a TB and then pair-fed for the remainder of the study. The remaining 6 sham-operated, or non-tumor-bearing rats, were ad-lib fed. Results: The tissue of the walnut-eating rats showed higher omega-3 fatty acid (immunonutrient) content which did not slow or prevent tumor growth or the loss of lean and fat mass typical of this TB model. In addition, blood glucose, insulin, IGF-1, and adiponectin levels were significantly lower in the TB, demonstrating metabolic dysregulation. Again, these changes were unaltered by consuming walnuts. Plasma proteomics identified six proteins elevated in the TB, but none could be connected with the observed metabolic dysregulation. Conclusion: Although walnuts' rich immunonutrient content prevented tumor growth in genetically programmed mice models, there was no effect in this model.

Key Area: Cancer

Dorans KS, Bazzano LA, Qi L, He H, Chen J, Appel LJ, Chen CS, Hsieh MH, Hu FB, Mills KT, Nguyen BT, O'Brien MJ, Samet JM, Uwaifo GI, He J. Effects of a Low-Carbohydrate Dietary Intervention on Hemoglobin A1c: A Randomized Clinical Trial.

JAMA Netw Open. 2022 Oct 3;5(10):e2238645. doi: 10.1001/jamanetworkopen.2022.38645. PMID: 36287562; PMCID: PMC9606840.

Abstract: Importance: Low-carbohydrate diets decrease hemoglobin A1c (HbA1c) among patients with type 2 diabetes at least as much as low-fat diets. However, evidence on the effects of low-carbohydrate diets on HbA1c among individuals with HbA1c in the range of prediabetes to diabetes not treated by diabetes medications is limited. Objective: To study the effect of a behavioral intervention promoting a low-carbohydrate diet compared with usual diet on 6-month changes in HbA1c among individuals with elevated untreated HbA1c. Design, setting, and participants: This 6-month randomized clinical trial with 2 parallel groups was conducted from September 2018 to June 2021 at an academic medical center in New Orleans, Louisiana. Laboratory analysts were blinded to assignment. Participants were aged 40 to 70 years with untreated HbA1c of 6.0% to 6.9% (42-52 mmol/mol). Data analysis was performed from November 2021 to September 2022. Interventions: Participants were randomized to a low-carbohydrate diet intervention (target <40 net grams of carbohydrates during the first 3 months; <60 net grams for months 3 to 6) or usual diet. The low-carbohydrate diet group received dietary counseling. Main outcomes and measures: Six-month change in HbA1c was the primary outcome. Outcomes were measured at 0, 3, and 6 months. Results: Of 2722 prescreened participants, 962 underwent screening, and 150 were enrolled (mean [SD] age, 58.9 [7.9] years; 108 women [72%]; 88 Black participants [59%]) and randomized to either the low-carbohydrate diet intervention (75 participants) or usual diet (75 participants) group. Six-month data were collected on 142 participants (95%). Mean (SD) HbA1c was 6.16% (0.30%) at baseline. Compared with the usual diet group, the low-carbohydrate diet intervention group had significantly greater 6-month reductions in HbA1c (net difference, -0.23%; 95% CI, -0.32% to -0.14%; $P < .001$), fasting plasma glucose (-10.3 mg/dL; 95% CI, -15.6 to -4.9 mg/dL; $P < .001$).

.001), and body weight (-5.9 kg; 95% CI, -7.4 to -4.4 kg; $P < .001$). Conclusions and relevance: In this randomized clinical trial, a low-carbohydrate dietary intervention led to improvements in glycemia in individuals with elevated HbA1c not taking glucose-lowering medication, but the study was unable to evaluate its effects independently of weight loss. This diet, if sustained, might be a useful dietary approach for preventing and treating type 2 diabetes, but more research is needed.

Key Area: Diabetes

Esselun C, Dieter F, Sus N, Frank J, Eckert GP. Walnut Oil Reduces A β Levels and Increases Neurite Length in a Cellular Model of Early Alzheimer Disease.

Nutrients. 2022 Apr 19;14(9):1694. doi: 10.3390/nu14091694. PMID: 35565661; PMCID: PMC9099939

Abstract (1) Background: Mitochondria are the cells' main source of energy. Mitochondrial dysfunction represents a key hallmark of aging and is linked to the development of Alzheimer's disease (AD). Maintaining mitochondrial function might contribute to healthy aging and the prevention of AD. The Mediterranean diet, including walnuts, seems to prevent age-related neurodegeneration. Walnuts are a rich source of α -linolenic acid (ALA), an essential n3-fatty acid and the precursor for n3-long-chain polyunsaturated fatty acids (n3-PUFA), which might potentially improve mitochondrial function. (2) Methods: We tested whether a lipophilic walnut extract (WE) affects mitochondrial function and other parameters in human SH-SY5Y cells transfected with the neuronal amyloid precursor protein (APP695). Walnut lipids were extracted using a Soxhlet Extraction System and analyzed using GC/MS and HPLC/FD. Adenosine triphosphate (ATP) concentrations were quantified under basal conditions in cell culture, as well as after rotenone-induced stress. Neurite outgrowth was investigated, as well as membrane integrity, cellular reactive oxygen species, cellular peroxidase activity, and citrate synthase activity. Beta-amyloid (A β) was quantified using homogenous time-resolved fluorescence. (3) Results: The main constituents of WE are linoleic acid, oleic acid, α -linolenic acid, and γ - and δ -tocopherol. Basal ATP levels following rotenone treatment, as well as citrate synthase activity, were increased after WE treatment. WE significantly increased cellular reactive oxygen species but lowered peroxidase activity. Membrane integrity was not affected. Furthermore, WE treatment reduced A β 1–40 and stimulated neurite growth. (4) Conclusions: WE might increase ATP production after induction of mitochondrial biogenesis. Decreased A β 1–40 formation and enhanced ATP levels might enhance neurite growth, making WE a potential agent to enhance neuronal function and to prevent the development of AD. In this sense, WE could be a promising agent for the prevention of AD.

Key Area: Cognitive Health

Gil-Zamorano J, Cofán M, López de las Hazas MC, García-Blanco T, García-Ruiz A, Doménech M, Serra-Mir M, Roth I, Valls-Pedret C, Rajaram S, Sabaté, J, Ros E, Dávalos A, Sala-Vila A. Interplay of Walnut Consumption, Changes in Circulating miRNAs and Reduction in LDL-Cholesterol in Elders.

Nutrients. 2022 Apr 1;14(7):1473. doi: 10.3390/nu14071473. PMID: 35406086; PMCID: PMC9003099.

Abstract: The mechanisms underlying the lipid-lowering effect of nuts remain elusive. This study explores whether one-year supplementation with walnuts decreases LDL-cholesterol (LDL-C) by affecting the expression of circulating microRNAs (c-miRNA). In this sub-study of the Walnuts and Healthy Aging (WAHA) trial, we obtained fasting serum at baseline and at 1 year from 330 free-living participants (63–79 year, 68% women), allocated into a control group (CG, abstinence from walnuts, $n = 164$) and a walnut group (WG, 15% of daily energy as walnuts, ~30–60 g/day, $n = 166$). Participants in the WG showed a 1 year decrease in LDL-C (-9.07 , (95% confidence interval: -12.87 ; -5.73) mg/dL; $p = 0.010$ versus changes in the CG). We conducted a miRNA array in eight randomly selected participants in the WG who decreased in LDL-C. This yielded 53 c-miRNAs with statistically significant changes, 27 of which survived the correction for multiple testing. When validating them in

the full population, statistical significance lasted for hsa-miR-551a, being upregulated in the WG. In mediation analysis, the change in hsa-miR-551a was unrelated to LDL-C decrease. Long-term supplementation with walnuts decreased LDL-C independently of the changes in c-miRNA. The hsa-miR-551a upregulation, which has been linked to a reduced cell migration and invasion in several carcinomas, suggests a novel mechanism of walnuts in cancer risk.

Key Area: Heart Health

Herselman MF, Bailey S, Deo P, Zhou X-F, Gunn KM, Bobrovskaya L. The Effects of Walnuts and Academic Stress on Mental Health, General Well-Being and the Gut Microbiota in a Sample of University Students: A Randomised Clinical Trial.

Nutrients. 2022; 14(22):4776. <https://doi.org/10.3390/nu14224776>.

Abstract: Poorer mental health is common in undergraduate students due to academic stress. An interplay between stress and diet exists, with stress influencing food choices. Nutritional interventions may be effective in preventing mental health decline due to complex bidirectional interactions between the brain, the gut and the gut microbiota. Previous studies have shown walnut consumption has a positive effect on mental health. Here, using a randomized clinical trial (Australian New Zealand Clinical Trials Registry, #ACTRN12619000972123), we aimed to investigate the effects of academic stress and daily walnut consumption in university students on mental health, biochemical markers of general health, and the gut microbiota. We found academic stress had a negative impact on self-reported mood and mental health status, while daily walnut consumption improved mental health indicators and protected against some of the negative effects of academic stress on metabolic and stress biomarkers. Academic stress was associated with lower gut microbial diversity in females, which was improved by walnut consumption. The effects of academic stress or walnut consumption in male participants could not be established due to small numbers of participants. Thus, walnut consumption may have a protective effect against some of the negative impacts of academic stress, however sex-dependent mechanisms require further study.

Key Area: Cognitive Health

Kaplan A, Zelicha H, Meir AY, Rinott E, Tsaban G, Levakov G, Prager O, Salti M, Yovell Y, Ofer J, Huhn S, Beyer F, Witte V, Villringer A, Meiran N, Emesh TB, Kovacs P, von Bergen M, Ceglarek U, Blüher M, Stumvoll M, Hu FB, Stampfer MJ, Friedman A, Shelef I, Avidan G, Shai I. The effect of a high-polyphenol Mediterranean diet (GREEN-MED) combined with physical activity on age-related brain atrophy: the DIRECT PLUS randomized controlled trial.

Am J Clin Nutr. 2022 Jan 11:nqac001. doi: 10.1093/ajcn/nqac001. Epub ahead of print. PMID: 35021194.

Abstract: Background: The effect of diet on age-related brain atrophy is largely unproven. Objective: To explore the effect of a Mediterranean diet higher in polyphenols and lower in red/processed meat (Green-MED diet) on age-related brain atrophy. Methods: This 18-month clinical trial longitudinally measured brain structure volumes by magnetic-resonance-imaging using hippocampal-occupancy (HOC) and lateral-ventricle-volume (LVV) expansion scores as neurodegeneration markers. Abdominally obese/dyslipidemic participants were randomly assigned to (1)-healthy dietary guidelines (HDG), (2)-Mediterranean (MED) diet, or (3)-Green-MED diet (MED diet higher in polyphenols and lower in red/processed meat). All subjects received free gym memberships and physical activity guidance. Both MED groups consumed 28g/day walnuts (+440 mg/d polyphenols). The Green-MED group consumed green-tea (3-4 cups/day) and Mankai (*Wolffia-globosa* strain, 100g frozen-cubes/day) green shake (+800mg/day polyphenols). Results: Among 284 participants (age = 51 years; 88% men; BMI = 31.2kg/m²; apolipoprotein E-ε4 genotype = 15.7%), 224 (79%) completed the trial with eligible whole-brain MRIs. The pallidum (-4.2%), third ventricle (+3.9%), and LVV (+2.2%) disclosed the largest volume changes. Compared to younger participants, atrophy was accelerated among those ≥ 50

years [HOC change = $-1.0 \pm 1.4\%$ vs. $-0.06 \pm 1.1\%$; 95% confidence-interval (CI): 0.6, 1.3; $p < 0.001$; LVV change = $3.2 \pm 4.5\%$ vs. $1.3 \pm 4.1\%$; 95%CI: -3.1, -0.8; $p = 0.001$]. In subjects ≥ 50 years, HOC decline and LVV expansion were attenuated in both MED groups, with the best outcomes among Green-MED diet participants, as compared to HDG (HOC: $-0.8 \pm 1.6\%$ vs. $-1.3 \pm 1.4\%$; 95%CI: -1.5, -0.02; $p = 0.042$, LVV: $2.3 \pm 4.7\%$ vs. $4.3 \pm 4.5\%$; 95%CI: 0.3, 5.2; $p = 0.021$). Similar patterns were observed among younger subjects. Improved insulin sensitivity over the trial was the strongest parameter associated with brain atrophy attenuation ($p < 0.05$). Greater Mankai, green-tea and walnuts intake and less red and processed meat were significantly and independently associated with reduced HOC decline ($p < 0.05$). Elevated urinary levels of the Mankai-derived polyphenols: urolithin-A ($r = 0.24$; $p = 0.013$) and tyrosol ($r = 0.26$; $p = 0.007$) were significantly associated with lower HOC decline. Conclusions: A Green-MED, high-polyphenol diet, rich in Mankai, green tea and walnuts and low in red/processed meat is potentially neuroprotective for age-related brain atrophy.

Key Area: Cognitive Health

Kleckner AS, Reschke JE, Kleckner IR, Magnuson A, Amitrano AM, Culakova E, Shayne M, Netherby-Winslow CS, Czap S, Janelins MC, Mustian KM, Peppone LJ. The Effects of a Mediterranean Diet Intervention on Cancer-Related Fatigue for Patients Undergoing Chemotherapy: A Pilot Randomized Controlled Trial.

Cancers. 2022; 14(17):4202. <https://doi.org/10.3390/cancers14174202>.

Abstract: Cancer-related fatigue is a common, burdensome symptom of cancer and a side-effect of chemotherapy. While a Mediterranean Diet (MedDiet) promotes energy metabolism and overall health, its effects on cancer-related fatigue remain unknown. In a randomized controlled trial, we evaluated a rigorous MedDiet intervention for feasibility and safety as well as preliminary effects on cancer-related fatigue and metabolism compared to usual care. Participants had stage I–III cancer and at least six weeks of chemotherapy scheduled. After baseline assessments, randomization occurred 2:1, MedDiet:usual care. Measures were collected at baseline, week 4, and week 8 including MedDiet adherence (score 0–14), dietary intake, and blood-based metabolic measures. Mitochondrial respiration from freshly isolated T cells was measured at baseline and four weeks. Participants ($n = 33$) were 51.0 ± 14.6 years old, 94% were female, and 91% were being treated for breast cancer. The study was feasible, with 100% completing the study and $>70\%$ increasing their MedDiet adherence at four and eight weeks compared to baseline. Overall, the MedDiet intervention vs. usual care had a small-moderate effect on change in fatigue at weeks 4 and 8 (ES = 0.31, 0.25, respectively). For those with a baseline MedDiet score <5 ($n = 21$), the MedDiet intervention had a moderate-large effect of 0.67 and 0.48 at weeks 4 and 8, respectively. The MedDiet did not affect blood-based lipids, though it had a beneficial effect on fructosamine (ES = -0.55). Fatigue was associated with mitochondrial dysfunction including lower basal respiration, maximal respiration, and spare capacity ($p < 0.05$ for FACIT-F fatigue subscale and BFI, usual fatigue). In conclusion, the MedDiet was feasible and attenuated cancer-related fatigue among patients undergoing chemotherapy, especially those with lower MedDiet scores at baseline.

Key Area: Cancer

Kopecky SL, Alias S, Klodas E, Jones PJH. Reduction in Serum LDL Cholesterol Using a Nutrient Compendium in Hyperlipidemic Adults Unable or Unwilling to Use Statin Therapy: A Double-Blind Randomized Crossover Clinical Trial.

J Nutr. 2022 Feb 8;152(2):458-465. doi: 10.1093/jn/nxab375. PMID: 35079806.

Abstract: Background: Many hyperlipidemic patients prescribed β -hydroxy- β -methylglutaryl coenzyme A reductase inhibitors (statins) are unable or unwilling to take them. A hedonically acceptable snack-based solution formulated from cholesterol-lowering food ingredients could represent a therapeutic alternative but has not been tested in this population. Objectives: To evaluate the effect of snacks containing a compendium of functional bioactives on fasting LDL cholesterol in statin candidates

unwilling to use or intolerant to ≥ 1 statin drug. Secondary outcomes included changes in circulating total cholesterol (TC), triglycerides, HDL cholesterol, fasting glucose, insulin, and high-sensitivity C-reactive protein concentrations, as well as effects of single-nucleotide polymorphisms (SNPs) on outcome. Methods: This multicenter, randomized, double-blind, free-living crossover study was composed of 2 regimented phases of 4 wk each, separated by a 4-wk washout. Eighteen men and 36 women, with a mean \pm SD age of 49 ± 12 y and mean \pm SD LDL cholesterol of 131 ± 32.1 mg/dL, were instructed to ingest a variety of ready-to-eat snacks twice daily as a substitute for something they were consuming already. Other behavior changes were actively discouraged. Treatment products provided ≥ 5 g fiber, 1000 mg ω -3 (n-3) fatty acids, 1000 mg phytosterols, and 1800 μ mol antioxidants per serving. Control products were calorie-matched like-items drawn from the general grocery marketplace. Serum lipids were measured at baseline and the end of each phase and compared using the ANOVA model. Compliance to study foods was confirmed by serum 18:3n-3 concentration assessment. Results: Comparing intervention phase endpoints, LDL cholesterol was reduced a mean \pm SD of $8.80 \pm 1.69\%$ ($P < 0.0001$), and TC was reduced a mean \pm SD of $5.08 \pm 1.12\%$ ($P < 0.0001$) by treatment foods compared with control foods, whereas effects on other analytes did not differ between treatments. SNPs were not significantly related to outcomes ($P \geq 0.230$). Compliance with study foods was 95%. Conclusions: Consumption of hedonically acceptable snacks containing a compendium of cholesterol-lowering bioactive compounds can rapidly and meaningfully reduce LDL cholesterol in adult patients unable or unwilling to take statin drugs.

Key Area: Heart Health

Lockyer S, de la Hunty AE, Steenson S, Spiro A, Stanner SA. Walnut consumption and health outcomes with public health relevance—a systematic review of cohort studies and randomized controlled trials published from 2017 to present.

Nutr Rev. 2022 Aug 1:nuac040. doi: 10.1093/nutrit/nuac040. Epub ahead of print. PMID: 35912883.

Abstract: Context: Considering the accumulation of recent studies investigating the health effects of walnut consumption, both including and beyond cardiovascular health effects, a systematic review of this literature to investigate the strength of the evidence is warranted. Objective: To investigate associations between walnut consumption and outcomes with public health relevance (specifically all-cause mortality, type 2 diabetes, CVD, metabolic syndrome, obesity, cancer, neurological and mental health, musculoskeletal, gastrointestinal, and maternal disorders) and the effect on associated disease risk markers, reported in studies published from 2017 to present. Data sources: MEDLINE, FSTA, CENTRAL, and Scopus were searched from 1 January 2017 to 5 May 2021. Data extraction: Human studies (cohort studies and RCTs) ≥ 3 weeks in duration comparing consumption of walnuts (whole, pieces, or 100% butter) to a control and measuring associations with relevant public health outcomes and disease risk markers were assessed. Key study characteristics were extracted independently by 2 investigators using a standardized table. The quality of the studies was assessed using the Cochrane Risk-of-Bias tool 2.0 and the Newcastle-Ottawa Scale. Data analysis: Only 1 RCT was considered to be at low risk of bias for any of its outcomes. The cohort studies were considered to be of moderate or high quality. The results were synthesized using vote counting, based on the direction of effect. Thirty-three articles, 23 describing RCTs (walnut dose ~ 10 –99 g/day, 1,948 subjects) and 10 describing cohort studies (~ 675 ,928 subjects), were included. Vote counting could be performed for the blood lipids, cardiovascular function, inflammation- and hemostatic-related factors, markers of glucose metabolism, and body weight and composition outcome groupings. The results are presented in effect direction plots. With respect to blood lipids, results from 8/8 RCTs favoured walnuts, in accordance with associations with a reduced risk of CVD suggested by cohort studies; results from 6/6 RCTs favoured control with respect to body weight and composition, although most of these effects were small. This was contrary to cohort study results suggesting small benefits of walnut consumption on body weight. There was no overall consistent direction of effect for cardiovascular function, markers of glucose metabolism, or

inflammation- and hemostatic-related factors. Conclusions: Evidence published since 2017 is consistent with previous research suggesting that walnut consumption improves lipid profiles and is associated with reduced CVD risk. Evidence is accumulating in other areas, such as cognitive health, although more research is needed to draw firm conclusions.

Key Area: Heart Health

Oliveras T, Lázaro I, Rueda F, Cediél G, Bhatt DL, Fitó M, Madrid-Gambin F, Pozo OJ, Harris WS, García-García C, Sala-Vila A, Bayés-Genís A. Circulating linoleic acid at the time of myocardial infarction and risk of primary ventricular fibrillation.

Sci Rep. 2022 Mar 14;12(1):4377. doi: 10.1038/s41598-022-08453-0. PMID: 35288655.

Abstract: Primary ventricular fibrillation (PVF) is a major driver of cardiac arrest in the acute phase of ST-segment elevation myocardial infarction (STEMI). Enrichment of cardiomyocyte plasma membranes with dietary polyunsaturated fatty acids (PUFA) reduces vulnerability to PVF experimentally, but clinical data are scarce. PUFA status in serum phospholipids is a valid surrogate biomarker of PUFA status in cardiomyocytes within a wide range of dietary PUFA. In this nested case-control study (n = 58 cases of STEMI-driven PVF, n = 116 control non-PVF STEMI patients matched for age, sex, smoking status, dyslipidemia, diabetes mellitus and hypertension) we determined fatty acids in serum phospholipids by gas-chromatography, and assessed differences between cases and controls, applying the Benjamini-Hochberg procedure on nominal P-values to control the false discovery rate (FDR). Significant differences between cases and controls were restricted to linoleic acid (LA), with PVF patients showing a lower level (nominal P = 0.002; FDR-corrected P = 0.027). In a conditional logistic regression model, each one standard deviation increase in the proportion of LA was related to a 42% lower prevalence of PVF (odds ratio = 0.58; 95% confidence interval, 0.37, 0.90; P = 0.02). The association lasted after the inclusion of confounders. Thus, regular consumption of LA-rich foods (nuts, oils from seeds) may protect against ischemia-driven malignant arrhythmias.

Key Area: Heart Health

Pinar-Martí A, Fernández-Barrés S, Gignac F, Persavento C, Delgado A, Romaguera D, Lázaro I, Ros E, López-Vicente M, Salas-Salvadó J, Sala-Vila A, Júlvez J. Red blood cell omega-3 fatty acids and attention scores in healthy adolescents.

Eur Child Adolesc Psychiatry 2022 Aug 12. doi: 10.1007/s00787-022-02064-w. Epub ahead of print. PMID: 35960396.

Abstract: Omega-3 fatty acids are critical for brain function. Adolescence is increasingly believed to entail brain vulnerability to dietary intake. In contrast to the abundant research on the omega-3 docosahexaenoic acid (DHA) in cognition, research on DHA and attention in healthy adolescents is scarce. In addition, the role of alpha-linolenic acid (ALA), the vegetable omega-3 fatty acid, is unexplored. We examined associations between DHA and ALA and attention function among a healthy young population. In this cross-sectional study conducted in 372 adolescents (13.8±0.9 years-old), we determined the red blood cell proportions of DHA and ALA by gas chromatography (objective biomarkers of their long-term dietary intake) and measured attention scores through the Attention Network Test. We constructed multivariable linear regression models to analyze associations, controlling for known confounders. Compared to participants at the lowest DHA tertile (reference), those at the highest DHA tertile showed significantly lower hit reaction time-standard error (higher attentiveness) (28.13 ms, 95% confidence interval [CI]= - 52.30; - 3.97), lower hit reaction time (- 38.30 ms, 95% CI= - 73.28; - 3.33) and lower executive conflict response (- 5.77 ms, 95% CI= - 11.44; - 0.09). In contrast, higher values were observed in those at the top tertile of ALA in hit reaction time compared to the lowest one (46.14 ms, 95% CI=9.90; 82.34). However, a beneficial association was observed for ALA, with decreasing impulsivity index across tertiles. Overall, our results suggest that DHA (reflecting its dietary intake) is associated with attention performance in typically developing adolescents. The role

of dietary ALA in attention is less clear, although higher blood levels of ALA appear to result in lower impulsivity. Future intervention studies are needed to determine the causality of these associations and to better shape dietary recommendations for brain health during the adolescence period.

Key Area: Cognitive Health

***Provatas AA, Ayers SA, Callas AA, Birk JW, Lacson TA, Rosenberg DW. Quantitative determination of selected urolithin metabolites in human urine by simple sample preparation and UPLC-MS/MS analysis**

Curr Top Anal Chem. 2021. Vol 13, pg 69-80.

Abstract: We report a simple, reliable, and validated method for the rapid screening and quantification of nine urolithin (UL) metabolites in human urine. Ultraperformance liquid chromatograph coupled with a tandem mass spectrometer (UPLC-MS/MS) was utilized for UL analysis following a simple sample preparation. Optimization of chromatographic and mass spectrometric conditions was performed to maximize the sensitivity and selectivity of the targeted analytes. A validation of the methodology was conducted to account for matrix interferences, linearity, method detection limits (MDLs), UL chemical stability, precision and accuracy of the ULs of interest. MDLs were achieved for the selected ULs ranging from 9.2-18.2 ng·mL⁻¹. Excellent linear coefficients of determination were obtained for the range of calibration standards of 5.0-5,000 ng·mL⁻¹, with R² values between 0.9991 and 0.9998. The surrogate compound, 6,7-dihydroxycoumarin, was used to monitor the extraction efficiency and chrysin as the quantitative internal standard. The recoveries of the analytes were 88-99% with surrogate recoveries greater than 82%. This analytical method was developed and validated for processing samples associated with a human study, where it is hypothesized that walnut supplementation improves colonic health and lowers colorectal cancer risk, in part through enhancing UL formation.

Key Area: Cancer

Rinott E, Meir AY, Tsaban G, Zelicha H, Kaplan A, Knights D, Tuohy K, Scholz MU, Koren O, Stampfer MJ, Wang DD, Shai I, Youngster I. The effects of the Green-Mediterranean diet on cardiometabolic health are linked to gut microbiome modifications: a randomized controlled trial.

Genome Med. 2022 Mar 10;14(1):29. doi: 10.1186/s13073-022-01015-z. PMID: 35264213

Abstract: Background Previous studies have linked the Mediterranean diet (MED) with improved cardiometabolic health, showing preliminary evidence for a mediating role of the gut microbiome. We recently suggested the Green-Mediterranean (Green-MED) diet as an improved version of the healthy MED diet, with increased consumption of plant-based foods and reduced meat intake. Here, we investigated the effects of MED interventions on the gut microbiota and cardiometabolic markers, and the interplay between the two, during the initial weight loss phase of the DIRECT-PLUS trial. Methods In the DIRECT-PLUS study, 294 participants with abdominal obesity/dyslipidemia were prospectively randomized to one of three intervention groups: healthy dietary guidelines (standard science-based nutritional counseling), MED, and Green-MED. Both isocaloric MED and Green-MED groups were supplemented with 28g/day walnuts. The Green-MED group was further provided with daily polyphenol-rich green tea and Mankai aquatic plant (new plant introduced to a western population). Gut microbiota was profiled by 16S rRNA for all stool samples and shotgun sequencing for a select subset of samples. Results Both MED diets induced substantial changes in the community structure of the gut microbiome, with the Green-MED diet leading to more prominent compositional changes, largely driven by the low abundant, “non-core,” microorganisms. The Green-MED diet was associated with specific microbial changes, including enrichments in the genus *Prevotella* and enzymatic functions involved in branched-chain amino acid degradation, and reductions in the genus *Bifidobacterium* and enzymatic functions responsible for branched-chain amino acid biosynthesis. The MED and Green-MED diets were also associated with stepwise beneficial changes in body weight and cardiometabolic biomarkers,

concomitantly with the increased plant intake and reduced meat intake. Furthermore, while the level of adherence to the Green-MED diet and its specific green dietary components was associated with the magnitude of changes in microbiome composition, changes in gut microbial features appeared to mediate the association between adherence to the Green-MED and body weight and cardiometabolic risk reduction. **Conclusions** Our findings support a mediating role of the gut microbiome in the beneficial effects of the Green-MED diet enriched with Mankai and green tea on cardiometabolic risk factors.

Key Area: Gut Health

Sala-Vila A, Fleming J, Kris-Etherton P, Ros E. Impact of Alpha-linolenic Acid, the Vegetable Omega-3 Fatty Acid, on Cardiovascular Disease and Cognition.

Adv Nutr. 2022 Feb 16:nmac016. doi: 10.1093/advances/nmac016. Epub ahead of print. PMID: 35170723.

Abstract: Given the evidence of the health benefits of plant-based diets and long-chain n-3 fatty acids, there is keen interest in better understanding the role of alpha-linolenic acid (ALA), a plant-derived n-3 fatty acid, on cardiometabolic diseases and cognition. There is increasing evidence for ALA largely based on its major food sources (i.e., walnuts and flaxseed); however, this lags behind our understanding of long-chain n-3 fatty acids. Meta-analyses of observational studies have shown that increasing dietary ALA is associated with a 10% lower risk of total cardiovascular disease and a 20% reduced risk of fatal coronary heart disease. Three randomized controlled trials (AlphaOmega trial, Prevención con Dieta Mediterránea [PREDIMED] trial, and Lyon Diet Heart Study) all showed benefits of diets high in ALA on cardiovascular-related outcomes, but the AlphaOmega trial, designed to specifically evaluate ALA effects, only showed a trend for benefit. Randomized controlled trials have shown that dietary ALA reduced total cholesterol, low-density-lipoprotein cholesterol, triglycerides, and blood pressure, and epidemiological studies and some trials also have shown an anti-inflammatory effect of ALA; which collectively account for, in part, the cardiovascular benefits of ALA. A meta-analysis reported a trend toward diabetes risk reduction with both dietary and biomarker ALA. For metabolic syndrome and obesity, the evidence for ALA benefits is inconclusive. The role of ALA in cognition is in the early stages but shows promising evidence of counteracting cognitive impairment. Much has been learned about the health benefits of ALA and with additional research we will be better positioned to make strong evidence-based dietary recommendations for the reduction of many chronic diseases.

Key Area: Heart Health

Yi SY, Steffen LM, Zhou X, Shikany JM, Jacobs Jr DR. Association of Nut Consumption with CVD risk factors in young to middle-aged adults: the Coronary Artery Risk Development in Young Adults (CARDIA) study.

Nutr Metab Cardiovasc Dis. 2022 Jul 31:S0939-4753(22)00302-7. doi: 10.1016/j.numecd.2022.07.013. Epub ahead of print. PMID: 35970686.

Abstract: **Background and Aims** Few studies have examined long-term associations of walnut, other nut, and no nut consumption with cardiovascular disease (CVD) risk factors. Results from prospective studies with long-term follow-up can provide further evidence for dietary guideline messaging to consume nuts. Therefore, we examined the associations of walnut, other nut, and no nut consumption with diet quality and CVD risk factors over 30 years of follow-up. **Methods and Results** Data were analyzed from 3,092 young adults enrolled in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Dietary intake, including walnuts and other nuts, was assessed 3 times over 20 years. CVD risk factors were measured at multiple exams. General linear regression evaluated the associations of walnut, other nut, and no nut consumption with CVD risk factors over 30 years (Y30) of follow-up. The 20-year cumulative mean intake of walnuts (0.74 oz/d), other nuts (1.6 oz/d), or no nut consumption was differentially associated with HEI-2015 and CVD risk factors by Y30. Generally, walnut consumers

had significantly higher HEI-2015, lower body mass index, waist circumference, blood pressure, and triglyceride concentration, and gained less weight since baseline than other nut consumers ($p \leq 0.05$ for all). Further, walnut consumers had lower fasting blood glucose than no nut consumers ($p \leq 0.05$). Conclusion Study findings that walnut and other nut consumption was associated with better CVD risk factors and diet quality aligns with the 2020-2025 U.S. Dietary Guidelines for Americans recommendation to consume nuts, such as walnuts, within the context of a healthy diet.

Key Area: Heart Health

Zelicha H, Kloting N, Kaplan A, Yaskolka Meir A, Rinott E, Tsaban G, Chassidim Y, Bluher M, Ceglarek U, Isermann B, Stumvoll M, Quayson RN, von Bergen M, Engelmann B, Rolle-Kampczyk UE, Haange SB, Tuohy KM, Diotallevi C, Shelef I, Hu FB, Stampfer MJ, Shai I. The effect of high-polyphenol Mediterranean diet on visceral adiposity: the DIRECT PLUS randomized controlled trial.

BMC Med. 2022 Sep 30;20(1):327. doi: 10.1186/s12916-022-02525-8. PMID: 36175997.

Abstract: Background Mediterranean (MED) diet is a rich source of polyphenols, which benefit adiposity by several mechanisms. We explored the effect of the green-MED diet, twice fortified in dietary polyphenols and lower in red/processed meat, on visceral adipose tissue (VAT). Methods In the 18-month Dietary Intervention Randomized Controlled Trial PoLyphenols UnproceSsed (DIRECT-PLUS) weight-loss trial, 294 participants were randomized to (A) healthy dietary guidelines (HDG), (B) MED, or (C) green-MED diets, all combined with physical activity. Both isocaloric MED groups consumed 28 g/day of walnuts (+ 440 mg/day polyphenols). The green-MED group further consumed green tea (3–4 cups/day) and *Wolffia globosa* (duckweed strain) plant green shake (100 g frozen cubes/day) (+ 800mg/day polyphenols) and reduced red meat intake. We used magnetic resonance imaging (MRI) to quantify the abdominal adipose tissues. Results Participants (age = 51 years; 88% men; body mass index = 31.2 kg/m²; 29% VAT) had an 89.8% retention rate and 79.3% completed eligible MRIs. While both MED diets reached similar moderate weight (MED: – 2.7%, green-MED: – 3.9%) and waist circumference (MED: – 4.7%, green-MED: – 5.7%) loss, the green-MED dieters doubled the VAT loss (HDG: – 4.2%, MED: – 6.0%, green-MED: – 14.1%; $p < 0.05$, independent of age, sex, waist circumference, or weight loss). Higher dietary consumption of green tea, walnuts, and *Wolffia globosa*; lower red meat intake; higher total plasma polyphenols (mainly hippuric acid), and elevated urine urolithin A polyphenol were significantly related to greater VAT loss ($p < 0.05$, multivariate models). Conclusions A green-MED diet, enriched with plant-based polyphenols and lower in red/processed meat, may be a potent intervention to promote visceral adiposity regression.

Key Area: Body Weight and Composition

2021

Cahoon D, Shertukde SP, Avendano EE, Tanprasertsuk J, Scott TM, Johnson EJ, Chung M, Nirmala N. Walnut intake, cognitive outcomes and risk factors: a systematic review and meta-analysis.

Ann Med. 2021 Dec;53(1):971-997. doi: 10.1080/07853890.2021.1925955. PMID: 34132152; PMCID: PMC8211141.

Abstract: Background Walnuts contain nutrients that are associated with improved cognitive health. To our knowledge, no review has systematically examined the effects of walnuts on cognitive function and risk for cognitive decline. Objective To conduct a systematic review and meta-analysis evaluating the effects of walnut intake on cognition-related outcomes and risk-factors for cognitive decline in adults. Methods Medline®, Commonwealth Agricultural Bureau, and Cochrane Central Register of Controlled Trials were searched for randomized controlled trials (RCTs) and observational studies published until April 2020 on walnut intake, cognition (e.g. cognitive function, stroke, and mood), and selected risk

factors for cognitive decline (e.g. glucose homeostasis and inflammation). Risk-of-bias and strength-of-evidence assessments were conducted using standard validated tools. Random-effects meta-analyses were conducted when ≥ 3 studies reported quantitative data for each outcome. Results 32 RCT and 7 observational study publications were included. Meta-analysis of cognition-related outcomes could not be conducted due to heterogeneity of tests. None of the 5 cognition RCTs found significant effects of walnuts on overall cognition, although 3 studies found improvements on subdomains and/or subgroups. All 7 observational studies found significant associations and a dose-response relationship between walnut intake and cognition-related outcomes. Meta-analyses of 27 RCTs reporting glucose homeostasis and inflammation outcomes, selected risk factors for cognitive decline, did not show significant effects of walnut intake. Conclusions Due to the non-uniformity of tests for cognition-related outcomes, definitive conclusions regarding the effect of walnut consumption on cognition could not be reached. Additionally, evidence does not show associations between walnut intake and glucose homeostasis or inflammation, cognitive decline risk-factors. High-quality studies with standardized measures are needed to clarify the role of walnuts in cognitive health.

Key Area: Cognitive Health

Dorans KS, Bazzano LA, Qi L, Hua H, Appel LJ, Samet JM, Chen J, Mills KT, Nguyen BT, O'Brien MJ, Uwaifo IU, He J. Low-carbohydrate dietary pattern on glycemic outcomes trial (ADEPT) among individuals with elevated hemoglobin A1c: study protocol for a randomized controlled trial.

Trials. 2021 Feb 1;22(1):108. doi: 10.1186/s13063-020-05001-x. PMID: 33522954; PMCID: PMC7848246

Abstract: Background: Type 2 diabetes mellitus (T2DM) is a major cause of morbidity and mortality globally. Strong evidence supports the importance of diet and other lifestyle factors in preventing T2DM. Among individuals with T2DM, low-carbohydrate diets lead to decreases in hemoglobin A1c (HbA1c). However, research on the effects of low-carbohydrate diets on glycemic outcomes among individuals not currently on glucose-lowering medications who have elevated HbA1c is limited. Methods: The objective of this randomized controlled trial is to study the effect of a healthy low-carbohydrate diet achieved through behavioral intervention and key food supplementation compared with usual diet on HbA1c and other metabolic risk factors among individuals with HbA1c from 6.0 to 6.9% who are not on glucose-lowering medications. In this parallel trial, 150 participants will be randomized to the intervention or control group for 6 months. The healthy low-carbohydrate diet target is < 40 g of net carbohydrates during the first 3 months and < 40 to 60 net grams for months 3 to 6. This diet is characterized by abundant unsaturated fat and protein, high-fiber foods such as non-starchy vegetables and nuts, and minimal refined carbohydrates. The primary outcome is the difference in HbA1c change from baseline to 6 months in the intervention compared with usual diet group. Secondary outcomes include differences between groups in 6-month changes in fasting glucose, systolic blood pressure, total-to-high-density lipoprotein (HDL) cholesterol ratio, and body weight. Exploratory outcomes include differences in 6-month changes in fasting insulin, homeostasis model assessment of insulin resistance, diastolic blood pressure, waist circumference, and 10-year cardiovascular disease risk. An intention-to-treat analysis will be used. Discussion: We expect that the results from this study will lead to new approaches for developing and implementing dietary approaches (other than the most commonly used reduced fat diet) that will substantially reduce risk of cardiometabolic disease among adults with or at high risk of T2DM. The study intervention involves behavioral counseling and promotes consumption of dietary components thought to reduce risk of cardiometabolic disease and has expected applicability in clinical practice.

Key Area: Diabetes

Fang Z, Wu Y, Li Y, Zhang X, Willett WC, Eliassen AH, Rosner B, Song M, Mucci LA, Giovannucci EL. Association of nut consumption with risk of total cancer and 5 specific cancers: evidence from 3 large prospective cohort studies.

Am J Clin Nutr. 2021 Sep 28:nqab295. doi: 10.1093/ajcn/nqab295. Epub ahead of print. PMID: 34582546.

Abstract: Background: The associations between nut consumption and cancer risk have not been extensively investigated. Objectives: We aimed to examine the associations between nut consumption, especially specific types of nuts (peanut, tree nut, walnut, and tree nut other than walnut), and cancer risk. Methods: Nut consumption was assessed by FFQ at baseline and updated every 2–4 y in the Nurses' Health Study (1980–2014), the Nurses' Health Study II (1991–2015), and the Health Professionals Follow-up Study (1986–2018). We examined the associations between the intake of total and specific types of nuts and risk of total cancer and common cancers, including lung, colorectal, breast, bladder, and aggressive prostate cancer. Cox proportional hazards models were used to obtain the HRs and 95% CIs in each cohort as well as pooled. Results: During 5,873,671 person-years of follow-up in 180,832 women and 45,560 men, we documented 44,561 incident cancer cases. As compared with nonconsumers, the pooled multivariable HRs of total nut consumption for ≥ 5 times/wk were 0.99 (95% CI: 0.94, 1.04; P-trend = 0.54) for total cancer, 0.88 (95% CI: 0.74, 1.04; P-trend = 0.18) for lung cancer, 1.07 (95% CI: 0.92, 1.26; P-trend = 0.89) for colorectal cancer, 0.90 (95% CI: 0.71, 1.14; P-trend = 0.65) for bladder cancer, 0.96 (95% CI: 0.85, 1.08; P-trend = 0.36) for breast cancer, and 1.18 (95% CI: 0.92, 1.51; P-trend = 0.52) for aggressive prostate cancer. Conclusions: In 3 large prospective cohorts, frequent nut consumption was not associated with risk of total cancer and common individual cancers.

Key Area: Cancer

Julvez J, Gignac F, Fernández-Barrés S, Romaguera D, Sala-Vila A, Ranzani OT., Persavento C, Delgado A, Carol A, Torrent J, Gonzalez J, Roso E, Barrera-Gómez J, López-Vicente M, Garcia-Esteban R, Boucher O, Fornis J, Burgaleta M, Sebastián N, Canals J, Arija V, Basagaña X, Ros E, Vendrell J, Salas-Salvadó J, Sunyer J. Walnuts, Long-Chain Polyunsaturated Fatty Acids, and Adolescent Brain Development: Protocol for the Walnuts Smart Snack Dietary Intervention Trial.

Front. Pediatr. 2021 Jun 8;9:593847. doi: 10.3389/fped.2021.593847. PMID: 34169045; PMCID: PMC8217431.

Abstract: Background: Adolescence, when the most complex behaviors are refined to adult sophistication, represents a major window of opportunity and vulnerability for neuropsychological development. To support and protect this complex and active brain growth, different nutritional components considered essential need to be acquired from the diet. For instance, omega-3 fatty acids are mainly obtained from seafood, seeds, and walnuts. Known for their rich lipid profile, walnuts contain sizable amounts of an essential fatty acid, alpha-linolenic acid (ALA), the vegetable omega-3 fatty acid that is the precursor of two longer-chain omega-3 polyunsaturated fatty acids (omega-3 PUFA): docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids. While there is growing evidence of neuropsychological improvements in the young developing brain associated with omega-3 PUFA intake, few studies have examined whether consuming walnuts during adolescence entails similar beneficial effects. There is a need to further explore the ways in which walnuts influence youthful brain function, particularly for the long-term. Thus, we designed the WALNUTs study (WSS), a population-based randomized controlled trial conducted in adolescents in Barcelona, Spain. We hypothesize that walnut intake will increase omega-3 PUFA tissue availability (particularly ALA) to a level that enhances the neuropsychological development during adolescence. Methodology/Design: We conducted a 6-month population-based randomized controlled trial in teenagers ($n = 800$) and we aimed to determine the effectiveness of the intervention (four walnuts per day, or 30 kernel g, ~ 1.5 g of ALA) in enhancing brain neuropsychological and socio-emotional development compared to a control group with no walnut

intervention. Before randomization, different neuropsychological tests were recorded for all participants, and blood samples (in a subsample of participants) were collected to measure omega-3 PUFA levels at baseline, and all again, after randomization and the intervention. The data is now collected and we will conduct linear regression models to assess the effect of the intervention. Discussion: The WALNUTs (WSS) study results will allow us to better understand the role of plant-based omega-3 PUFA intake from regular walnut consumption on neuropsychological development during adolescence. Results could be translated into nutritional public health recommendations targeting teenagers.

Key Area: Cognitive Health

Lackey KA and Fleming SA (2021) Brief Research Report: Estimation of the Protein Digestibility-Corrected Amino Acid Score of Defatted Walnuts.

Front. Nutr. 8:702857. doi: 10.3389/fnut.2021.702857

Abstract: Introduction: Walnuts are considered a good source of essential fatty acids, which is unique among tree nuts. Walnuts are also composed of about 10–15% protein, but the quality of this protein has not been evaluated. Pistachios and almonds have been evaluated for their protein content using a protein digestibility-corrected amino acid score (PDCAAS), but it is unclear how the quality of protein in walnuts relates to that in other commonly consumed tree nuts. The objective of this study was to substantiate the protein quality of walnuts by determining their PDCAAS. Methods: A small, 10-day dietary intervention trial was conducted using male Sprague-Dawley rats (n = 8, 4 per group) with two diets: a nitrogen-free diet and a diet containing protein exclusively from defatted walnuts. Feed intake and fecal output of nitrogen were measured to estimate the true protein digestibility, and the amino acid compositions of walnuts compared to child and adult populations were used to calculate amino acid scores (AAS) and PDCAAS. Results: The true protein digestibility score of raw walnuts was calculated to be 86.22%. Raw walnuts contained 15.6 g protein/g walnut with AAS of 0.45 and 0.63 for children aged 6 months to 3 years and 3–10 years, respectively. For each population, a PDCAAS of 39 and 46% was calculated, respectively, using a protein conversion constant of 5.30. Using a protein constant of 6.25, a PDCAAS of 39% (6 months - 3 years) or 46% (3-10 years) was calculated. Conclusions: This is the first known assessment of the PDCAAS of walnuts. Like almonds, appear to have a low-to-moderate score, indicating they are not a quality source of protein.

Key Area: Nutrient and Bioactive Composition

Levakov G, Kaplan A, Yaskolka Meir A, Rinott E, Tsaban G, Zelicha H, Meiran N, Shelef I, Shai I, Avidan G. Neural correlates of future weight loss reveal a possible role for brain-gastric interactions.

Neuroimage. 2021 Jan 1;224:117403. doi: 10.1016/j.neuroimage.2020.117403. Epub 2020 Sep 23.

Abstract: Lifestyle dietary interventions are an essential practice in treating obesity, hence neural factors that may assist in predicting individual treatment success are of great significance. Here, in a prospective, open-label, three arms study, we examined the correlation between brain resting-state functional connectivity measured at baseline and weight loss following 6 months of lifestyle intervention in 92 overweight participants. We report a robust subnetwork composed mainly of sensory and motor cortical regions, whose edges correlated with future weight loss. This effect was found regardless of intervention group. Importantly, this main finding was further corroborated using a stringent connectivity-based prediction model assessed with cross-validation thus attesting to its robustness. The engagement of senso-motor regions in this subnetwork is consistent with the over-sensitivity to food cues theory of weight regulation. Finally, we tested an additional hypothesis regarding the role of brain-gastric interaction in this subnetwork, considering recent findings of a cortical network synchronized with gastric activity. Accordingly, we found a significant spatial overlap with the subnetwork reported in the present study. Moreover, power in the gastric basal electric frequency within our reported subnetwork negatively correlated with future weight loss. This finding

was specific to the weight loss related subnetwork and to the gastric basal frequency. These findings should be further corroborated by combining direct recordings of gastric activity in future studies. Taken together, these intriguing results may have important implications for our understanding of the etiology of obesity and the mechanism of response to dietary intervention.

Key Area: Gut Health

Liu X, Guasch-Ferré M, Tobias DK, Li Y. Association of Walnut Consumption with Total and Cause-Specific Mortality and Life Expectancy in U.S. Adults

Nutrients 2021 Aug 4;13(8):2699. doi: 10.3390/nu13082699. PMID: 34444859; PMCID: PMC8401409.

Abstract: Walnut consumption is associated with health benefits. We aimed to (1) examine the association between walnut consumption and mortality and (2) estimate life expectancy in relation to walnut consumption in U.S. adults. We included 67,014 women of the Nurses' Health Study (1998–2018) and 26,326 men of the Health Professionals Follow-up Study (1998–2018) who were free of cancer, heart disease, and stroke at baseline. We used Cox regression models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). During up to 20 years of follow-up, we documented 30,263 deaths. The hazard ratios for total mortality across categories of walnut intake (servings/week), as compared to non-consumers, were 0.95 (95% confidence interval (CI), 0.91, 0.98) for <1 serving/week, 0.94 (95% CI, 0.89, 0.99) for 1 serving/week, 0.87 (95% CI, 0.82, 0.93) for 2–4 servings/week, and 0.86 (95% CI, 0.79, 0.93) for ≥5 servings/week (p for trend <0.0001). A greater life expectancy at age 60 (1.30 years in women and 1.26 years in men) was observed among those who consumed walnuts more than 5 servings/week compared to non-consumers. Higher walnut consumption was associated with a lower risk of total and CVD mortality and a greater gained life expectancy among U.S. elder adults.

Key Area: Heart Health

Park JM, Han YM, Lee HJ, Hwang SJ, Kim SJ, Hahm KB. Transcriptome profiling analysis of the response to walnut polyphenol extract in *Helicobacter pylori*-infected cells.

J Clin Biochem Nutr. 2021 Feb;https://doi.org/10.3164/jcbn.20-128

Abstract: Dietary intervention to prevent *Helicobacter pylori* (*H. pylori*)-associated gastric diseases seems to be ideal with no risk of bacterial resistance, safe long-term intervention, and correcting pathogenic mechanisms including rejuvenation of precancerous atrophic gastritis and anti-mutagenesis. A transcriptome as set of all RNAs transcribed by certain tissues or cells demonstrates gene functions and reveals the molecular mechanism of specific biological processes against diseases. Here, we have performed RNAseq and bioinformatic analysis to explain proof of concept that walnut intake can rescue from *H. pylori* infection and explore unidentified mode of actions of walnut polyphenol extract (WPE). As results, BIRC3, SLC25A4, f3 transcription, VEGFA, AZU1, HMOX1, RAB3A, RELBTNIP1, ETFB, INPP5J, PPME1, RHOB, TPI1, FOSL1, JUND.RELB, KLF2, MUC1, NDRG1, ALDOA, ENO1, PFKP, GPI, GDF15, and NRTN genes were newly discovered to be enriched with WPE, whereas CCR4, BLNK, CCR7, CXCR4, CDO1, KLSG1, SELE, RASGRP2, PIK3R3, TSPAN32, HOXC-AS3, HCG8, BTNL8, and CXCL3 genes as inhibitory targets by WPE in *H. pylori* infection. We identified additional genes what WPE afforded actions of avoiding *H. pylori*-driven onco-inflammation and rejuvenating precancerous atrophic gastritis. Conclusively, after applying RNAseq analysis in order to document walnut intake for precision medicine against *H. pylori* infection, significant transcriptomic profiling applicable for validation were drawn.

Key Area: Gut Health

Park JM, Han YM, Park, YJ, Hahm KB. Dietary intake of walnut prevented *Helicobacter pylori*-associated gastric cancer through rejuvenation of chronic atrophic gastritis.

J Clin Biochem Nutr. 2021 Jan; 68(1): 37–50. Published online 2020 Oct 13. doi: 10.3164/jcbn.20-103

Abstract: The fact that Fat-1 transgenic mice producing n-3 polyunsaturated fatty acids via overexpressed 3-desaturase significantly mitigated *Helicobacter pylori* (*H. pylori*)-associated gastric tumorigenesis through rejuvenation of chronic atrophic gastritis (CAG) led us to study whether dietary intake of walnut plentiful of n-3 PUFAs can be nutritional intervention to prevent *H. pylori*-associated gastric cancer. In our model that *H. pylori*-initiated, high salt diet-promoted gastric carcinogenesis, pellet diet containing 100 mg/kg and 200 mg/kg walnut was administered up to 36 weeks. As results, control mice (24 weeks) developed significant chronic CAG, in which dietary walnuts significantly ameliorated chronic atrophic gastritis. Expressions of COX-2/PGE2/NF- κ B/c-Jun, elevated in 24 weeks control group, were all significantly decreased with walnut ($p < 0.01$). Tumor suppressive enzyme, 15-PGDH, was significantly preserved with walnut. Control mice (36 weeks) all developed significant tumors accompanied with severe CAG. However, significantly decreased tumorigenesis was noted in group treated with walnuts, in which expressions of COX-2/PGE2/NF- κ B/IL-6/STAT3, all elevated in 36 weeks control group, were significantly decreased with walnut. Defensive proteins including HO-1, Nrf2, and SOCS-1 were significantly increased in walnut group. Proliferative index as marked with Ki-67 and PCNA was significantly regulated with walnut relevant to 15-PGDH preservation. Conclusively, walnut can be an anticipating nutritional intervention against *H. pylori*.

Key Area: Cancer

Rajaram,S, Cofán M, Sala-Vila A, Haddad E, Serra-Mir M, Bitok E, Roth I, Freitas-Simoes TM, Kaur A, Valls-Pedret C, Doménech M, Oda K, Corella D, Sabaté J, Ros E. Effects of walnut consumption for 2 years on lipoprotein subclasses among healthy elders: Findings from the Walnuts and Healthy Aging (WAHA) randomized controlled trial.

Circulation. 2021 Sep 28;144(13):1083-1085. doi: 10.1161/CIRCULATIONAHA.121.054051. Epub 2021 Aug 30. PMID: 34455809; PMCID: PMC8478315.

Abstract: Background: Frequent consumption of nuts, an important component of plant-based diets, is associated with 15% lower total cardiovascular disease (CVD) and 23% lower CVD mortality rates. Small, short-term randomized controlled trials (RCTs) indicate that diets supplemented with nuts have a consistent cholesterol-lowering effect; however, no trials of nut-enriched diets for lipid changes focused on elderly individuals, recruited participants from diverse geographical locations, or lasted 2 years. Also, there is little information concerning effects of nuts on lipoprotein subclasses.

Objective: We hypothesized that incorporating walnuts into the usual diet would improve the lipid profile irrespective of differences in geographical and dietary background. Methods: The Walnuts and Healthy Aging (WAHA) study is a two-center (Barcelona, Spain and California, USA), 2-year, parallel-group RCT testing the effects of walnut supplemented diets in healthy elders. Lipoprotein changes were a pre-specified secondary outcome. Eligible candidates were cognitively healthy elders (63-79 years-old) without major comorbidities. Participants (n=708) were allocated to either a walnut-free (control) or walnut-supplemented diet ($\approx 15\%$ of energy, 30-60g/day). In 2-monthly visits, compliance, tolerance, medication changes, and body weight were recorded. At each visit, 8-week allotments of raw, pieced walnuts were delivered to the corresponding group. Results: 636 participants completed the study (90% retention rate) and 628 had full data for lipoprotein analyses (mean age 69 years, 67% women, 32% treated with statins). Mean baseline LDL-C and triglycerides were 117 and 105 mg/dL, respectively. The walnut diet significantly decreased (mg/dL) total cholesterol (mean -8.5 [95% CI, -11.2, -5.4]), LDL-C (mean -4.3 [-6.6, -1.6]), and intermediate-density lipoprotein (IDL)-C (-1.3 [-1.5, -1.0]), corresponding to reductions of 4.4%, 3.6%, and 16.8%, respectively, while triglycerides and HDL-C were unaffected (Figures-B, C). Total LDL particles and small LDL particle number decreased by 4.3% and 6.1%, respectively (Figure-D). Results were not different by study site. Lipid responses to the walnut diet differed by sex: LDL-C was reduced by 7.9% in men and by 2.6% in women (P-interaction=0.007). Conclusions: The results demonstrate that incorporating daily doses of walnuts ($\approx 15\%$ of energy) to the habitual diet of free-living elders with an essentially normal lipid

profile resulted in a mean 4.3 mg/dL LDL-C reduction, which is modest, although greater responses have been observed among individuals with hypercholesterolemia. Our data also support a beneficial effect of the walnut diet on NMR-assessed lipoprotein subfractions, with reductions of IDL-C (a sizable contributor to remnant-C) and total LDL particles. Prospective studies have reported that LDL particle number consistently outperforms LDL-C in CVD risk prediction and that remnant-C causally relates to CVD independent of LDL-C. That lipid responses were not different in two cohorts consuming diverse diets strengthens the generalization of our results. WAHA is the largest and longest nut trial to date, overcoming the limitations of prior smaller and shorter nut studies. The novel finding of sexual dimorphism in LDL-C response to walnut supplementation needs confirmation. WAHA was conducted in free-living individuals, who chose their daily foods, which may be viewed as desirable since it is closer to real life than the situation in controlled feeding studies. On the basis of associations ascertained in cohort studies, the observed shift of the lipoprotein subclass phenotype suggests a reduction of lipoprotein-related CVD risk by long-term consumption of walnuts, which provides novel mechanistic insight for their potential cardiovascular benefit beyond effects on the standard lipid panel. Our data reinforce the notion that regular walnut consumption may be a useful part of a multi-component dietary intervention or dietary pattern to lower atherogenic lipids and improve CVD risk.

Key Area: Heart Health

Tsaban G, Yaskolka Meir A, Zelicha H, Rinott E, Kaplan A, Shalev A, Katz A, Brikner D, Blüher M, Ceglarek U, Stumvoll M, Stampfer MJ, Shai I. Diet-induced fasting ghrelin elevation reflects the recovery of insulin sensitivity and visceral adiposity regression.

J Clin Endocrinol Metab. 2021 Oct 13:dgab681. doi: 10.1210/clinem/dgab681. Epub ahead of print. PMID: 34643713.

Abstract: Aims: Lower fasting-ghrelin-levels (FGL) are associated with obesity and metabolic syndrome. We aimed to explore the dynamics of FGL during weight-loss and its metabolic and adiposity-related manifestations beyond weight-loss. Methods: A secondary analysis of a clinical trial where we randomized participants with abdominal-obesity/dyslipidemia to one of three diets: healthy-dietary-guidelines (HDG), Mediterranean diet (MED), or green-MED diet, all combined with physical activity (PA). Both MED diets were similarly hypocaloric and included 28g/day walnuts. The green-MED group further consumed green tea (3-4 cups/day) and a *Wolffia-globosa* (Mankai) plant green-shake. We measured FGL and quantified body fat depots by Magnetic-Resonance-Imaging at baseline and after 18-months. Results: Among 294 participants [body-mass-index=31.3kg/m²;FGL=504±208pg/mL; retention rate=89.8%], lower FGL were associated with unfavorable cardiometabolic parameters as higher visceral-adipose-tissue (VAT), intra-hepatic fat, leptin, and blood pressure (p<0.05 for all; multivariate models). ΔFGL18-month differed between men (+7.3+26.6%) and women (-9.2+21.3%,p=0.001). After 18-months of moderate and similar weight loss among the MED-groups, FGL increased by 1.3%, 5.4%, and 10.5% in HDG, MED, and green-MED groups, respectively (p=0.03 for green-MED vs. HDG), sex-stratified analysis revealed similar changes in men only. Among men, FGL18-month elevation was associated with favorable changes in insulin resistance profile and VAT regression, after adjusting for relative weight-loss (HbA1c:r=-0.216; homeostatic-model-of insulin-resistance:r=-0.154; HDL-c:r=0.147;VAT:r=-0.221;p<0.05 for all). , Insulin resistance and VAT remained inversely related with FGL elevation, beyond which was explained by weight-loss (residual regression analyses;p<0.05). Conclusions: Diet-induced FGL elevation may reflect insulin sensitivity recovery and VAT regression beyond weight-loss, specifically among men. Green-MED diet is associated with greater FGL elevation.

Key Area: Body Weight and Composition

Tuccinardi D, Perakakis N, Farr OM, Upadhyay J, Mantzoros CS. Branched-Chain Amino Acids in relation to food preferences and insulin resistance in obese subjects consuming walnuts: A cross-over, randomized, double-blind, placebo-controlled inpatient physiology study.

Clin Nutr. 2021 May;40(5):3032-3036. doi: 10.1016/j.clnu.2021.01.020. Epub 2021 Jan 23. PMID: 33541836; PMCID: PMC8172419.

Abstract: Summary Background&aims To assess whether the concentrations of circulating Branched-Chain Amino Acids (BCAAs) change after walnut consumption and, whether these changes are associated with alterations in markers of insulin resistance and food preferences.

Methods In a crossover, randomized, double-blind, placebo-controlled study, ten subjects participated in two 5-day inpatient study admissions, during which they had a smoothie containing 48 g walnuts or a macronutrient-matched placebo smoothie without nuts every morning. Between the two phases there was a 1-month washout period. Results Fasting valine and isoleucine levels were reduced ($p = .047$ and $p < .001$) and beta-hydroxybutyrate levels were increased after 5-days of walnut consumption compared to placebo ($p = .023$). Fasting valine and isoleucine correlated with HOMA-IR while on walnut ($r = 0.709$, $p = .032$ and $r = 0.679$, $p = .044$). The postprandial area under the curve (AUC) of leucine in response to the smoothie consumption on day 5 was higher after walnut vs placebo ($p = .023$) and correlated negatively with the percentage of Kcal from carbohydrate and protein consumed during an ad libitum buffet meal consumed the same day for lunch ($r = -0.661$, $p = .037$; $r = -0.628$, $p = .05$, respectively). Conclusion The fasting and postabsorptive profiles of BCAAs are differentially affected by walnut consumption. The reduction in fasting valine and isoleucine may contribute to the longer-term benefits of walnuts on insulin resistance, cardiovascular risk and mortality, whereas the increase in postabsorptive profiles with walnuts may influence food preference.

Key Area: Body Weight and Composition

Wilson T, DeVaan LS, LaCasse ME, Gile EM, Weis MJ, Ahmann MD, Schnellman GI, Lenz MT, Hooks TL. Effect of Walnut Predinner Snack on Mealtime Hunger and Nutrient Intake Among University Students.

J Med Food. 2021 Oct 29. doi: 10.1089/jmf.2021.0092. Epub ahead of print. PMID: 34714144.

Abstract: Freshman-15 is a phenomenon of first-year university students resulting in weight gain partly due to new cafeteria eating patterns and stress. This study determined if a premeal walnut snack alters planned eating behavior and mealtime nutrient intake during a subsequent buffet-model meal. Healthy university students ($n = 36$; 18.1 ± 0.5 years; body mass index: 23.6 ± 3.9) received three treatments (90 min premeal) in randomized order on 3 consecutive days: (1) snack of 190 Cal (1 oz) of walnuts (WS), (2) snack of 190 Cal of gummy candy (GS), or (3) no snack (NS; control) before a standard cafeteria dinner (1760 Cal). Visual analog scale (VAS) surveys were administered before and after dinner, and caloric intake was determined. Premeal VAS desire to eat was lower after WS and GS than NS, whereas the sense of hunger and sense of fullness were higher after WS and GS compared with NS. Postmeal VAS was not different between treatments. Mealtime calories, total fat, saturated fat, cholesterol, protein, sodium, fiber, and sugar consumed after WS were significantly less than NS. Total fat and sodium consumed after GS did not significantly differ from NS. Mealtime total fat, sodium, and fiber for WS were significantly less than GC, and a trend was observed for total calories. Differences in calorie intake were not observed between treatments when snack calories were included as part of the mealtime caloric intake. These findings could be helpful for promoting WS and to a lesser degree GS for increased satiety before meals possibly leading to reduced food intake during dinner by university students.

Key Area: Body Weight and Composition

Yaskolka Meir A, Tuohy K, von Bergen M, Krajmalnik-Brown R, Heinig U, Zelicha H, Tsaban G, Rinott E, Kaplan A, Aharoni A, Zeibich L, Chang D, Dirks B, Dotallevi C, Arapitsas P, Vrhovsek

U, Ceglarek U, Haange S-B, Rolle-Kampczyk U, Engelmann B, Lapidot M, Colt M, Sun Q, Shai I. The Metabolomic-Gut-Clinical Axis of Mankai Plant-Derived Dietary Polyphenols.

Nutrients. 2021; 13(6):1866. <https://doi.org/10.3390/nu13061866>.

Abstract: Background: Polyphenols are secondary metabolites produced by plants to defend themselves from environmental stressors. We explored the effect of *Wolffia globosa* ‘Mankai’, a novel cultivated strain of a polyphenol-rich aquatic plant, on the metabolomic-gut clinical axis in vitro, in-vivo and in a clinical trial. Methods: We used mass-spectrometry-based metabolomics methods from three laboratories to detect Mankai phenolic metabolites and examined predicted functional pathways in a Mankai artificial-gut bioreactor. Plasma and urine polyphenols were assessed among the 294 DIRECT-PLUS 18-month trial participants, comparing the effect of a polyphenol-rich green-Mediterranean diet (+1240 mg/polyphenols/day, provided by Mankai, green tea and walnuts) to a walnuts-enriched (+440 mg/polyphenols/day) Mediterranean diet and a healthy controlled diet. Results: Approximately 200 different phenolic compounds were specifically detected in the Mankai plant. The Mankai-supplemented bioreactor artificial gut displayed a significantly higher relative-abundance of 16S-rRNA bacterial gene sequences encoding for enzymes involved in phenolic compound degradation. In humans, several Mankai-related plasma and urine polyphenols were differentially elevated in the green Mediterranean group compared with the other groups ($p < 0.05$) after six and 18 months of intervention (e.g., urine hydroxy-phenyl-acetic-acid and urolithin-A; plasma Naringenin and 2,5-diOH-benzoic-acid). Specific polyphenols, such as urolithin-A and 4-ethylphenol, were directly involved with clinical weight-related changes. Conclusions: The Mankai new plant is rich in various unique potent polyphenols, potentially affecting the metabolomic-gut-clinical axis.

Key Area: Body Weight and Composition

Yaskolka Meir A, Rinott E, Tsaban G, Zelicha H, Kaplan A, Rosen P, Shelef I, Youngster I, Shalev A, Blüher M, Ceglarek U, Stumvoll M, Tuohy K, Diotallevi C, Vrhovsek U, Hu F, Stampfer M, Shai I. Effect of green-Mediterranean diet on intrahepatic fat: the DIRECT PLUS randomised controlled trial.

Gut. 2021 Nov;70(11):2085-2095. doi: 10.1136/gutjnl-2020-323106. Epub 2021 Jan 18. PMID: 33461965; PMCID: PMC8515100.

Abstract: Objective To examine the effectiveness of green-Mediterranean (MED) diet, further restricted in red/ processed meat, and enriched with green plants and polyphenols on non-alcoholic fatty liver disease (NAFLD), reflected by intrahepatic fat (IHF) loss. Design For the DIRECT-PLUS 18-month randomized clinical trial, we assigned 294 participants with abdominal obesity/dyslipidaemia into healthy dietary guidelines (HDG), MED and green-MED weight-loss diet groups, all accompanied by physical activity. Both isocaloric MED groups consumed 28 g/day walnuts (+440 mg/day polyphenols provided). The green-MED group further consumed green tea (3–4 cups/day) and Mankai (a *Wolffia globosa* aquatic plant strain; 100 g/ day frozen cubes) green shake (+1240 mg/day total polyphenols provided). IHF% 18-month changes were quantified continuously by proton magnetic resonance spectroscopy (MRS). Results Participants (age=51 years; 88% men; body mass index=31.3 kg/m²; median IHF%=6.6%; mean=10.2%; 62% with NAFLD) had 89.8% 18 month retention-rate, and 78% had eligible follow-up MRS. Overall, NAFLD prevalence declined to: 54.8% (HDG), 47.9% (MED) and 31.5% (green-MED), $p=0.012$ between groups. Despite similar moderate weight-loss in both MED groups, green-MED group achieved almost double IHF% loss (–38.9% proportionally), as compared with MED (–19.6% proportionally; $p=0.035$ weight loss adjusted) and HDG (–12.2% proportionally; $p<0.001$). After 18 months, both MED groups had significantly higher total plasma polyphenol levels versus HDG, with higher detection of Naringenin and 2-5-dihydroxybenzoic- acid in green-MED. Greater IHF% loss was independently associated with increased Mankai and walnuts intake, decreased red/processed meat consumption, improved serum folate and adipokines/lipids biomarkers, changes in microbiome composition (beta-diversity) and specific bacteria ($p<0.05$ for all). Conclusion The new

suggested strategy of green-Mediterranean diet, amplified with green plant-based proteins/polyphenols as Mankai, green tea, and walnuts, and restricted in red/processed meat can double IHF loss than other healthy nutritional strategies and reduce NAFLD in half.

Key Area: Body Weight and Composition

2020

Al Abdrabalnabi A, Rajaram S, Bitok E, Oda K, Beeson WL, Kaur A, Cofán M, Serra-Mir M, Roth I, Ros E, Sabaté J. Effects of Supplementing the Usual Diet with a Daily Dose of Walnuts for Two Years on Metabolic Syndrome and Its Components in an Elderly Cohort.

Nutrients. 2020 Feb 11;12(2). pii: E451. doi: 10.3390/nu12020451.

Abstract: Accumulating evidence links nut consumption with an improved risk of metabolic syndrome (MetS); however, long-term trials are lacking. We examined the effects of a daily dose of walnuts for two years on MetS in a large elderly cohort. A total of 698 healthy elderly participants were randomly assigned to either a walnut supplemented or a control diet. The participants in the walnut group were provided with packaged walnuts (1, 1.5, or 2 oz. or ~15% of energy) and asked to incorporate them into their daily habitual diet. The participants in the control group were asked to continue with their habitual diet and abstain from eating walnuts and other tree nuts. Intake of n-3 fatty acid supplements was not permitted in either group. Fasting blood chemistries, blood pressure, and anthropometric measurements were obtained at baseline and at the end of intervention. A total of 625 participants (67% women, mean age 69.1 y) completed this two-year study (90% retention rate). Triglycerides decreased in both walnut (-0.94 mg/dl) and control (-0.96 mg/dl) groups, with no significant between-group differences. There was a non-significant decrease in systolic and diastolic blood pressure in the walnut group (-1.30 and -0.71 mm Hg, respectively) and no change in the control group. Fasting blood glucose decreased by ~1 point in both the walnut and control groups. There were no significant between-group differences in the development or reversion of MetS. In conclusion, supplementing the diet of older adults with a daily dose of walnuts had no effect on MetS status or any of its components, although the walnut group tended to have lower blood pressure.

Key Area: Metabolic Syndrome

An JM, Kim EH, Lee H, Lee HJ, Hahm KB. Dietary walnut as food factor to rescue from NSAID-induced gastrointestinal mucosal damages

Arch Biochem Biophys. 2020;689:108466. doi:10.1016/j.abb.2020.108466

Abstract: Nuclear factor erythroid-derived 2-like 2 (Nrf-2) is transcription factor implicated in the antioxidant response element-mediated induction of endogenous antioxidant enzyme such as heme oxygenase-1 (HO-1), glutamylcysteine ligase, and NAD(P)H quinone dehydrogenase 1, among which HO-1 is an enzyme catalyzing the degradation of heme, producing biliverdin, ferrous iron, and carbon monoxide. In the stomach, as much as regulating gastric acid secretions, well-coordinated establishment of defense system stands for maintaining gastric integrity. In previous study, author et al. for the first time discovered HO-1 induction was critical in affording faithful gastric defense against various irritants including *Helicobacter pylori* infection, stress, alcohol, nonsteroidal anti-inflammatory drugs (NSAIDs), aspirin, and toxic bile acids. In this review article, we can add the novel evidence that dietary walnut intake can be reliable way to rescue from NSAIDs-induced gastrointestinal damages via the induction of HO-1 transcribed with Nrf-2 through specific inactivation of Keap-1. From molecular exploration to translational animal model of indomethacin-induced gastrointestinal damages, significant induction of HO-1 contributed to rescuing from damages. In addition to HO-1 induction action relevant to walnut, we added the description the general actions of walnut extracts or dietary intake of walnut regarding cytoprotection and why we have focused on to NSAID damages.

Key Area: Gut Health

Bishop, N., & Zuniga, K. (2020). Investigating walnut consumption and cognitive trajectories in a representative sample of older US adults.

Public Health Nutr. 2021 May;24(7):1741-1752. doi: 10.1017/S1368980020001287. Epub 2020 Jul 3. PMID: 32618237.

Abstract: Objective: Existing research suggests walnut intake may be associated with better cognitive function in older adults, yet few studies utilise longitudinal data from observational studies of ageing populations. Our objective was to estimate the association between whole walnut intake and cognitive change in a representative sample of older Americans. Design: Secondary analysis of the Health and Retirement Study and Health Care and Nutrition Study. Walnut consumption was defined as a categorical measure (none, low intake (0·01–0·08 1 oz. servings per day) and moderate intake (>0·08 1 oz. servings per day)) and cognitive function was measured using the Telephone Interview for Cognitive Status. Latent growth modelling estimated the association between walnut consumption and trajectories of cognitive status over a 4-year observational period. Sensitivity analyses assessing non-random dropout and Monte Carlo power analyses were conducted to contextualise results. Setting: The USA. Participants: A sample of 3632 US adults aged 65 years and older. Results: Those reporting any walnut consumption had greater cognitive scores at baseline than those not consuming walnuts (low walnut consumption, $b = 1.53$, $se = 0.21$, $P < 0.001$; moderate walnut consumption, $b = 2.22$, $se = 0.27$, $P < 0.001$), but walnut consumption was not associated with cognitive change. Walnut consumption was positively associated with socioeconomic status and health behaviours as well as intake of nutrients identified to have neuroprotective benefits. Conclusions: We identified an association between walnut consumption and cognitive function in older adults, although we did not find that walnut consumption was protective against age-related cognitive decline.

Key Area: Cognitive Health

Carey AN, Fisher DR, Bielinski DF, Cahoon DS, Shukitt-Hale B. Walnut-associated fatty acids inhibit LPS-induced activation of BV-2 microglia.

Inflammation. 2020 Feb;43(1):241-250. doi: 10.1007/s10753-019-01113-y.

Abstract: Walnuts have high levels of the omega-3 fatty acid alpha-linolenic acid (C18:3n-3, ALA) and the omega-6 fatty acid linoleic acid (C18:2n-6, LA). Previous research has demonstrated that pre-treatment of BV-2 microglia with walnut extract inhibited lipopolysaccharide (LPS)-induced activation of microglia. As an extension of that study, the effects of walnut-associated fatty acids on BV-2 microglia were assessed. BV-2 murine microglia cells were treated with LA, ALA, or a combination of LA+ALA prior to or after exposure to LPS. Nitric oxide (NO) and tumor necrosis factor-alpha (TNF-alpha) were measured in cell-conditioned media. Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression were assessed in BV-2 microglia. Both LA and ALA protected against LPS-induced increases in NO, iNOS, COX-2, and TNF-alpha when used before LPS exposure. When BV-2 microglia were treated with fatty acids after LPS, only COX-2 and TNF-alpha were significantly attenuated by the fatty acids. There was no synergism of LA+ALA, as the LA+ALA combination was no more effective than LA or ALA alone. Fatty acids, like those found in walnuts, may protect against production of cytotoxic intermediates and cell-signaling molecules from microglia and may prove beneficial for preventing age- or disease-related neurodegeneration.

Key Area: Cognitive Health

Chen Y, Nakanishi M, Bautista EJ, Qendro V, Sodergren E, Rosenberg DW, Weinstock GM. Colon cancer prevention with walnuts: a longitudinal study in mice from the perspective of a gut enterotype-like cluster.

Cancer Prev Res (Phila). 2020 Jan;13(1):15-24. doi: 10.1158/1940-6207.CAPR-19-0273. Epub 2019 Dec 9. PMID: 31818852.

Abstract: There is limited understanding of how walnut consumption inhibits the development of colorectal cancer. A possible mechanism may involve alterations to the gut microbiota. In this study, the effects of walnut on gut microbiota were tested in a mouse tumor bioassay using the colonotropic carcinogen, azoxymethane (AOM) added to the total Western diet (TWD). 16S rRNA pyrosequencing identified three enterotype-like clusters (E1, E2, and E3) in this murine model. E1, E2, and E3 are associated with AOM exposure, walnut consumption, and TWD diet, respectively. E2 and E3 showed distinct taxonomic and functional characteristics, while E1 represented an intermediate state. At the family level, E1 and E3 were both enriched with Bacteroidaceae, but driven by two different operational taxonomic units (OTU; OTU-2 for E1, OTU-4 for E3). E2 was overrepresented with Porphyromonadaceae and Lachnospiraceae, with OTU-3 (family Porphyromonadaceae) as the "driver" OTU for this cluster. Functionally, E3 is overrepresented with genes of glycan biosynthesis and metabolism, xenobiotic metabolism, and lipid metabolism. E2 is enriched with genes associated with cell motility, replication and repair, and amino acid metabolism. Longitudinally, E2 represents the gut microbial status of early life in these mice. In comparison with E1 and E3, E2 is associated with a moderate lower tumor burden ($P = 0.12$). Our results suggest that walnuts may reduce the risk of colorectal cancer within a Western diet by altering the gut microbiota. Our findings provide further evidence that colorectal cancer risk is potentially modifiable by diet via alterations to the microbiota.

Key Area: Cancer

Cofán M, Rajaram S, Sala-Vila A, Valls-Pedret C, Serra-Mir M, Roth I, Freitas-Simoes TM, Bitok E, Sabaté J, Ros E. Effects of 2-Year Walnut-Supplemented Diet on Inflammatory Biomarkers
J Am Coll Cardiol. 2020 Nov, 76 (19) 2282–2284

Robust epidemiological evidence suggests that regular nut consumption is associated with lower cardiovascular disease (CVD) risk. As summarized in a recent meta-analysis of 19 prospective studies (1), when comparing extreme quantiles of total nut consumption (2.5 to 28 g/day), total CVD and CVD mortality were 15% and 23% lower, respectively. Walnut consumption independent from other nuts revealed similar inverse associations with CVD in 3 studies

Key Area: Heart Health

Esselun C, Dilberger B, Silaidos CV, Koch E, Schebb NH, Eckert GP. A Walnut Diet in Combination with Enriched Environment Improves Cognitive Function and Affects Lipid Metabolites in Brain and Liver of Aged NMRI Mice.

Neuromolecular Med. 2020 Dec 26. doi: 10.1007/s12017-020-08639-7. Epub ahead of print. PMID: 33367957.

Abstract: This in vivo study aimed to test if a diet enriched with 6% walnuts alone or in combination with physical activity supports healthy ageing by changing the oxylipin profile in brain and liver, improving motor function, cognition, and cerebral mitochondrial function. Female NMRI mice were fed a 6% walnut diet starting at an age of 12 months for 24 weeks. One group was additionally maintained in an enriched environment, one group without intervention served as control. After three months, one additional control group of young mice (3 weeks old) was introduced. Motor and cognitive functions were measured using Open Field, Y-Maze, Rotarod and Passive Avoidance tests. Lipid metabolite profiles were determined using RP-LC-ESI(-)-MS/MS in brain and liver tissues of mice. Cerebral mitochondrial function was characterized by the determination of ATP levels, mitochondrial membrane potential and mitochondrial respiration. Expression of genes involved with mito- and neurogenesis, inflammation, and synaptic plasticity were determined using qRT-PCR. A 6% walnut-enriched diet alone improved spatial memory in a Y-Maze alternation test ($p < 0.05$) in mice. Additional physical

enrichment enhanced the significance, although the overall benefit was virtually identical. Instead, physical enrichment improved motor performance in a Rotarod experiment ($p^* < 0.05$) which was unaffected by walnuts alone. Bioactive oxylipins like hydroxy-polyunsaturated fatty acids (OH-PUFA) derived from linoleic acid (LA) were significantly increased in brain ($p^{**} < 0.01$) and liver ($p^{***} < 0.0001$) compared to control mice, while OH-PUFA of α -linolenic acid (ALA) could only be detected in the brains of mice fed with walnuts. In the brain, walnuts combined with physical activity reduced arachidonic acid (ARA)-based oxylipin levels ($p < 0.05$). Effects of walnut lipids were not linked to mitochondrial function, as ATP production, mitochondrial membrane potential and mitochondrial respiration were unaffected. Furthermore, common markers for synaptic plasticity and neuronal growth, key genes in the regulation of cytoprotective response to oxidative stress and neuronal growth were unaffected. Taken together, walnuts change the oxylipin profile in liver and brain, which could have beneficial effects for healthy ageing, an effect that can be further enhanced with an active lifestyle. Further studies may focus on specific nutrient lipids that potentially provide preventive effects in the brain.

Key Area: Cognitive Health

Freitas-Simoes TM, Wagner M, Samieri C, Sala-Vila A, Grodstein F. Consumption of Nuts at Midlife and Healthy Aging in Women.

J Aging Res. 2020 Jan 7;2020:5651737. doi: 10.1155/2020/5651737. PMID: 32399296; PMCID: PMC7199627.

Abstract: Background. Nut consumption may reduce age-related diseases and lead to better health and well-being in aging. Many conditions of aging develop over decades, and thus earlier lifestyle factors may particularly influence later health. Methods. In 1998 and 2002, we administered food frequency questionnaires to assess nut consumption (peanuts, walnuts, and other nuts and peanut butter) in women in the Nurses' Health Study in their 50 s/early 60 s. In 2012, those who survived beyond 65 years with no chronic diseases, no reported memory impairment, no physical disabilities, and intact mental health were considered "healthy agers." We used multivariable logistic regression to estimate odds ratios for healthy versus usual aging, controlled for sociodemographic, behavioral, dietary, and other potential confounding factors. Results. Of 33,931 participants at midlife, 16% became "healthy agers." After age adjustment, we observed a significant association between total nut consumption at midlife and higher odds of healthy aging, with strongest associations observed excluding peanut butter (odds ratio (OR) = 1.46, 95% confidence interval (CI) 1.32–1.62, ≥ 3 servings/week versus none). Findings were attenuated after further control for covariates, including overall diet quality (OR = 1.14, 95% CI 1.02–1.28, P trend = 0.05). For nut types, we found statistically significantly higher odds of healthy aging across peanuts, walnuts, and other nuts after age adjustment. After full control for confounders, only walnut consumption remained associated with healthy aging (P trend = 0.0001); for example, the OR was 1.20 (95% CI 1.00–1.44) for ≥ 2 servings/week versus none. Conclusions. Women consuming nuts at midlife have a greater likelihood of overall health and well-being at older ages. Nut consumption may represent a simple intervention to explore and promote healthy aging.

Key Area: Cognitive Health

Guan V, Neale E, Tapsell L Probst Y. Identifying usual food choice combinations with walnuts: Analysis of a 2005-2015 clinical trial cohort of overweight and obese adults

Front Nutr. 2020 Sep 23;7:149. doi: 10.3389/fnut.2020.00149. eCollection 2020.

Abstract: Consumption of nuts has been associated with a range of favorable health outcomes. Evidence is now emerging to suggest that walnuts may also play an important role in supporting the consumption of a healthy dietary pattern. However, limited studies have explored how walnuts are eaten at different meal occasions. The aim of this study was to explore the food choices in relation to walnuts at meal occasions as reported by a sample of overweight and obese adult participants of weight loss

clinical trials. Baseline usual food intake data were retrospectively pooled from four food-based clinical trials (n=758). A nut-specific food composition database was applied to determine walnut consumption within the food intake data. The Apriori algorithm of association rules was used to identify food choices associated with walnuts at different meal occasions using a nested hierarchical food group classification system. The proportion of participants who were consuming walnuts was 14.5% (n=110). The median walnut intake was 5.14 (IQR 1.10 - 11.45) grams per day. A total of 128 food items containing walnuts were identified for walnut consumers. The proportion of participants who reported consuming unsalted raw walnut was 80.5% (n=103). There were no identified patterns to food choices in relation to walnut at the breakfast, lunch or dinner meal occasions. A total of 24 clusters of food choices related to walnuts were identified at others (meals). By applying a novel food composition database, the present study was able to map the precise combinations of foods associated with walnuts intakes at mealtimes using data mining. This study offers insights into the role of walnuts for the food choices of overweight adults and may support guidance and dietary behavior change strategies.

Key Area: Body Weight and Composition

Guasch-Ferré M, Hernández-Alonso P, Drouin-Chartier JP, Ruiz-Canela M, Razquin C, Toledo E, Li J, Dennis C, Wittenbecher C, Corella D, Estruch R, Fitó M, Ros E, Babio N, Bhupathiraju SN, Clish CB, Liang L, Martínez-González MA, Hu FB, Salas-Salvadó J. Walnut Consumption, Plasma Metabolomics, and Risk of Type 2 Diabetes and Cardiovascular Disease.

J Nutr. 2020 Dec 31:nxaa374. doi: 10.1093/jn/nxaa374. Epub ahead of print. PMID: 33382410.

Abstract: Background: Walnut consumption is associated with lower risk of type 2 diabetes (T2D) and cardiovascular disease (CVD). However, it is unknown whether plasma metabolites related to walnut consumption are also associated with lower risk of cardiometabolic diseases. Objectives: The study aimed to identify plasma metabolites associated with walnut consumption and evaluate the prospective associations between the identified profile and risk of T2D and CVD. Methods: The discovery population included 1833 participants at high cardiovascular risk from the PREvención con DIeta MEDiterránea (PREDIMED) study with available metabolomics data at baseline. The study population included 57% women (baseline mean BMI (in kg/m²): 29.9; mean age: 67 y). A total of 1522 participants also had available metabolomics data at year 1 and were used as the internal validation population. Plasma metabolomics analyses were performed using LC-MS. Cross-sectional associations between 385 known metabolites and walnut consumption were assessed using elastic net continuous regression analysis. A 10-cross-validation (CV) procedure was used, and Pearson correlation coefficients were assessed between metabolite weighted models and self-reported walnut consumption in each pair of training-validation data sets within the discovery population. We further estimated the prospective associations between the identified metabolite profile and incident T2D and CVD using multivariable Cox regression models. Results: A total of 19 metabolites were significantly associated with walnut consumption, including lipids, purines, acylcarnitines, and amino acids. Ten-CV Pearson correlation coefficients between self-reported walnut consumption and the plasma metabolite profile were 0.16 (95% CI: 0.11, 0.20) in the discovery population and 0.15 (95% CI: 0.10, 0.20) in the validation population. The metabolite profile was inversely associated with T2D incidence (HR per 1 SD: 0.83; 95% CI: 0.71, 0.97; P = 0.02). For CVD incidence, the HR per 1-SD was 0.71 (95% CI: 0.60, 0.85; P < 0.001). Conclusions: A metabolite profile including 19 metabolites was associated with walnut consumption and with a lower risk of incident T2D and CVD in a Mediterranean population at high cardiovascular risk.

Key Area: Diabetes

Lázaro I, Rueda F, Cediél G, Ortega E, García-García C, Sala-Vila A, Bayés-Genís A. Circulating Omega-3 Fatty Acids and Incident Adverse Events in Patients With Acute Myocardial Infarction

J Am Coll Cardiol. 2020 Nov 3;76(18):2089-2097. doi: 10.1016/j.jacc.2020.08.073. PMID: 33121716.

Abstract: Background Dietary omega-3 eicosapentaenoic acid (EPA) has multiple cardioprotective properties. The proportion of EPA in serum phosphatidylcholine (PC) mirrors dietary EPA intake during previous weeks. Circulating EPA in ST-segment elevation myocardial infarction (STEMI) relates to smaller infarct size and preserved long-term ventricular function. Objectives The authors investigated whether serum-PC EPA (proxy for marine omega-3 consumption) levels at the time of STEMI were associated with a lower incidence of major adverse cardiovascular events (MACE), all-cause mortality, and readmission for cardiovascular (CV) causes at 3 years' follow-up. We also explored the association of alpha-linolenic acid (ALA, proxy for vegetable omega-3 intake) with all-cause mortality and MACE. Methods The authors prospectively included 944 consecutive patients with STEMI (mean age 61 years, 209 women) undergoing primary percutaneous coronary intervention. We determined serum-PC fatty acids with gas chromatography. Results During follow-up, 211 patients had MACE, 108 died, and 130 were readmitted for CV causes. A Cox proportional hazards model adjusted for known clinical predictors showed that serum-PC EPA at the time of STEMI was inversely associated with both incident MACE and CV readmission (hazard ratio [HR]: 0.76; 95% confidence interval [CI]: 0.62 to 0.94, and HR: 0.74; 95% CI: 0.58 to 0.95, respectively, for a 1-standard deviation [SD] increase). Serum-PC ALA was inversely related to all-cause mortality (HR: 0.65; 95% CI: 0.44 to 0.96, for a 1-SD increase). Conclusions Elevated serum-PC EPA and ALA levels at the time of STEMI were associated with a lower risk of clinical adverse events. Consumption of foods rich in these fatty acids might improve the prognosis of STEMI.

Key Area: Heart Health

Liu X, Guasch-Ferré M, Drouin-Chartier JP, Tobias DK, Bhupathiraju SN, Rexrode KM, Willett WC, Sun Q, Li Y. Changes in Nut Consumption and Subsequent Cardiovascular Disease Risk Among US Men and Women: 3 Large Prospective Cohort Studies.

J Am Heart Assoc. 2020;9(7):e013877. doi:10.1161/JAHA.119.013877

Abstract: Background we aim to evaluate the association of within-individual changes in consumption of total and specific types of nuts and the subsequent risk of incident cardiovascular disease (CVD) in US men and women. Methods and Results We included 34 103 men from the HPFS (Health Professionals Follow-Up Study) (1986-2012), 77 815 women from the NHS (Nurses' Health Study) (1986-2012), and 80 737 women from the NHS II (1991-2013). We assessed nut consumption every 4 years using validated food frequency questionnaires. We used multivariable Cox proportional hazards regression models to examine the association between 4-year changes in nut consumption and risk of confirmed CVD end points in the subsequent 4 years. Per 0.5 serving/day increase in total nut consumption was associated with lower risk of CVD (relative risk [RR], 0.92; 95% CI, 0.86-0.98), coronary heart disease (RR, 0.94; 95% CI, 0.89-0.99), and stroke (RR, 0.89; 95% CI, 0.83-0.95). Compared with individuals who remained nonconsumers in a 4-year interval, those who had higher consumption of total nuts (≥ 0.5 servings/day) had a lower risk of CVD (RR, 0.75; 95% CI, 0.67-0.84), coronary heart disease (RR, 0.80; 95% CI, 0.69-0.93), and stroke (RR, 0.68; 95% CI, 0.57-0.82) in next 4 years. Individuals who decreased nut consumption by ≥ 0.50 servings/day had a higher risk of developing CVD (RR, 1.14; 95% CI, 0.99-1.32), coronary heart disease (RR, 1.06; 95% CI, 0.88-1.28), and stroke (RR, 1.28; 95% CI, 1.02-1.60) when compared with those who maintained their nut consumption. Conclusions Increasing total consumption of nuts and intake of individual types of nuts (eg, walnuts, other tree nuts, and peanuts) was associated with a subsequent lower risk of CVD. These data support the role of nut intake in the primary prevention of CVD.

Key Area: Heart Health

McArthur B, Mattes, R. Energy extraction from nuts: walnuts, almonds, pistachios.

Br J Nutr. 2020;123(4):361-371. doi:10.1017/S0007114519002630

Abstract: The bioaccessibility of fat has implications for satiety and postprandial lipidemia. The prevailing view holds that the integrity of plant cell wall structure is the primary determinant of energy and nutrient extraction from plant cells as they pass through the gastrointestinal tract. However, comparisons across nuts (walnuts, almonds, pistachios) with varying physical properties do not support this view. In this study, masticated samples of three nuts from healthy adults were exposed to a static model of gastric digestion followed by simulated intestinal digestion. Primary outcomes were particle size and lipid release at each phase of digestion. Walnuts produced a significantly larger particle size post-mastication compared to almonds. Under gastric and intestinal conditions, the particle size was larger for walnuts compared to pistachios and almonds ($P < 0.05$). However, the masticated and digested particle sizes were not related to the integrity of cell walls nor lipid release. The total lipid release was comparable between nuts after the in vitro intestinal phase ($P > 0.05$). Microstructural examination showed ruptured and fissured cell walls that would allow digestion of cellular contents and this may be governed by internal cellular properties such as oil body state. Furthermore, the cell walls of walnuts tend to rupture rather than separate and as walnut tissue passes through the gastrointestinal track, lipids tend to coalesce reducing digestion efficiency.

Key Area: Body Weight and Composition

Park JM, An JM, Han YM, Surh YJ, Hwang SJ, Kim SJ, Hahm KB. Walnut polyphenol extracts inhibit *Helicobacter pylori*-induced STAT3Tyr705 phosphorylation through activation of PPAR- γ and SOCS1 induction.

J Clin Biochem Nutr. 2020 Nov;67(3):248-256. doi: 10.3164/jcbn.20-89. Epub 2020 Sep 30. PMID: 33293765; PMCID: PMC7705089.

Abstract: The health beneficial effects of walnut plentiful of n-3 polyunsaturated fatty acid had been attributed to its anti-inflammatory and anti-oxidative properties against various clinical diseases. Since we have published Fat-1 transgenic mice overexpressing 3-desaturase significantly mitigated *Helicobacter pylori* (*H. pylori*)-associated gastric pathologies including rejuvenation of chronic atrophic gastritis and prevention of gastric cancer, in this study, we have explored the underlying molecular mechanisms of walnut against *H. pylori* infection. Fresh walnut polyphenol extracts (WPE) were found to suppress the phosphorylation and nuclear translocation of signal transducer and activator of transcription 3 (STAT3) induced by *H. pylori* infection in RGM-1 gastric mucosal cells. Notably, *H. pylori* infection significantly decreased suppressor of cytokine signaling 1 (SOCS1), but WPE induced expression of SOCS1, by which the suppressive effect of walnut extracts on STAT3Tyr705 phosphorylation was not seen in SOCS1 KO cells. WPE induced significantly increased nuclear translocation of PPAR- γ in RGM1 cells, by which PPAR- γ KO inhibited transcription of SOCS1 and suppressive effect of WPE on p-STAT3Tyr705 was not seen. WPE inhibited the expression of c-Myc and IL-6/IL-6R signaling, which was attenuated in the RGM1 cells harboring SOCS1 specific siRNA. Conclusively, WPE inhibits *H. pylori*-induced STAT3 phosphorylation in a PPAR- γ and SOCS1-dependent manner.

Key Area: Cancer

Neale E, Guan V, Tapsell L, Probst YC. Effect of walnut consumption on markers of blood glucose control: A systematic review and meta-analysis.

Br J Nutr. 2020 Oct 14;124(7):641-653. doi: 10.1017/S0007114520001415. Epub 2020 Apr 21. PMID: 32312354.

Abstract: Type 2 diabetes mellitus is a chronic disease increasing in global prevalence. Although habitual consumption of walnuts is associated with reduced risk of CVD, there is inconsistent evidence for the impact of walnut consumption on markers of glycaemic control. This systematic review and meta-analysis aimed to examine the effect of walnut consumption on markers of blood glucose control. A systematic search of Medline, PubMed, CINAHL and Cochrane databases (to 2 March 2019) was

conducted. Inclusion criteria were randomised controlled trials conducted with adults which assessed the effect of walnut consumption on fasting blood glucose and insulin, glycated Hb and homeostatic model assessment of insulin resistance. Random effects meta-analyses were conducted to assess the weighted mean differences (WMD) for each outcome. Risk of bias in studies was assessed using the Cochrane Risk of Bias tool 2.0. Sixteen studies providing eighteen effect sizes were included in the review. Consumption of walnuts did not result in significant changes in fasting blood glucose levels (WMD: 0.331 mg/dl; 95 % CI -0.817, 1.479) or other outcome measures. Studies were determined to have either 'some concerns' or be at 'high risk' of bias. There was no evidence of an effect of walnut consumption on markers of blood glucose control. These findings suggest that the known favourable effects of walnut intake on CVD are not mediated via improvements in glycaemic control. Given the high risk of bias observed in the current evidence base, there is a need for further high-quality randomised controlled trials.

Key Area: Diabetes

Rinott E, Youngster I, Meir AY, Tsaban G, Zelicha H, Kaplan A, Knights D, Tuohy K, Fava F, Scholz MU, Ziv O, Reuven E, Tirosh A, Rudich A, Blüher M, Stumvoll M, Ceglarek U, Clement K, Koren O, Wang DD, Hu FB, Stampfer MJ, Shai I. Effects of Diet-Modulated Autologous Fecal Microbiota Transplantation on Weight Regain.

Gastroenterology 2021 Jan;160(1):158-173.e10. doi: 10.1053/j.gastro.2020.08.041. Epub 2020 Aug 26. PMID: 32860791; PMCID: PMC7755729.

ABSTRACT Background & Aims: We evaluated the efficacy and safety of diet-modulated autologous fecal microbiota transplantation (aFMT) for treatment of weight regain after the weight loss phase. **Methods:** In the DIRECT-PLUS weight loss trial (May 2017 through July 2018), abdominally obese or dyslipidemic participants in Israel were randomly assigned to (1) healthy dietary guidelines, (2) Mediterranean diet, and (3) green-Mediterranean diet weight-loss groups. All groups received free gym membership and physical activity guidelines. Both iso-caloric Mediterranean groups consumed 28g/day walnuts (+440mg/d polyphenols provided). The green-Mediterranean dieters further consumed green tea (3-4 cups/day) and a *Wolffia-globosa* (Mankai strain;100g/day) green shake (+800mg/day polyphenols provided). After 6 months (weight-loss phase), 90 eligible participants (mean age, 52 years; mean weight loss, 8.3 kg) provided a fecal sample that was processed into aFMT by frozen, opaque and odorless capsules. The participants were then randomly assigned to groups that received 100 capsules containing their own fecal microbiota or placebo until month 14. The primary outcome was regain of the lost weight over the expected weight regain phase (months 6–14). Secondary outcomes were gastrointestinal symptoms, waist-circumference, glycemic status and changes in the gut microbiome, as measured by metagenomic sequencing and 16s-rRNA. We validated the results in a parallel in-vivo study of mice specifically fed with Mankai, as compared to control chow diet. **Results:** Of the 90 participants in the aFMT trial, 96% ingested at least 80 of 100 oral aFMT or placebo frozen capsules over the transplantation period. No aFMT-related adverse events or symptoms were observed. For the primary outcome, although no significant differences in weight regain were observed among the participants in the different lifestyle interventions during months 6–14 (aFMT, 30.4% vs. placebo, 40.6%; $P=.28$), aFMT significantly attenuated weight regain in the green Mediterranean group (aFMT, 17.1%, vs placebo, 50%; $P=.02$), but not in the dietary guidelines ($P=.57$) or Mediterranean diet ($P=.64$) groups (P for the interaction= $.03$). Accordingly, aFMT attenuated waist circumference gain (aFMT, 1.89cm vs placebo, 5.05cm; $P=.01$) and insulin rebound (aFMT, $1.46\pm 3.6\mu\text{IU/ml}$ vs placebo, $1.64\pm 4.7\mu\text{IU/ml}$; $P=.04$) in the green Mediterranean group but not in the dietary guidelines or Mediterranean diet (P for the interaction= $.04$ and $.03$, respectively). The green-Mediterranean diet was the only intervention to induce a significant change in microbiome composition during the weight loss phase, and to prompt preservation of weight loss-associated specific bacteria and microbial metabolic pathways (mainly microbial sugar transport) following the aFMT. In mice, Mankaimodulated aFMT in

the weight loss phase, compared with control diet aFMT, significantly prevented weight regain, and resulted in better glucose tolerance, during a high-fat-diet induced regain phase ($P < .05$ for all). Conclusions: Autologous FMT, collected during the weight loss phase and administered in the regain phase, might preserve weight loss and glycemic control and is associated with specific microbiome signatures. High-polyphenols, green plant-based or Mankai diet better optimizes the microbiome for an aFMT procedure.

Key Area: Gut Health

Sala-Vila A, Valls-Pedret C, Rajaram S, Coll-Adrós N, Cofán M, Serra-Mir M, Pérez-Heras AM, Roth I, Freitas-Simoes TM, Doménech M, Calvo C, López-Illamola A, Bitok E, Buxton NK, Huey L, Arechiga A, Oda K, Lee GJ, Corella D, Vaqué-Alcázar L, Sala-Llloch R, Bartrés-Faz D, Sabaté J, Ros E. Effect of a 2-year diet intervention with walnuts on cognitive decline. The Walnuts And Healthy Aging (WAHA) study: a randomized controlled trial.

Am J Clin Nutr. 2020 ;111(3):590-600. doi:10.1093/ajcn/nqz328

Abstract: BACKGROUND: Walnut consumption counteracts oxidative stress and inflammation, 2 drivers of cognitive decline. Clinical data concerning effects on cognition are lacking. OBJECTIVES: The Walnuts And Healthy Aging study is a 2-center (Barcelona, Spain; Loma Linda, CA) randomized controlled trial examining the cognitive effects of a 2-y walnut intervention in cognitively healthy elders. METHODS: We randomly allocated 708 free-living elders (63-79 y, 68% women) to a diet enriched with walnuts at ~15% energy (30-60 g/d) or a control diet (abstention from walnuts). We administered a comprehensive neurocognitive test battery at baseline and 2 y. Change in the global cognition composite was the primary outcome. We performed repeated structural and functional brain MRI in 108 Barcelona participants. RESULTS:

A total of 636 participants completed the intervention. Besides differences in nutrient intake, participants from Barcelona smoked more, were less educated, and had lower baseline neuropsychological test scores than those from Loma Linda. Walnuts were well tolerated and compliance was good. Modified intention-to-treat analyses ($n = 657$) uncovered no between-group differences in the global cognitive composite, with mean changes of -0.072 (95% CI: -0.100, -0.043) in the walnut diet group and -0.086 (95% CI: -0.115, -0.057) in the control diet group ($P = 0.491$). Post hoc analyses revealed significant differences in the Barcelona cohort, with unadjusted changes of -0.037 (95% CI: -0.077, 0.002) in the walnut group and -0.097 (95% CI: -0.137, -0.057) in controls ($P = 0.040$). Results of brain fMRI in a subset of Barcelona participants indicated greater functional network recruitment in a working memory task in controls. CONCLUSIONS: Walnut supplementation for 2 y had no effect on cognition in healthy elders. However, brain fMRI and post hoc analyses by site suggest that walnuts might delay cognitive decline in subgroups at higher risk. These encouraging but inconclusive results warrant further investigation, particularly targeting disadvantaged populations, in whom greatest benefit could be expected.

Key Area: Cognitive Health

Sela I, Yaskolka Meir A, Brandis A, Krajmalnik-Brown R, Zeibich L, Chang D, Dirks B, Tsaban G, Kaplan A, Rinott E, Zelicha H. Wolffia globosa–Mankai Plant-Based Protein Contains Bioactive Vitamin B12 and Is Well Absorbed in Humans.

Nutrients. 2020 Oct;12(10):3067. doi: 10.3390/nu12103067

Abstract: Background: Rare plants that contain corrinoid compounds mostly comprise cobalamin analogues, which may compete with cobalamin (vitamin B12 (B12)) metabolism. We examined the presence of B12 in a cultivated strain of an aquatic plant: Wolffia globosa (Mankai), and predicted functional pathways using gut-bioreactor, and the effects of long-term Mankai consumption as a partial meat substitute, on serum B12 concentrations. Methods: We used microbiological assay, liquid-chromatography/electrospray-ionization-tandem-mass-spectrometry (LC-MS/MS), and anoxic

bioreactors for the B12 experiments. We explored the effect of a green Mediterranean/low-meat diet, containing 100 g of frozen Mankai shake/day, on serum B12 levels during the 18-month DIRECT-PLUS (ID:NCT03020186) weight-loss trial, compared with control and Mediterranean diet groups. Results: The B12 content of Mankai was consistent at different seasons ($p = 0.76$). Several cobalamin congeners (Hydroxocobalamin(OH-B12); 5-deoxyadenosylcobalamin(Ado-B12); methylcobalamin(Me-B12); cyanocobalamin(CN-B12)) were identified in Mankai extracts, whereas no pseudo B12 was detected. A higher abundance of 16S-rRNA gene amplicon sequences associated with a genome containing a KEGG ortholog involved in microbial B12 metabolism were observed, compared with control bioreactors that lacked Mankai. Following the DIRECT-PLUS intervention ($n = 294$ participants; retention-rate = 89%; baseline B12 = 420.5 ± 187.8 pg/mL), serum B12 increased by 5.2% in control, 9.9% in Mediterranean, and 15.4% in Mankai-containing green Mediterranean/low-meat diets ($p = 0.025$ between extreme groups). Conclusions: Mankai plant contains bioactive B12 compounds and could serve as a B12 plant-based food source.

Key Area: Gut Health

Son Y, Cox JM, Stevenson JL, Cooper JA, Paton CM.

Angiopoietin-1 protects 3T3-L1 preadipocytes from saturated fatty acid-induced cell death.

Nutr Res. 2020;76:20-28. doi:10.1016/j.nutres.2020.02.007

Abstract: Cross talk between endothelial cells and adipocytes is vital to adipocyte functions, but little is known about the mechanisms or factors controlling the process. Angiogenesis is a critical component linking the endothelium to healthy adipogenesis, yet it is not known if or how it is involved in adipocyte physiology. Therefore, the purpose of this study was to determine the effect of angiopoietin-1 (Ang-1) and -2 (Ang-2) as well as their receptor, Tie-2, on adipocyte physiology. 3T3-L1 pre- and mature adipocytes were found to express Ang-1, Ang-2, and Tie-2, which decrease upon polyunsaturated fatty acid treatment. Furthermore, 3T3-L1 cells treated with recombinant Ang-1 or Ang-2 increased expression of the antiapoptotic gene Bcl-x and decreased expression of the proapoptotic gene Casp-8. Next, preadipocytes were treated with saturated fatty acids (SFAs) to induce cell stress. SFA-mediated splicing of X-box-binding protein-1 was reduced by co-treatment with Ang-1, and cell viability was improved in the presence of SFAs + Ang-1. Taken together, these results indicate that Ang-1 may protect preadipocytes from SFA-induced apoptosis and endoplasmic reticulum stress.

Key Area: Heart Health

Steffen LM, Yi SY, Duprez D, Zhou X, Shikany JM, Jacobs Jr DR. Walnut Consumption and Cardiac Phenotypes: the Coronary Artery Risk Development in Young Adults(CARDIA) Study.

Nutr Metab Cardiovasc Dis. 2020 Sep 5;S0939-4753(20)30381-1. doi: 10.1016/j.numecd.2020.09.001. Online ahead of print.

Abstract: Background and Aims. Observational studies and clinical trials have shown cardiovascular benefits of nut consumption, including walnuts. However, the relations of walnut consumption with systolic and diastolic function, risk factors for heart failure, are unknown. We examined the associations of walnut consumption with cardiac structure and function parameters in black and white adults enrolled in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Methods and Results. After exclusions, the study population included 3,341 participants. Dietary intake was assessed using the CARDIA Diet History questionnaire at baseline, year 7 and year 20 exams. Cardiac structure and function were measured by echocardiography at year 25. Multivariable linear regression evaluated the associations of walnut consumption with blood pressure (BP), heart rate, and cardiac phenotypes, adjusting for age, sex, race, lifestyle habits, and clinical characteristics. We found the majority of walnut consumers compared to non-consumers were females, whites, and more highly educated, and had lower waist circumference, diastolic BP, and heart rate, and higher diet quality score. Even though cardiac

structure and function measures were generally within normal ranges among participants, walnut consumers had significantly better values for diastolic function parameters A wave, E/A ratio, septal and lateral e' than non-consumers. Further adjustment for body mass index and diabetes status did not materially change the significance between walnut consumer groups. Systolic function parameters did not differ by walnut group. Conclusion. Compared to non-consumers, walnut consumption is associated with better diastolic dysfunction in young to middle-aged adults.

Key Area: Heart Health

Tindall AM, Kris-Etherton PM, Petersen KS. Replacing Saturated Fats with Unsaturated Fats from Walnuts or Vegetable Oils Lowers Atherogenic Lipoprotein Classes Without Increasing Lipoprotein(a).

J Nutr. 2020;150(4):818-825. doi:10.1093/jn/nxz313

Abstract: BACKGROUND: Walnuts have established lipid-/lipoprotein-lowering properties; however, their effect on lipoprotein subclasses has not been investigated. Furthermore, the mechanisms by which walnuts improve lipid/lipoprotein concentrations are incompletely understood. OBJECTIVES: We aimed to examine, as exploratory outcomes of this trial, the effect of replacing SFAs with unsaturated fats from walnuts or vegetable oils on lipoprotein subclasses, cholesterol efflux, and proprotein convertase subtilisin/kexin type 9 (PCSK9). METHODS: A randomized, crossover, controlled-feeding study was conducted in individuals at risk of cardiovascular disease (CVD) (n = 34; 62% men; mean \pm SD age 44 ± 10 y; BMI: 30.1 ± 4.9 kg/m²). After a 2-wk run-in diet (12% SFAs, 7% PUFAs, 12% MUFAs), subjects consumed the following diets, in randomized order, for 6 wk: 1) walnut diet (WD) [57-99 g/d walnuts, 7% SFAs, 16% PUFAs (2.7% α -linolenic acid (ALA)), 9% MUFAs]; 2) walnut fatty acid-matched diet [7% SFAs, 16% PUFAs (2.6% ALA), 9% MUFAs]; and 3) oleic acid replaces ALA diet (ORAD) [7% SFAs, 14% PUFAs (0.4% ALA); 12% MUFAs] (all percentages listed are of total kilocalories). Serum collected after the run-in (baseline) and each diet period was analyzed for lipoprotein classes and subclasses (vertical auto profile), cholesterol efflux, and PCSK9. Linear mixed models were used for data analysis. RESULTS: Compared with the ORAD, total cholesterol (mean \pm SEM -8.9 ± 2.3 mg/dL; -5.1% ; $P < 0.001$), non-HDL cholesterol (-7.4 ± 2.0 mg/dL; -5.4% ; $P = 0.001$), and LDL cholesterol (-6.9 ± 1.9 mg/dL; -6.5% ; $P = 0.001$) were lower after the WD; no other pairwise differences existed. There were no between-diet differences for HDL-cholesterol or LDL-cholesterol subclasses. Lipoprotein(a) [Lp(a)], cholesterol efflux, and PCSK9 were unchanged after the diets. CONCLUSIONS: In individuals at risk of CVD, replacement of SFAs with unsaturated fats from walnuts or vegetable oils improved lipid/lipoprotein classes, including LDL-cholesterol, non-HDL cholesterol, and total cholesterol, without an increase in Lp(a). These improvements were not explained by changes in cholesterol efflux capacity or PCSK9.

Key Area: Heart Health

Tindall AM, McLimans CJ, Petersen KS, Kris-Etherton PM, Lamendella R. Walnuts and Vegetable Oils Containing Oleic Acid Differentially Affect the Gut Microbiota and Associations with Cardiovascular Risk Factors: Follow-up of a Randomized, Controlled, Feeding Trial in Adults at Risk for Cardiovascular Disease.

J Nutr. 2020;150(4):806-817. doi:10.1093/jn/nxz289

Abstract: BACKGROUND: It is unclear whether the favorable effects of walnuts on the gut microbiota are attributable to the fatty acids, including α -linolenic acid (ALA), and/or the bioactive compounds and fiber. OBJECTIVE: This study examined between-diet gut bacterial differences in individuals at increased cardiovascular risk following diets that replace SFAs with walnuts or vegetable oils. METHODS: Forty-two adults at cardiovascular risk were included in a randomized, crossover, controlled-feeding trial that provided a 2-wk standard Western diet (SWD) run-in and three 6-wk isocaloric study diets: a diet containing whole walnuts (WD; 57-99 g/d walnuts; 2.7% ALA), a fatty acid-matched diet devoid of walnuts (walnut fatty acid-matched diet; WFMD; 2.6% ALA), and a diet replacing ALA with oleic acid without walnuts (oleic acid replaces ALA diet; ORAD; 0.4% ALA). Fecal samples were collected following the run-in and study diets to assess gut microbiota with 16S rRNA sequencing and Qiime2 for amplicon sequence variant picking. RESULTS: Subjects had elevated BMI (30 ± 1 kg/m²), blood pressure ($121 \pm 2/77 \pm 1$ mmHg), and LDL cholesterol (120 ± 5 mg/dL). Following the WD, Roseburia [relative abundance (RA) = 4.2%, linear discriminant analysis (LDA) = 4], Eubacterium eligensgroup (RA = 1.4%, LDA = 4), LachnospiraceaeUCG001 (RA = 1.2%, LDA = 3.2), Lachnospiraceae UCG004 (RA = 1.0%, LDA = 3), and Leuconostocaceae (RA = 0.03%, LDA = 2.8) were most abundant relative to taxa in the SWD ($P \leq 0.05$ for all). The WD was also enriched in Gordonibacter relative to the WFMD. Roseburia (3.6%, LDA = 4) and Eubacterium eligensgroup (RA = 1.5%, LDA = 3.4) were abundant following the WFMD, and Clostridialesvadin BB60group (RA = 0.3%, LDA = 2) and gutmetagenome (RA = 0.2%, LDA = 2) were most abundant following the ORAD relative to the SWD ($P \leq 0.05$ for all). Lachnospiraceae were inversely correlated with blood pressure and lipid/lipoprotein measurements following the WD. CONCLUSIONS:

The results indicate similar enrichment of Roseburia following the WD and WFMD, which could be explained by the fatty acid composition. Gordonibacter enrichment and the inverse association between Lachnospiraceae and cardiovascular risk factors following the WD suggest that the gut microbiota may contribute to the health benefits of walnut consumption in adults at cardiovascular risk. This trial was registered at clinicaltrials.gov as NCT02210767.

Key Area: Gut Health

Tsaban G, Yaskolka Meir A, Rinott E, Zelicha H, Kaplan A, Shalev A, Katz A, Rudich A, Tirosh A, Shelef I, Youngster I, Lebovitz S, Israeli N, Shabat M, Brikner D, Pupkin E, Stumvoll M, Thiery J, Ceglarek U, Heiker JT, Körner A, Landgraf K, von Bergen M, Blüher M, Stampfer MJ, Shai I. Effect of green Mediterranean diet on cardiometabolic risk; a randomised controlled trial

Heart. 2020 Nov 23;heartjnl-2020-317802. doi: 10.1136/heartjnl-2020-317802. Epub ahead of print. PMID: 33234670.

Abstract: Background A Mediterranean diet is favourable for cardiometabolic risk. Objective To examine the residual effect of a green Mediterranean diet, further enriched with green plant-based foods and lower meat intake, on cardiometabolic risk. Methods For the DIRECT-PLUS parallel, randomised clinical trial we assigned individuals with abdominal obesity/dyslipidaemia 1:1:1 into three diet groups: healthy dietary guidance (HDG), Mediterranean and green Mediterranean diet, all combined with physical activity. The Mediterranean diets were equally energy restricted and included 28 g/day walnuts. The green Mediterranean diet further included green tea (3–4 cups/day) and a Wolffia globosa (Mankai strain; 100 g/day frozen cubes) plant-based protein shake, which partially substituted animal protein. We examined the effect of the 6-month dietary induction weight loss phase on cardiometabolic state. Results Participants (n=294; age 51 years; body mass index 31.3 kg/m²; waist circumference 109.7 cm; 88% men; 10 year Framingham risk score 4.7%) had a 6-month retention rate of 98.3%. Both Mediterranean diets achieved similar weight loss ((green Mediterranean –6.2 kg; Mediterranean –5.4 kg) vs the HDG group –1.5 kg; p<0.001), but the green Mediterranean group had a greater reduction in waist circumference (–8.6 cm) than the Mediterranean (–6.8 cm; p=0.033) and HDG (–4.3 cm; p<0.001) groups. Stratification by gender showed that these differences were significant only among men. Within 6 months the green Mediterranean group achieved greater decrease in low-density lipoprotein cholesterol (LDL-C; green Mediterranean –6.1 mg/dL (–3.7%), –2.3 (–0.8%), HDG –0.2 mg/dL (+1.8%); p=0.012 between extreme groups), diastolic blood pressure (green Mediterranean –7.2 mm Hg, Mediterranean –5.2 mm Hg, HDG –3.4 mm Hg; p=0.005 between extreme groups), and homeostatic model assessment for insulin resistance (green Mediterranean –0.77, Mediterranean –0.46, HDG –0.27; p=0.020 between extreme groups). The LDL-C/high-density lipoprotein cholesterol (HDL-C) ratio decline was greater in the green Mediterranean group (–0.38) than in the Mediterranean (–0.21; p=0.021) and HDG (–0.14; p<0.001) groups. High-sensitivity C-reactive protein reduction was greater in the green Mediterranean group (–0.52 mg/L) than in the Mediterranean (–0.24 mg/L; p=0.023) and HDG (–0.15 mg/L; p=0.044) groups. The green Mediterranean group achieved a better improvement (–3.7% absolute risk reduction) in the 10-year Framingham Risk Score (Mediterranean –2.3%; p=0.073, HDG –1.4%; p<0.001). Conclusions The green MED diet, supplemented with walnuts, green tea and Mankai and lower in meat/poultry, may amplify the beneficial cardiometabolic effects of Mediterranean diet.

Key Area: Heart Health

2019

Arab L, Guo R, Elashoff D. Lower depression scores among walnut consumers in NHANES.

Nutrients. 2019 Jan 26;11(2):275. doi: 10.3390/nu11020275.

Abstract: Background: Multiple studies have shown a Mediterranean diet, characterized by their olive oil and nut consumption, to be correlated with lower depression risk. Objective: To examine whether part of this reduced risk in the United States is attributable to walnut consumption, we analyzed data on walnut consumption and depression scores from the National Health and Nutrition Examination Survey (NHANES). Methods: NHANES survey data for 2005 through 2014 was pooled for adults with 24 h recall dietary data. Depression scores were based on PHQ-9 self-report responses. A total of 26,656 participants were characterized as reporting the consumption of walnuts with

high certainty, walnuts with other nuts, other nuts, or no nuts. Results: After an adjustment for covariates, walnut consumers showed lower depression scores compared to non-nut consumers. The least square mean for total depression score was 26% lower for walnut with high certainty consumers than for non-nut consumers ($p < 0.0001$), and the association was stronger among women (32%, $p < 0.0001$) than men (21%, $p = 0.05$). The significant contributors to this difference were due to walnut consumers reporting greater interest in doing things ($p = 0.003$), less hopelessness ($p = 0.02$), and feeling more energetic ($p = 0.05$) than non-nut consumers. Non-nut consumers were more likely to have trouble concentrating ($p = 0.02$), to feel they were moving or speaking abnormally ($p = 0.03$), and to have thought they were better off dead ($p = 0.002$). Conclusion: Depression scores were significantly lower among nut consumers and particularly walnut consumers as compared to non-nut consumers. After controlling for potential covariates, walnut users had scores significantly lower than other nut consumers. The difference was strongest among women, who are more likely than men to report higher depression scores.

Key Area: Cognitive Health

Borkowski K, Yim SJ, Holt RR, Hackman RM, Keen CL, Newman JW, Shearer GC. Walnuts change lipoprotein composition suppressing TNF α -stimulated cytokine production by diabetic adipocyte.

J Nutr Biochem. 2019 Jun;68:51-58. doi: 10.1016/j.jnutbio.2019.03.004.

Abstract: Walnut consumption can provide both vascular and metabolic health benefits, and walnut-induced changes in lipoprotein particle chemical payloads may be responsible for these health benefits. To explore this possibility with a focus on metabolic health, this study investigated the impact of walnut consumption on lipoprotein lipid composition and changes in LDL anti-inflammatory properties, as reported by inflamed adipocyte. Hypercholesterolemic, postmenopausal females were treated with 40 g/day (i.e., 1.6 servings/day; $n=15$) of walnuts for 4 weeks. Fatty acids and their oxygenated metabolites, i.e., oxylipins, were quantified in isolated lipoproteins. Human primary adipocytes were exposed to LDL and TNF α -stimulated adipokine production was measured. Walnut treatment elevated α -linolenic acid and its epoxides in all lipoproteins and depleted mid-chain alcohols in VLDL and LDL, but not HDL. Walnuts also reduced TNF α -induced diabetic adipocyte production of IL-6 (−48%, $P=.0006$) and IL-8 (−30%, $P=.01$), changes inversely correlated with levels of α -linolenic acid-derived epoxides but not α -linolenic acid itself. In conclusion, modest walnut consumption can alter lipoprotein lipid profiles and enhance their ability to inhibit TNF α -dependent pro-inflammatory responses in human diabetic primary adipocytes. Moreover, this study suggests the oxylipins, rather than the parent fatty acids, mediate LDL action of adipocytes.

Key Area: Heart Health

Choi J, Shin PK, Kim Y, Hong CP, Choi SW. Metabolic influence of walnut phenolic extract on mitochondria in a colon cancer stem cell model.

Eur J Nutr. 2019;58(4):1635-1645. doi:10.1007/s00394-018-1708-z

Abstract: PURPOSE: Walnut phenolic extract (WPE) reduces proliferation and enhances differentiation of colon cancer stem cells (CSCs). The present study investigated the metabolic influence of WPE on the mitochondrial function of colon CSCs to determine its underlying mechanism. METHODS: CD133+CD44+ HCT116 colon cancer cells were selected by fluorescence-activated cell sorting and were treated with or without 40 μ g/mL WPE. RNA-sequencing (RNA-Seq) was performed to identify differentially expressed genes (DEGs), which were further validated with RT-PCR. WPE-induced alterations in mitochondrial function were investigated through a mitochondrial stress test by determining cellular oxygen consumption rate (OCR), an indicator of mitochondrial respiration, and extracellular acidification rate (ECAR), an indicator of glycolysis, which were further confirmed by glucose uptake and lactate production tests. RESULTS: RNA-Seq analysis identified two major functional clusters: metabolic and mitochondrial clusters. WPE treatment shifted the metabolic profile of cells towards the glycolysis pathway (Δ ECAR = 36.98 mpH/min/ptn, $p = 0.02$) and oxidative pathway (Δ OCR = 29.18 pmol/min/ptn, $p = 0.00001$). Serial mitochondrial stimulations using respiration modulators, oligomycin, carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone, and rotenone/antimycin A, found an increased potential of mitochondrial respiration (Δ OCR = 111.5 pmol/min/ptn, $p = 0.0006$). WPE treatment also increased glucose uptake ($\Delta = 0.39$ pmol/ μ L, $p = 0.002$) and lactate production ($\Delta = 0.08$ nmol/ μ L, $p = 0.005$). CONCLUSIONS: WPE treatment shifts the

mitochondrial metabolism of colon CSC towards more aerobic glycolysis, which might be associated with the alterations in the characteristics of colon CSC.

Key Area: Cancer

Domènech M, Serra-Mir M, Roth I, Freitas-Simoes T, Valls-Pedret C, Cofán M, López A, Sala-Vila A, Calvo C, Rajaram S, Sabaté J. Effect of a walnut diet on office and 24-hour ambulatory blood pressure in elderly individuals: findings From the WAHA randomized trial.

Hypertension. 2019;73(5):1049-1057. doi:10.1161/HYPERTENSIONAHA.118.12766

Abstract: Nut consumption lowers blood cholesterol and is associated with reduced cardiovascular disease, but effects on blood pressure (BP) are inconsistent. We assessed the 2-year effects of a walnut diet versus a control diet on office BP and 24-hours ambulatory BP in free-living elders participating in the Walnuts and Healthy Aging study, a randomized trial testing the effects of walnuts at $\approx 15\%$ energy on age-related disorders. In a prespecified analysis, we enrolled 305 participants, of whom 236 (75%) completed the study (65% women; age, 69 years; 60% with mild hypertension). Walnuts were well tolerated, and compliance was $>98\%$. Mean baseline office BP was 128/79 mm Hg. Adjusted changes from baseline in mean office systolic BP were -4.61 mm Hg (95% CI, -7.43 to -1.79 mm Hg) in the walnut group and -0.59 mm Hg (-3.38 to 2.21 mm Hg) in controls ($P=0.051$). Respective changes in mean systolic 24-hour ambulatory BP were -3.86 mm Hg (CI, -5.45 to -2.26 mm Hg) and -2.00 mm Hg (CI, -3.58 to -0.42 mm Hg; $P=0.111$). No changes in diastolic BP were observed. In participants in the upper tertile of baseline 24-hour ambulatory systolic BP (>125 mm Hg), mean 2-year systolic 24-hour BP was -8.5 mm Hg (CI, -12 to -5.0 mm Hg) in the walnut group and -2.5 mm Hg (CI, -6.3 to 1.3 mm Hg) in controls ($P=0.034$). During the trial, participants in the walnut group required less uptitration of antihypertensive medication and had better overall BP regulation than controls. Walnut consumption reduces systolic BP in elderly subjects, particularly in those with mild hypertension.

Key Area: Heart Health

Gepner Y, Shelef I, Komy O, Cohen N, Schwarzfuchs D, Bril N, Rein M, Serfaty D, Kenigsbuch S, Zelicha H, Meir AY. The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content.

J Hepatol. 2019;71(2):379-388. doi:10.1016/j.jhep.2019.04.013

Abstract: BACKGROUND & AIM: It is unclear if a reduction in hepatic fat content (HFC) is a major mediator of the cardiometabolic benefit of lifestyle intervention, and whether it has prognostic significance beyond the loss of visceral adipose tissue (VAT). In the present sub-study, we hypothesized that HFC loss in response to dietary interventions induces specific beneficial effects independently of VAT changes. METHODS: In an 18-month weight-loss trial, 278 participants with abdominal obesity/dyslipidemia were randomized to low-fat (LF) or Mediterranean/low-carbohydrate (MED/LC + 28 g walnuts/day) diets with/without moderate physical activity. HFC and abdominal fat-depots were measured using magnetic resonance imaging at baseline, after 6 (sub-study, $n = 158$) and 18 months. RESULTS: Of 278 participants (mean HFC 10.2% [range: 0.01%-50.4%]), the retention rate was 86.3%. The %HFC substantially decreased after 6 months (-6.6% absolute units [-41% relatively]) and 18 months (-4.0% absolute units [-29% relatively]; $p < 0.001$ vs. baseline). Reductions of HFC were associated with decreases in VAT beyond weight loss. After controlling for VAT loss, decreased %HFC remained independently associated with reductions in serum gamma glutamyltransferase and alanine aminotransferase, circulating chemerin, and glycated hemoglobin ($p < 0.05$). While the reduction in HFC was similar between physical activity groups, MED/LC induced a greater %HFC decrease ($p = 0.036$) and greater improvements in cardiometabolic risk parameters ($p < 0.05$) than the LF diet, even after controlling for VAT changes. Yet, the greater improvements in cardiometabolic risk parameters induced by MED/LC were all markedly attenuated when controlling for HFC changes. CONCLUSIONS: %HFC is substantially reduced by diet-induced moderate weight loss and is more effectively reduced by the MED/LC diet than the LF diet, independently of VAT changes. The beneficial effects of the MED/LC diet on specific cardiometabolic parameters appear to be mediated more by decreases in %HFC than VAT loss. LAY SUMMARY: High hepatic fat content is associated with metabolic syndrome, type 2 diabetes mellitus, and coronary heart disease. In the CENTRAL 18-month intervention trial, a Mediterranean/low-carbohydrate diet induced a greater decrease in hepatic fat content than a low-fat diet, conferring beneficial health effects that were beyond the favorable effects of visceral fat loss. ClinicalTrials.gov Identifier: NCT01530724.

Key Area: Body Weight and Composition

H Al Wattar B, Dodds J, Placzek A, Beresford L, Spyrelli E, Moore A, Carreras FJ, Austin F, Murugesu N, Roseboom TJ, Bes-Rastrollo M. Mediterranean-style diet in pregnant women with metabolic risk factors (ESTEEM): A pragmatic multicentre randomised trial.

PLoS Med. 2019 Jul 23;16(7):e1002857. doi: 10.1371/journal.pmed.1002857. PMID: 31335871; PMCID: PMC6650045.

Abstract: Background Pregnant women with metabolic risk factors are at high risk of complications. We aimed to assess whether a Mediterranean-style diet reduces adverse pregnancy outcomes in high-risk women. Methods and findings We conducted a multicentre randomised trial in 5 maternity units (4 in London and 1 in Birmingham) between 12 September 2014 and 29 February 2016. We randomised inner-city pregnant women with metabolic risk factors (obesity, chronic hypertension, or hypertriglyceridaemia) to a Mediterranean-style diet with high intake of nuts, extra virgin olive oil, fruits, vegetables, nonrefined grains, and legumes; moderate to high consumption of fish; low to moderate intake of poultry and dairy products; low intake of red and processed meat; and avoidance of sugary drinks, fast food, and food rich in animal fat versus usual care. Participants received individualised dietary advice at 18, 20, and 28 weeks' gestation. The primary endpoints were composite maternal (gestational diabetes or preeclampsia) and composite offspring (stillbirth, small for gestational age, or admission to neonatal care unit) outcomes prioritised by a Delphi survey. We used an intention-to-treat (ITT) analysis with multivariable models and identified the stratification variables and prognostic factors a priori. We screened 7,950 and randomised 1,252 women. Baseline data were available for 593 women in the intervention (93.3% follow-up, 553/593) and 612 in the control (95.6% follow-up, 585/612) groups. Over a quarter of randomised women were primigravida (330/1,205; 27%), 60% (729/1,205) were of Black or Asian ethnicity, and 69% (836/1,205) were obese. Women in the intervention arm consumed more nuts (70.1% versus 22.9%; adjusted odds ratio [aOR] 6.8, 95% confidence interval [CI] 4.3–10.6, $p \leq 0.001$) and extra virgin olive oil (93.2% versus 49.0%; aOR 32.2, 95% CI 16.0–64.6, $p \leq 0.001$) than controls; increased their intake of fish ($p < 0.001$), white meat ($p < 0.001$), and pulses ($p = 0.05$); and reduced their intake of red meat ($p < 0.001$), butter, margarine, and cream ($p < 0.001$). There was no significant reduction in the composite maternal (22.8% versus 28.6%; aOR 0.76, 95% CI 0.56–1.03, $p = 0.08$) or composite offspring (17.3% versus 20.9%; aOR 0.79, 95% CI 0.58–1.08, $p = 0.14$) outcomes. There was an apparent reduction in the odds of gestational diabetes by 35% (aOR 0.65, 95% CI 0.47–0.91, $p = 0.01$) but not in other individual components of the composite outcomes. Mothers gained less gestational weight (mean 6.8 versus 8.3 kg; adjusted difference -1.2 Kg, 95% CI -2.2 to -0.2 , $p = 0.03$) with intervention versus control. There was no difference in any of the other maternal and offspring complications between both groups. When we pooled findings from the Effect of Simple, Targeted Diet in Pregnant Women With Metabolic Risk Factors on Pregnancy Outcomes (ESTEEM) trial with similar trials using random effects meta-analysis, we observed a significant reduction in gestational diabetes (odds ratio [OR] 0.67, 95% CI 0.53–0.84, $I^2 = 0\%$), with no heterogeneity (2 trials, 2,397 women). The study's limitations include the use of participant reported tools for adherence to the intervention instead of objective biomarkers. Conclusions A simple, individualised, Mediterranean-style diet in pregnancy did not reduce the overall risk of adverse maternal and offspring complications but has the potential to reduce gestational weight gain and the risk of gestational diabetes.

Key Area: Reproductive Health

Hagan KA, Grodstein F. The Alternative Healthy Eating Index and physical function impairment in men.

J Nutr Health Aging. 2019;23(5):459-465. doi:10.1007/s12603-019-1185-y

Abstract: Objectives Physical function is increasingly recognized as integral to healthy aging, in particular as a core component of mobility and independent living in older adults. Thus, it is important to identify strategies for the prevention of physical function decline. Design Longitudinal cohort study.

Setting and Participants A total of 12,658 men from the Health Professionals Follow-Up Study were followed from 2008–2012. **Measurements** We examined the association between the Alternative Healthy Eating Index-2010 (AHEI), a measure of diet quality combining 11 dietary components (vegetables, fruits, nuts and legumes, red and processed meats, sugar-sweetened beverages and fruit juices, alcohol, whole grains, omega-3 fatty acids, polyunsaturated fatty acids, trans fatty acids, sodium), and impairment in physical function, as measured by the SF-36. Multivariable logistic regression models were used to estimate the odds ratios (OR) and 95% confidence intervals (CI) of impairment in physical function. **Results** In the multivariable-adjusted model, each 10-point increase in total AHEI score was associated with a 10% lower odds of impairment in physical function (OR=0.90, 95% CI: 0.86,0.95), and in the categorical analysis, men with AHEI scores in the top quintile had a 26% lower odds (OR=0.74, 95% CI:0.63,0.86) compared with men in the bottom quintile. For individual AHEI components, higher intake of vegetables (p-trend=0.01), nuts and legumes (p-trend<0.01), polyunsaturated fatty acids (p-trend<0.01) and lower intake of red and processed meats (p-trend=0.03) and sugar-sweetened beverages (p-trend=0.01) were significantly associated with lower odds of physical impairment. For specific foods, higher consumption of lettuce, broccoli, blueberries, peanuts, walnuts and other nuts were associated with lower odds of impairment. **Conclusions** In this large cohort of older men, better overall diet quality was significantly associated with a lower odds of impairment in physical function. Given the value of physical function to healthy aging and quality of life, this may represent a particularly compelling public health rationale for older men to improve their diet.

Key Area: Cognitive Health

Hardman WE, Primerano DA, Legenza MT, Morgan J, Fan J, Denvir J. Dietary walnut altered gene expressions related to tumor growth, survival, and metastasis in breast cancer patients: a pilot clinical trial.

Nutr Res. 2019 Jun;66:82-94. doi: 10.1016/j.nutres.2019.03.004

Abstract: Consumption of walnuts has slowed breast cancer growth and/or reduced the risk of mammary cancer in mice. The benefit against cancer was associated with altered expression of genes for cancer growth and survival. We hypothesized that walnut consumption would alter gene expression in pathologically confirmed breast cancers of women in a direction that would be expected to decrease breast cancer growth and survival, as was seen in mice. The study was a non-placebo, two-arm, clinical trial. Women with breast lumps large enough for research and pathology biopsies were recruited and randomized to walnut consuming or control groups. Immediately after biopsy collection, women in the walnut group began to consume two ounces of walnuts per day until follow-up surgery. Pathological studies confirmed that lumps were breast cancer in all women who remained in the trial. At surgery, about two weeks after biopsy, additional specimens were taken from the breast cancers. Changes in gene expression in the surgical specimen compared to baseline were determined in each individual woman in walnut-consuming (n=5) and control (n=5) groups. RNA-Seq expression profiling revealed that expression of 456 identified genes was significantly changed in the tumor due to walnut consumption. Ingenuity Pathway Analysis showed activation of pathways that promote apoptosis and cell adhesion, and inhibition of pathways that promote cell proliferation and migration. These results support the hypothesis that, in humans, walnut consumption could suppress growth and survival of breast cancers.

Key Area: Cancer

Hardman WE, Primerano DA, Legenza MT, Morgan J, Fan J, Denvir J. mRNA expression data in breast cancers before and after consumption of walnut by women.

Data in Brief. 2019 May 23;25:104050. doi: 10.1016/j.dib.2019.104050

Abstract: This article contains supporting data for the research paper entitled: ‘Dietary walnut altered gene expressions related to tumor growth, survival, and metastasis in breast cancer patients: a pilot clinical trial’ [1] Hardman et al., 2019. Included are tables for all mapped genes and all unmapped loci

identifications that were significantly changed in breast cancers by consumption of walnut for about 2 weeks. All gene networks that were identified by Ingenuity Pathway Analyses as modified are shown in table 3. Files containing the raw reads, along with a shell script describing the complete data analysis pipeline, were deposited to the Gene Expression Omnibus (GEO) at the National Center for Biotechnology Information (NCBI) and can be obtained via accession number GSE111073.

Key Area: Cancer

Hwang HJ, Liu Y, Kim HS, Lee H, Lim Y, Park H. Daily walnut intake improves metabolic syndrome status and increases circulating adiponectin levels: randomized controlled crossover trial.

Nutr Res Pract. 2019 Apr;13(2):105–114. doi: 10.4162/nrp.2019.13.2.105

Abstract: BACKGROUND/OBJECTIVES: Several previous studies have investigated whether regular walnut consumption positively changes heart-health-related parameters. The aim of this study was to investigate the effects of daily walnut intake on metabolic syndrome (MetS) status and other metabolic parameters among subjects with MetS. MATERIALS/METHODS: This study was a two-arm, randomized, controlled crossover study with 16 weeks of each intervention (45 g of walnuts or iso-caloric white bread) with a 6 week washout period between interventions. Korean adults with MetS (n = 119) were randomly assigned to one of two sequences; 84 subjects completed the trial. At each clinic visit (at 0, 16, 22, and 38 weeks), MetS components, metabolic parameters including lipid profile, hemoglobin A1c (HbA1c), adiponectin, leptin, and apolipoprotein B, as well as anthropometric and bioimpedance data were obtained. RESULTS: Daily walnut consumption for 16 weeks improved MetS status, resulting in 28.6%-52.8% reversion rates for individual MetS components and 51.2% of participants with MetS at baseline reverted to a normal status after the walnut intervention. Significant improvements after walnut intake, compared to control intervention, in high-density lipoprotein cholesterol (HDL-C) (P = 0.028), fasting glucose (P = 0.013), HbA1c (P = 0.021), and adiponectin (P = 0.019) were observed after adjustment for gender, age, body mass index, and sequence using a linear mixed model. CONCLUSION: A dietary supplement of 45 g of walnuts for 16 weeks favorably changed MetS status by increasing the concentration of HDL-C and decreasing fasting glucose level. Furthermore, consuming walnuts on a daily basis changed HbA1c and circulating adiponectin levels among the subjects with MetS.

Key Area: Metabolic Syndrome

Kaviani S, Taylor CM, Stevenson JL, Cooper JA, Paton CM. A 7-day high-PUFA diet reduces angiotensin-like protein 3 and 8 responses and postprandial triglyceride levels in healthy females but not males: a randomized control trial.

BMC Nutr. 2019 Jan 6;5(1). doi:10.1186/s40795-018-0262-7.

Abstract: Background Polyunsaturated fatty acids (PUFAs) have beneficial effects on hypertriglyceridemia although their effect on angiotensin-like proteins (ANGPTLs), specifically ANGPTL3, ANGPTL4 and ANGPTL8 is unknown. Objective: To determine whether a high-PUFA diet improves postprandial triglyceride (TG) levels through reducing ANGPTL responses following high saturated fat (SFA) meals. Methods: Twenty-six adults were randomized into a PUFA diet (n = 16) or a control diet group (n = 10). Participants completed a pre-diet visit (v1) where they were given two SFA-rich, high-fat meals. Blood draws were taken at fasting and every 2 h postprandially for a total of 8 h. After v1, participants completed a 7d diet of the same macronutrient proportions (50% carbohydrate, 35% fat, 15% protein) but with different fatty acid (FA) compositions (PUFA = 21% of total energy from PUFAs vs. Control = 7% of total energy from PUFA). All participants then completed the post-diet visit (v2) identical to v1. Results: In the PUFA group, females, but not males, reduced TG concentrations (Area under the curve (AUC): 141.2 ± 18.7 vs. 80.7 ± 6.5 mg/dL/h, p = 0.01, for v1 vs. v2, respectively). Fasting and postprandial AUC levels of ANGPTL3 and 8, but not ANGPTL4, also

decreased from v1 to v2 in PUFA females, but not males. No changes from v1 to v2 were seen in either sex in the control group. Conclusions: A PUFA-rich diet improves TG levels in response to high-SFA meals with reductions in ANGPTL3 and ANGPTL8. PUFAs may be more protective against hypertriglyceridemia in females, compared to males since no diet effect was observed in males.

Key Area: Heart Health

Koh SJ, Choi YI, Kim Y, Kim YS, Choi SW, Kim JW, Kim BG, Lee KL. Walnut phenolic extract inhibits nuclear factor kappaB signaling in intestinal epithelial cells, and ameliorates experimental colitis and colitis-associated colon cancer in mice.

Eur J Nutr. 2019;58(4):1603-1613. doi:10.1007/s00394-018-1704-3

Abstract: **PURPOSE:** Walnuts (*Juglans regia*) are known to have anti-cancer and immunomodulatory effects. However, little information is available on the effects of walnut phenolic extract (WPE) on intestinal inflammation and colitis-associated colon cancer. **METHODS:** COLO205 cells were pretreated with WPE and then stimulated with tumor necrosis factor (TNF)- α . In the acute colitis model, wild type mice (C57BL/6) were administered 4% dextran sulfate sodium (DSS) for 5 days. In the chronic colitis model, interleukin (IL)-10-/- mice were administered with either the vehicle or WPE (20 mg/kg) by oral gavage daily for 2 weeks. In an inflammation-associated tumor model, wild type mice were administered a single intraperitoneal injection of azoxymethane followed by three cycles of 2% DSS for 5 days and 2 weeks of free water consumption. **RESULTS:** WPE significantly inhibited IL-8 and IL-1 α expression in COLO205 cells. WPE attenuated both the TNF- α -induced I κ B phosphorylation/degradation and NF- κ B DNA binding activity. The administration of oral WPE significantly reduced the severity of colitis in both acute and chronic colitis models, including the IL-10-/- mice. In immunohistochemical staining, WPE attenuated NF- κ B signaling in the colons of both colitis models. Finally, WPE also significantly reduced tumor development in a murine model of colitis-associated colon cancer (CAC). **CONCLUSIONS:** WPE ameliorates acute and chronic colitis and CAC in mice, suggesting that WPE may have potentials for the treatment of inflammatory bowel disease.

Key Area: Cancer

Liu X, Li Y, Guasch-Ferré M, Willett WC, Drouin-Chartier JP, Bhupathiraju SN, Tobias DK. Changes in nut consumption influence long-term weight change in US men and women.

BMJ Nutr Prev Health. 2019 Sep 23;2(2):90-99. doi: 10.1136/bmjnp-2019-000034. PMID: 33235963; PMCID: PMC7664489.

Abstract: Background Nut consumption has increased in the US but little evidence exists on the association between changes in nut consumption and weight change. We aimed to evaluate the association between changes in total consumption of nuts and intakes of different nuts (including peanuts) and long-term weight change, in three independent cohort studies. Methods and findings Data collected in three prospective, longitudinal cohorts among health professionals in the US were analysed. We included 27 521 men (Health Professionals Follow-up Study, 1986 to 2010), 61 680 women (Nurses' Health Study, 1986 to 2010), and 55 684 younger women (Nurses' Health Study II, 1991 to 2011) who were free of chronic disease at baseline in the analyses. We investigated the association between changes in nut consumption over 4-year intervals and concurrent weight change over 20–24 years of follow-up using multivariate linear models with an unstructured correlation matrix to account for within-individual repeated measures. 21 322 individuals attained a body mass index classification of obesity (BMI \geq 30 kg/m²) at the end of follow-up. Average weight gain across the three cohorts was 0.32 kg each year. Increases in nut consumption, per 0.5 servings/day (14 g), was significantly associated with less weight gain per 4-year interval ($p < 0.01$ for all): -0.19 kg (95% CI -0.21 to -0.17) for total consumption of nuts, -0.37 kg (95% CI -0.45 to -0.30) for walnuts, -0.36 kg (95% CI -0.40 to -0.31) for other tree nuts, and -0.15 kg (95% CI -0.19 to -0.11) for peanuts. Increasing intakes of nuts, walnuts, and other tree nuts by 0.5 servings/day was associated with a lower risk of obesity. The multivariable adjusted RR for total nuts, walnuts, and other tree nuts was 0.97 (95% CI 0.96 to 0.99, $p = 0.0036$), 0.85 (95% CI 0.81 to 0.89,

$p=0.0002$), and 0.89 (95% CI 0.87 to 0.91, $p<0.0001$), respectively. Increasing nut consumption was also associated with a lower risk of gaining ≥ 2 kg or ≥ 5 kg (RR 0.89–0.98, $p<0.01$ for all). In substitution analyses, substituting 0.5 servings/day of nuts for red meat, processed meat, French fries, desserts, or potato, chips (crisps) was associated with less weight gain ($p<0.05$ for all). Our cohorts were largely composed of Caucasian health professionals with relatively higher socioeconomic status; thus the results may not be generalisable to other populations. Conclusion Increasing daily consumption of nuts is associated with less long-term weight gain and a lower risk of obesity in adults. Replacing 0.5 servings/day of less healthful foods with nuts may be a simple strategy to help prevent gradual long-term weight gain and obesity.

Key Area: Body Weight and Composition

Nakanishi M, Matz A, Klemashevich C, Rosenberg DW. Dietary walnut supplementation alters mucosal metabolite profiles during DSS-induced colonic ulceration.

Nutrients. 2019 May 20;11(5). pii: 1118. doi: 10.3390/nu11051118

Abstract: Walnuts contain a complex array of natural compounds and phytochemicals that exhibit a wide range of health benefits, including protection against inflammation and colon cancer. In this study, we assess the effects of dietary supplementation with walnuts on colonic mucosal injury induced in mice by the ulcerogenic agent, dextran sodium sulfate (DSS). C57Bl/6J mice were started on the Total Western Diet supplemented with freshly-ground whole walnuts (0, 3.5, 7 and 14% g/kg) 2 weeks prior to a 5-day DSS treatment and walnut diets were continued throughout the entire experimental period. Mice were examined at 2 days or 10 days after withdrawal of DSS. In a separate study, a discovery-based metabolite profiling analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed on fecal samples and colonic mucosa following two weeks of walnut supplementation. Dietary walnut supplementation showed significant effects in the 10-day post-DSS recovery-phase study, in which the extent of ulceration was significantly reduced (7.5% vs. 0.3%, $p < 0.05$) with 14% walnuts. In the metabolite-profiling analysis, walnuts caused a significant increase in several polyunsaturated fatty acids (PUFAs), including docosahexaenoic acid (DHA) and 9-oxo-10(E),12(E)-octadecadienoic acid (9-oxoODA), as well as kynurenic acid. In colon tissue samples, walnuts caused a significant increase in the levels of S-adenosylhomocysteine (SAH) and betaine, important components of fatty acid β -oxidation. These metabolite changes may contribute in part to the observed protection against DSS-induced inflammatory tissue injury.

Key Area: Cancer

Njike VY, Costales VC, Petraro P, Annam A, Yarandi N, Katz DL. The resulting variation in nutrient intake with the inclusion of walnuts in the diets of adults at risk for type 2 diabetes: A randomized, controlled, crossover trial.

Am J Health Promot. 2019 Mar;33(3):430-438. doi: 10.1177/0890117118791120. Epub 2018 Aug 1.

Abstract: PURPOSE: We previously demonstrated that including walnuts in the diets of adults at risk for type 2 diabetes mellitus (T2DM) led to improved overall diet quality. This report examines the specific changes in their nutrient intake. DESIGN: This was a randomized, controlled, modified Latin square parallel design trial with 2 treatment arms. Participants were randomized to walnut intake with, or without, dietary advice to regulate caloric intake. Within each treatment arm, they were further randomized to one of 2 sequence permutations (walnut-included/walnut-excluded or walnut-excluded/walnut-included diet), with a 3-month washout between treatment phases. SETTING: Community hospital in Lower Naugatuck Valley in Connecticut. PARTICIPANTS: Cohort of 112 participants (31 men and 81 women) at risk for T2DM. INTERVENTION: Participants included 56 g (366 kcal) of walnuts in their daily diets for 6 months. MEASURES: Nutrient intake was assessed using

web-based Automated Self-Administered 24-Hour Dietary Assessment. ANALYSIS: Data were analyzed using generalized linear models. RESULTS: Walnut inclusion led to increased intake of total fat, calcium, magnesium, thiamin, total saturated fatty acids, and monounsaturated and polyunsaturated fatty acids (379.0 ± 90.3 g vs -136.5 ± 92.7 g, $P < .01$; 230.7 ± 114.2 mg vs -95.2 ± 117.4 mg, $P = .05$; 111.0 ± 33.9 mg vs -32.3 ± 34.9 mg, $P < .01$; 0.28 ± 0.2 mg vs -0.47 ± 0.2 mg, $P = .02$; 8.6 ± 3.4 g vs -1.1 ± 3.5 g, $P = .05$; 6.3 ± 3.9 g vs -6.3 ± 4.0 g, $P = .03$; and 25.4 ± 4.0 vs -6.6 ± 4.2 g, $P < .01$, respectively). Vitamin C intake decreased (-65.3 ± 55.3 mg vs 98.9 ± 56.8 mg, $P = .04$). Protein intake increased from baseline with the inclusion of walnuts (20.0 ± 8.8 g, $P < .05$). Walnut inclusion led to an increase in total calories consumed when caloric intake is not regulated. CONCLUSION: Including walnuts in the diets of these adults led to increased dietary intake of some nutrients associated with lower risk of developing T2DM and other cardiometabolic risk factors.

Key Area: Diabetes

Senevirathne, A., Neale, E., Peoples, G. and Tapsell, L. Relationship between long-chain omega-3 polyunsaturated fatty acid intake and ankle brachial index, pulse wave velocity and resting heart rate in a sample of overweight adults: A secondary analysis of baseline data in the HealthTrack study.

Nutr Diet. 2019 Feb; 76(1):95-103. doi: 10.1111/1747-0080.12479 Epub 2018 Oct 11.

Abstract: AIM: The present study aimed to explore the association between dietary long-chain omega-3 polyunsaturated fatty acid (LCn3PUFA) intake and cardiovascular risk indicators (ankle brachial index, resting heart rate and brachial-ankle pulse wave velocity) in a clinical sample of overweight and obese participants volunteering for a weight loss trial. METHODS: This was a secondary analysis of baseline data from the HealthTrack study ($n = 351$). LCn3PUFA intake was calculated via a diet history and the association with ankle brachial index, resting heart rate and brachio-ankle pulse wave velocity was explored using linear regression after controlling for covariates. RESULTS: LCn3PUFA intake was inversely associated with ankle brachial index ($R(2)$ change = 0.021, F change (1, 339) = 8.864, $P < 0.05$) and resting heart rate ($R(2)$ change = 0.014, F change (1, 342) = 5.337, $P < 0.05$) but not with brachio-ankle pulse wave velocity ($R(2)$ change = 0.001, F change (1, 339) = 0.725, $P > 0.05$). CONCLUSIONS: In this clinical sample of overweight adults, LCn3PUFA consumption was significantly associated with a lower resting heart rate, adding to the current evidence on the potential benefits of LCn3PUFA consumption. It also supports the value of targeting a diet rich in this nutrient when planning future dietetic approaches. Relationships with ankle brachial index and pulse wave velocity require further investigation. Future research should assess the effect of changes in dietary LCn3PUFA intake on novel cardiovascular risk indicators.

Key Area: Heart Health

Shin PK, Zoh Y, Choi J, Kim MS, Kim Y, Choi SW. Walnut phenolic extracts reduce telomere length and telomerase activity in a colon cancer stem cell model.

Nutr Res Pract. 2019 Feb;13(1):58-63. doi: 10.4162/nrp.2019.13.1.58. Epub 2018 Dec 3.

Abstract: BACKGROUND/OBJECTIVES: Telomeres are located at the chromosomal ends and progressively shortened during each cell cycle. Telomerase, which is regulated by hTERT and c-MYC, maintains telomeric DNA sequences. Especially, telomerase is active in cancer and stem cells to maintain telomere length for replicative immortality. Recently we reported that walnut phenolic extract (WPE) can reduce cell viability in a colon cancer stem cell (CSC) model. We, therefore, investigated the effect of WPE on telomere maintenance in the same model. MATERIALS/METHODS CD133+CD44+ cells from HCT116, a human colon cancer cell line, were sorted by Fluorescence-activated cell sorting (FACS) and treated with WPE at the concentrations of 0, 10, 20, and 40 $\mu\text{g/mL}$ for 6 days. Telomere lengths were assessed by quantitative real-time PCR (qRT-PCR) using telomere specific primers and DNA extracted from the cells, which was further adjusted with single-copy gene and reference DNA

(ddCt). Telomerase activity was also measured by qRT-PCR after incubating the PCR mixture with cell protein extracts, which was adjusted with reference DNA (dCt). Transcriptions of hTERT and c-MYC were determined using conventional RT-PCR. **RESULTS** Telomere length of WPE-treated cells was significantly decreased in a dose-dependent manner (5.16 ± 0.13 at $0 \mu\text{g/mL}$, 4.79 ± 0.12 at $10 \mu\text{g/mL}$, 3.24 ± 0.08 at $20 \mu\text{g/mL}$ and 3.99 ± 0.09 at $40 \mu\text{g/mL}$; $P = 0.0276$). Telomerase activities concurrently decreased with telomere length (1.47 ± 0.04 , 1.09 ± 0.01 , 0.76 ± 0.08 , and 0.88 ± 0.06 ; $P = 0.0067$). There was a positive correlation between telomere length and telomerase activity ($r = 0.9090$; $P < 0.0001$). Transcriptions of both hTERT and c-MYC were also significantly decreased in the same manner. **CONCLUSIONS** In the present cell culture model, WPE reduced telomere maintenance, which may provide a mechanistic link to the effect of walnuts on the viability of colon CSCs.

Key Area: Cancer

Tindall AM, Petersen KS, Skulas-Ray AC, Richter CK, Proctor DN, Kris-Etherton PM. Replacing saturated fat with walnuts or vegetable oils improves central blood pressure and serum lipids in adults at risk for cardiovascular disease: a randomized controlled-feeding trial.

J Am Heart Assoc. 2019 May 7;8(9):e011512. doi: 10.1161/JAHA.118.011512

Abstract: Background Walnuts have beneficial effects on cardiovascular risk factors, but it is unclear whether these effects are attributable to the fatty acid (FA) content, including α -linolenic acid (ALA), and/or bioactives. Methods and Results A randomized, controlled, 3-period, crossover, feeding trial was conducted in individuals at risk for cardiovascular disease ($n=45$). Following a 2-week standard Western diet run-in (12% saturated FAs [SFA], 7% polyunsaturated FAs, 12% monounsaturated FAs), participants consumed 3 isocaloric weight-maintenance diets for 6 weeks each: a walnut diet (WD; 7% SFA, 16% polyunsaturated FAs, 3% ALA, 9% monounsaturated FAs); a walnut FA-matched diet; and an oleic acid-replaced-ALA diet (7% SFA, 14% polyunsaturated FAs, 0.5% ALA, 12% monounsaturated FAs), which substituted the amount of ALA from walnuts in the WD with oleic acid. This design enabled evaluation of the effects of whole walnuts versus constituent components. The primary end point, central systolic blood pressure, was unchanged, and there were no significant changes in arterial stiffness. There was a treatment effect ($P=0.04$) for central diastolic blood pressure; there was a greater change following the WD versus the oleic acid-replaced-ALA diet (-1.78 ± 1.0 versus 0.15 ± 0.7 mm Hg, $P=0.04$). There were no differences between the WD and the walnut fatty acid-matched diet (-0.22 ± 0.8 mm Hg, $P=0.20$) or the walnut FA-matched and oleic acid-replaced-ALA diets ($P=0.74$). The WD significantly lowered brachial and central mean arterial pressure. All diets lowered total cholesterol, LDL (low-density lipoprotein) cholesterol, HDL (high-density lipoprotein) cholesterol, and non-HDL cholesterol. Conclusions Cardiovascular benefits occurred with all moderate-fat, high-unsaturated-fat diets. As part of a low-SFA diet, the greater improvement in central diastolic blood pressure following the WD versus the oleic acid-replaced-ALA diet indicates benefits of walnuts as a whole-food replacement for SFA.

Key Area: Heart Health

Tuccinardi D, Farr OM, Upadhyay J, Oussaada SM, Klapa MI, Candela M, Rampelli S, Lehoux S, Lázaro I, Sala-Vila A, Brigidi P, Cummings RD, Mantzoros CS. Mechanisms underlying the cardiometabolic protective effect of walnut consumption in obese subjects: a cross-over, randomized, double-blinded, controlled inpatient physiology study.

Diabetes Obes Metab. 2019 Sep;21(9):2086-2095. doi: 10.1111/dom.13773. Epub 2019 Jul 2.

Abstract: AIMS: To assess the effects of walnuts on cardiometabolic outcomes in obese subjects and to explore underlying mechanisms using novel methods including metabolomic, lipidomic, glycomic, and microbiome analysis integrated with lipid particle fractionation, appetite-regulating hormones and hemodynamic measurements. MATERIALS AND METHODS: 10 obese subjects were enrolled in this cross-over, randomized, double-blind, placebo-controlled clinical trial. Patients participated in two 5-day inpatient stays during which they consumed a smoothie containing 48g walnuts or a macronutrient-matched placebo smoothie without nuts, with a one-month washout period between the two visits. RESULTS: Walnut consumption improved aspects of the lipid profile, i.e. reduced fasting small and dense LDL particles ($p<.02$) and increased postprandial large HDL particles ($p<.01$). Lipoprotein Insulin Resistance Score, glucose and insulin AUC decreased significantly after walnut consumption ($p<.01$, $p<.02$, $p<.04$, respectively). Consuming walnuts significantly increased 10 N-glycans, with 8 of them carrying a fucose core. Lipidomic analysis showed a robust reduction in harmful ceramides, hexosylceramides and sphingomyelins, which have been shown to mediate effects on cardiometabolic risk. Peptide YY AUC significantly increased after walnut consumption ($p<.03$). No major significant changes in hemodynamic, metabolomic analysis or in host health-promoting bacteria such as *Faecalibacterium* were found. CONCLUSIONS: These data provide a more comprehensive mechanistic perspective of the effect of dietary walnut consumption on cardiometabolic parameters. Lipidomic and lipid nuclear magnetic resonance spectroscopy analysis showed an early but significant

reduction in ceramides and other atherogenic lipids with walnut consumption that may explain the longer-term benefits of walnuts on insulin resistance, cardiovascular risk and mortality.

Key Area: Body Weight and Composition

Yaskolka Meir A, Tsaban G, Zelicha H, Rinott E, Kaplan A, Youngster I, Rudich A, Shelef I, Tirosh A, Brikner D, Pupkin E, Sarusi B, Blüher M, Stümmvoll M, Thiery J, Ceglarek U, Stampfer MJ, Shai I. A Green-Mediterranean Diet, Supplemented with Mankai Duckweed, Preserves Iron-Homeostasis in Humans and Is Efficient in Reversal of Anemia in Rats.

J Nutr. 2019 Jun 1;149(6):1004-1011. doi: 10.1093/jn/nxy321. PMID: 30915471.

Abstract: Background: Decreased dietary meat may deplete iron stores, as plant-derived iron bioavailability is typically limited. Objectives: We explored the effect of a low-meat Mediterranean (green-MED) diet, supplemented with *Wolffia globosa* duckweed (Mankai: rich in protein and iron) as a food source for humans, on iron status. We further examined the iron bioavailability of Mankai in rats. Methods: Two hundred and ninety-four abdominally obese/dyslipidemic [mean age = 51.1 y; body mass index (kg/m²) = 31.3; 88% men] nonanemic participants were randomly assigned to physical activity (PA), PA + MED diet, or PA + green-MED diet. Both isocaloric MED groups consumed 28 g walnuts/d and the low-meat green-MED group further consumed green tea (800 mL/d) and Mankai (100 g green shake/d). In a complementary animal experiment, after 44 d of an iron deficiency anemia-inducing diet, 50 female rats (age = 3 wk; Sprague Dawley strain) were randomly assigned into: iron-deficient diet (vehicle), or vehicle + iso-iron: ferrous gluconate (FG) 14, Mankai 50, and Mankai 80 versions (1.7 mg · kg⁻¹ · d⁻¹ elemental iron), or FG9.5 and Mankai 50-C version (1.15 mg · kg⁻¹ · d⁻¹ elemental iron). The specific primary aim for both studies was changes in iron homeostasis parameters. Results: After 6 mo of intervention, iron status trajectory did not differ between the PA and PA + MED groups. Hemoglobin modestly increased in the PA + green-MED group (0.23 g/dL) compared with PA (-0.1 g/dL; P < 0.001) and PA + MED (-0.1 g/dL; P < 0.001). Serum iron and serum transferrin saturation increased in the PA + green-MED group compared with the PA group (8.21 µg/dL compared with -5.23 µg/dL and 2.39% compared with -1.15%, respectively; P < 0.05 for both comparisons), as did folic acid (P = 0.011). In rats, hemoglobin decreased from 15.7 to 9.4 mg/dL after 44 d of diet-induced anemia. After depletion treatment, the vehicle-treated group had a further decrease of 1.3 mg/dL, whereas hemoglobin concentrations in both FG and Mankai iso-iron treatments similarly rebounded (FG14: +10.8 mg/dL, Mankai 50: +6.4 mg/dL, Mankai 80: +7.3 mg/dL; FG9.5: +5.1 mg/dL, Mankai 50-C: +7.1 mg/dL; P < 0.05 for all vs. the vehicle group). Conclusions: In humans, a green-MED low-meat diet does not impair iron homeostasis. In rats, iron derived from Mankai (a green-plant protein source) is bioavailable and efficient in reversal of anemia.

Key Area: Nutrient & Bioactive Composition

2018

Arab L, Dhaliwal SK, Martin CJ, Larios AD, Jackson NJ, Elashoff D. Association between walnut consumption and diabetes risk in NHANES.

Diabetes Metab Res Rev. 2018 Oct; 34(7):e3031. doi: 10.1002/dmrr.3031. Epub 2018 Jul 11.

Abstract: BACKGROUND: Dietary interventions and cohort studies relating tree nut consumption to blood glucose levels suggest a possible effect of walnuts. OBJECTIVE: To examine the associations between walnut consumption and diabetes risk using data from the National Health and Nutrition Examination Survey (NHANES). METHODS: NHANES survey data on adults conducting 24 hour dietary recall was pooled across the years 1999 through 2014. Diabetes status or risk was based on self-report, medication use, fasting plasma (FPG) glucose levels and hemoglobin A1c (HbA1c) levels. Individuals were characterized based on reported consumption of walnuts, mixed-nuts, or no nuts.

RESULTS: After adjustment for covariates, walnut consumers showed lower risk for diabetes compared to non-nut consumers based on self-report (odds ratio of 0.47 95% confidence interval 0.31-0.72) as well as fasting blood glucose (RRR 0.32 CI 0.17-0.58) and HbA1c (RRR 0.51 CI 0.27-0.99). For each standard deviation of increase in walnut intake, prevalence of diabetes dropped 47%. The gender by walnut interaction suggests the effect may be more potent among women than men (dose response $p=0.061$). **CONCLUSIONS:** Both among individuals with known diabetes and those diagnosed based on elevated diabetes blood markers, the prevalence of individuals with diabetes was significantly lower among the walnut consumers. A possible gender specific effect invites further attention.

Key Area: Diabetes

Badri NW, Flatt SW, Barkai HS, Pakiz B, Heath DD, Rock CL. Insulin resistance improves more in women than in men in association with a weight loss intervention.

J Obes Weight Loss Ther. 2018;8(1). pii: 365. doi: 10.4172/2165-7904.1000365.

Abstract: **BACKGROUND:** Fasting glucose and homeostatic model assessment-insulin resistance (HOMA-IR) are important measures of the risk for metabolic syndrome and diabetes. Weight loss interventions are considered part of the first line of therapy for those who develop disease states associated with insulin resistance, such as pre-diabetes, diabetes, or metabolic syndrome. Sex differences in insulin resistance have been extensively reported, but sex differences in the ability to improve insulin sensitivity are not well-established. This study sought to identify factors that predict change in HOMA-IR in response to weight loss. **METHODS:** Non-diabetic subjects who were overweight/obese ($n=100$) were randomly assigned to a walnut-enriched reduced-energy diet or a standard reduced-energy-density diet in a 6-month weight loss intervention. There were no significant differences in weight change, glucose, insulin, or HOMA-IR between the two diet groups. These subjects were combined into a single cohort and analyzed with multivariate analysis. **RESULTS:** The combined groups lost an average of 8.7 kg ($p<0.0001$), decreased serum glucose by an average 0.2 mmol/L ($p<0.001$), and decreased HOMA-IR by an average of 1.4 ($p<0.0001$). Change in HOMA-IR ($R^2=0.69$) was positively associated with weight change ($p<0.0001$) and male sex ($p<0.01$), and negatively associated with baseline HOMA-IR ($p<0.0001$). **CONCLUSION:** Findings from this study suggest that men may have a more difficult time improving insulin sensitivity as compared with women with an equivalent weight loss and baseline HOMA-IR. One hypothesis to explain the differences across sexes may be due to sex differences in visceral adipose fat (VAT). This may mean that insulin resistant men require more aggressive intervention than women to prevent progression to metabolic syndrome or diabetes.

Key Area: Body Weight and Composition

Bamberger C, Rossmeier A, Lechner K, Wu L, Waldmann E, Fischer S, Stark RG, Altenhofer J, Henze K, Parhofer KG. A walnut-enriched diet affects gut microbiome in healthy Caucasian subjects: A randomized, controlled trial.

Nutrients. 2018 Feb 22; 10(2). pii: E244. doi: 10.3390/nu10020244

Abstract: Regular walnut consumption is associated with better health. We have previously shown that eight weeks of walnut consumption (43 g/day) significantly improves lipids in healthy subjects. In the same study, gut microbiome was evaluated. We included 194 healthy subjects (134 females, 63 ± 7 years, BMI 25.1 ± 4.0 kg/m²) in a randomized, controlled, prospective, cross-over study. Following a nut-free run-in period, subjects were randomized to two diet phases (eight weeks each); 96 subjects first followed a walnut-enriched diet (43 g/day) and then switched to a nut-free diet, while 98 subjects followed the diets in reverse order. While consuming the walnut-enriched diet, subjects were advised to either reduce fat or carbohydrates or both to account for the additional calories. Fecal samples were collected from 135 subjects at the end of the walnut-diet and the control-diet period for microbiome analyses. The 16S rRNA gene sequencing data was clustered with a 97% similarity into Operational

Taxonomic Units (OTUs). UniFrac distances were used to determine diversity between groups. Differential abundance was evaluated using the Kruskal-Wallis rank sum test. All analyses were performed using Rhea. Generalized UniFrac distance shows that walnut consumption significantly affects microbiome composition and diversity. Multidimensional scaling (metric and non-metric) indicates dissimilarities of approximately 5% between walnut and control ($p = 0.02$). The abundance of Ruminococcaceae and Bifidobacteria increased significantly ($p < 0.02$) while Clostridium sp. cluster XIVa species (Blautia; Anaerostipes) decreased significantly ($p < 0.05$) during walnut consumption. The effect of walnut consumption on the microbiome only marginally depended on whether subjects replaced fat, carbohydrates or both while on walnuts. Daily intake of 43 g walnuts over eight weeks significantly affects the gut microbiome by enhancing probiotic- and butyric acid-producing species in healthy individuals. Further evaluation is required to establish whether these changes are preserved during longer walnut consumption and how these are linked to the observed changes in lipid metabolism.

Key Area: Gut Health

Bitok E, Rajaram S, Jaceldo-Siegl K, Oda K, Sala-Vila A, Serra-Mir M, Ros E, Sabaté J. Effects of long-term walnut supplementation on body weight in free-living elderly: results of a randomized controlled trial.

Nutrients. 2018 Sep 18;10(9). pii: E1317. doi: 10.3390/nu10091317.

Abstract: OBJECTIVE: To assess the effects of chronic walnut consumption on body weight and adiposity in elderly individuals. METHODS: The Walnuts and Healthy Aging study is a dual-center (Barcelona, Spain and Loma Linda University (LLU)), 2-year randomized parallel trial. This report concerns only the LLU cohort. Healthy elders (mean age 69 year, 67% women) were randomly assigned to walnut ($n = 183$) or control diets ($n = 173$). Subjects in the walnut group received packaged walnuts (28–56 g/day), equivalent to $\approx 15\%$ of daily energy requirements, to incorporate into their habitual diet, while those in the control group abstained from walnuts. Adiposity was measured periodically, and data were adjusted for in-trial changes in self-reported physical activity. Results: After 2 years, body weight significantly decreased ($p = 0.031$), while body fat significantly increased ($p = 0.0001$). However, no significant differences were observed between the control and walnut groups regarding body weight (-0.6 kg and -0.4 kg, respectively, $p = 0.67$) or body fat ($+0.9\%$ and $+1.3\%$, respectively, $p = 0.53$). Lean body mass, waist circumference, and waist-to-hip ratio remained essentially unchanged. Sensitivity analyses were consistent with the findings of primary analysis. CONCLUSION: Our findings indicate that walnuts can be incorporated into the daily diet of healthy elders without concern for adverse effects on body weight or body composition.

Key Area: Body Weight and Composition

Farr OM, Tuccinardi D, Upadhyay J, Oussaada SM, Mantzoros CS. Walnut consumption increases activation of the insula to highly desirable food cues: A randomized, double-blind, placebo-controlled, cross-over fMRI study.

Diabetes Obes Metab. 2018 Jan;20(1):173-177. doi: 10.1111/dom.13060, Epub 2017 Aug 17.

Abstract AIMS: The use of walnuts is recommended for obesity and type 2 diabetes, although the mechanisms through which walnuts may improve appetite and/or glycemic control remain largely unknown. MATERIALS AND METHODS: To determine whether short-term walnut consumption could alter the neural control of appetite using functional magnetic resonance imaging, we performed a randomized, placebo-controlled, double-blind, cross-over trial of 10 patients who received, while living in the controlled environment of a clinical research center, either walnuts or placebo (using a validated smoothie delivery system) for 5 days each, separated by a wash-out period of one month. RESULTS: Walnut consumption decreased feelings of hunger and appetite assessed using visual analog scales and increased the activation of the right insula to highly desirable food cues. CONCLUSIONS: These

findings suggest that walnut consumption may increase salience and cognitive control processing of highly desirable food cues, leading to the beneficial metabolic effects observed.

Key Area: Body Weight and Composition

Freitas-Simoes TM, Cofán M, Blasco MA, Soberón N, Foronda M, Serra-Mir M, Roth I, Valls-Pedret C, Doménech M, Ponferrada-Ariza E, Calvo C, Rajaram S, Sabaté J, Ros E, Sala-Vila A. Walnut consumption for two years and leukocyte telomere attrition in Mediterranean elders: results of a randomized controlled trial.

Nutrients. 2018 Dec 4;10(12). pii: E1907. doi: 10.3390/nu10121907

Abstract: Randomized controlled trials on diet and shortening of leukocyte telomere length (LTL) mostly focus on marine-derived n-3 polyunsaturated fatty acids (PUFA). Walnuts are a sustainable source of n-3 PUFA. We investigated whether inclusion of walnuts (15% of energy) in the diet for 2 years would maintain LTL in cognitively healthy elders (63–79 years old) compared to a control group (habitual diet, abstaining from walnuts). This opportunistic sub-study was conducted within the Walnuts and Healthy Aging study, a dual-centre (Barcelona, Spain and Loma Linda University, California) parallel trial. A sub-set of the Barcelona site participants were randomly assigned to the walnut (n = 80) or control group (n = 69). We assessed LTL at baseline and at 2 years and we conducted repeated-measures ANCOVA with 2 factors: time (baseline, 2 years) and group (control, walnut) and their interaction. Adjusted means (95% confidence interval) of LTL (in kb) in controls were 7.360 (7.084,7.636) at baseline and 7.061 (6.835,7.288) after 2 years; corresponding values in the walnut group were 7.064 (6.807,7.320) and 7.074 (6.864,7.284). The time × intervention interaction was nearly significant (p = 0.079), suggestive of a trend of walnut consumption in preserving LTL. This exploratory research finding should be confirmed in trials with adequate statistical power.

Key Area: Cognitive Health

Freitas-Simoes TM, Cofán M, Blasco MA, Soberón N, Foronda M, Corella D, Asensio EM, Serra-Mir M, Roth I, Calvo C, Valls-Pedret C, Casaroli-Marano RP, Doménech M, Rajaram S, Sabaté J, Ros E, Sala-Vila A. The red blood cell proportion of arachidonic acid relates to shorter leukocyte telomeres in Mediterranean elders: A secondary analysis of a randomized controlled trial.

Clin Nutr. 2018 Apr;38(2):958-961. doi: 10.1016/j.clnu.2018.02.011. Epub 2018 Feb 17.

Abstract: BACKGROUND & AIMS: Shortening of leukocyte telomere length (LTL) is a biomarker of aging. Epidemiologic studies of LTL in relation to dietary fatty acids have reported conflicting results. The red blood cell (RBC) fatty acid status is a valid objective biomarker of long-term dietary intake of C18:2n-6, C18:3n-3 and long-chain n-3 polyunsaturated fatty acids (C20:5n-3 and C22:6n-3). In healthy older individuals, we investigated whether LTL relates to the RBC proportions of the main dietary polyunsaturated fatty acids (PUFA), and to the RBC proportion of arachidonic acid (C20:4n-6), a fatty acid that can generate pro-inflammatory lipid mediators once released from cell membranes. DESIGN: Cross-sectional study in 344 subjects (mean age 68.8 y, 68.6% women) who participated in a randomized controlled trial testing whether a diet enriched in walnuts can delay the onset of age-related diseases (<https://clinicaltrials.gov/ct2/show/NCT01634841>). At baseline, we assessed LTL by high-throughput quantitative fluorescence and determined fatty acids in RBCs by gas chromatography. RESULTS: In multivariate models adjusted for age and gender, the RBC proportions of dietary PUFA were unrelated to LTL. In contrast, the RBC proportion of arachidonic acid inversely related to LTL (regression coefficient [95% confidence interval], -0.10 (-0.19 to -0.01), P = 0.023). CONCLUSION: An increasing proportion of C20:4n-6 in RBCs is associated with shorter telomeres. Further research is needed to investigate the role of this fatty acid and its derived lipid mediators in the aging process.

Key Area: Heart Health

Gepner Y, Shelef I, Schwarzfuchs D, Zelicha H, Tene L, Yaskolka Meir A, Tsaban G, Cohen N, Bril N, Rein M, Serfaty D, Kenigsbuch S, Komy O, Wolak A, Chassidim Y, Golan R, Avni-Hassid H, Bilitzky A, Sarusi B, Goshen E, Shemesh E, Henkin Y, Stumvoll M, Blüher M, Thiery J, Ceglarek U, Rudich A, Stampfer MJ, Shai I. Effect of distinct lifestyle interventions on mobilization of fat storage pools: The CENTRAL MRI randomized controlled trial.

Circulation. 2018 Mar 13;137(11):1143-1157. doi: 10.1161/CIRCULATIONAHA.117.030501. Epub 2017 Nov 15.

Abstract: BACKGROUND: We aimed to assess whether distinct lifestyle strategies can differentially affect specific body adipose depots. METHODS: We performed an eighteen-month randomized controlled trial among 278 sedentary adults with abdominal obesity (75%) or dyslipidemia in an isolated workplace with a monitored provided lunch. Participants were randomized to iso-caloric low-fat (LF) or Mediterranean/low-carbohydrate (MED/LC) diet+28g walnuts/day with/without added moderate physical activity (PA;80% aerobic; supervised/free gym membership). Overall primary outcome was body fat re-distribution, and the main specific endpoint was visceral adipose tissue (VAT). We further followed the dynamics of different fat depots [deep/superficial subcutaneous (D/SSAT), liver, pericardial, muscle, pancreas and renal-sinus] by magnetic-resonance-imaging. Results -Of 278 participants (age=48y; 89%men, body-mass-index=30.8kg/m²), 86% completed the trial, with good adherence. The LF group preferentially decreased reported fat intake (-21.0% vs. -11.5% for the MED/LC; P<0.001), and the MED/LC group decreased reported carbohydrates intake (-39.5%vs. -21.3% for the LF;P<0.001). The PA+ groups significantly increased the metabolic-equivalents (METs)/week vs. the PA- groups (19.0 vs. 2.1;P=0.009). Whereas final moderate weight loss was indifferent, exercise attenuated the waist circumference rebound with the greatest effect in MED/LCPA+ group (P<0.05). VAT (-22%), intra-hepatic (-29%), and Intra-pericardial (-11%) fats declines were higher than pancreatic and femur intermuscular fats (1-2%) loss. Independent of weight loss, PA+ with either diet had a significantly greater effect on decreasing VAT [mean-of-difference=-6.67cm²;95%CI:(-14.8 to -0.45) compared with PA-]. The MED/LC diet was superior to LF in decreasing intra-hepatic, intra-pericardial and pancreatic fats (P<0.05 for all). In contrast, renal-sinus and femoral-intermuscular fats were not differentially altered by lifestyle interventions, but by weight loss per-se. In multivariate models, further adjusted for weight loss, losing VAT or intra-hepatic fat were independently associated with improved lipid profile, losing deep-SAT with improved insulin sensitivity and losing superficial-SAT remained neutral except of association with decreased leptin. CONCLUSIONS: Moderate weight loss alone inadequately reflects the significant lifestyle effects on atherogenic and diabetogenic fat depots. The MED/LC diet mobilizes specific ectopic fat depots, and exercise has an independent contribution to VAT loss. Fat depots exhibit diverse responsiveness and are differentially related to cardiometabolic markers. Distinct lifestyle protocols may uniquely induce fat mobilization from specific anatomical sites.

Key Area: Body Weight and Composition

Guan F, Tabrizian T, Novaj A, Nakanishi M, Rosenberg DW, Huffman D. Dietary walnuts protect against obesity-driven intestinal stem cell decline and tumorigenesis.

Front. Nutr. 2018 May 31;5:37. doi: 10.3389/fnut.2018.00037. eCollection 2018.

Abstract: Obesity can negatively impact intestinal homeostasis, and increase colon cancer risk and related mortality. Thus, given the alarmingly high rates of obesity in the US and globally, it is critical to identify practical strategies that can break the obesity-cancer link. Walnuts have been increasingly recognized to mitigate cancer risk, and contain many bioactive constituents with antioxidant and anti-inflammatory properties that could potentially counteract pathways thought to be initiators of obesity-related cancer. Therefore, the purpose of this study was to determine if walnuts could preserve intestinal homeostasis, and attenuate tumorigenesis and growth in the context of obesity and a high calorie diet. To this end, we studied effects of walnuts on these parameters under different dietary

conditions in wildtype mice, two independent *Apc* models (*Apc*^{1638N/+} and *Apc*^{Δ14}), and in MC38 colon cancer cells in vivo, respectively. Walnuts did not alter the metabolic phenotype or intestinal morphology in normal mice fed either a low-fat diet (LFD), LFD with 6% walnuts (LFD+W), high-fat diet (HFD), or HFD with 7.6% walnuts (HFD+W). However, walnuts did lead to a significant reduction in circulating CCL5 and preserved intestinal stem cell (ISC) function under HFD-fed conditions. Furthermore, walnuts reduced tumor multiplicity in *Apc*^{1638N/+} male HFD+W animals, as compared to HFD controls (3.7 ± 0.5 vs. 2.5 ± 0.3 ; $P = 0.015$), tended to reduce the number of adenocarcinomas (0.67 ± 0.16 vs. 0.29 ± 0.12 ; $P = 0.07$), and preferentially limited tumor growth in *Apc*^{Δ14} male mice ($P = 0.019$) fed a high-calorie western-style diet. In summary, these data demonstrate that walnuts confer significant protection against intestinal tumorigenesis and growth and preserve ISC function in the context of a high-calorie diet and obesity. Thus, these data add to the accumulating evidence connecting walnuts as a potentially effective dietary strategy to break the obesity-colon cancer link.

Key Area: Cancer

Guan VX, Probst YC, Neale EP, Batterham MJ, Tapsell LC. Identifying usual food choices at meals in overweight and obese study volunteers: implications for dietary advice.

Br J Nutr. 2018;120(4):472-480. doi: 10.1017/S0007114518001587. Epub 2018 Jul 17.

Abstract: Understanding food choices made for meals in overweight and obese individuals may aid strategies for weight loss tailored to their eating habits. However, limited studies have explored food choices at meal occasions. The aim of this study was to identify the usual food choices for meals of overweight and obese volunteers for a weight-loss trial. A cross-sectional analysis was performed using screening diet history data from a 12-month weight-loss trial (the HealthTrack study). A descriptive data mining tool, the Apriori algorithm of association rules, was applied to identify food choices at meal occasions using a nested hierarchical food group classification system. Overall, 432 breakfasts, 428 lunches, 432 dinners and 433 others (meals) were identified from the intake data (n 433 participants). A total of 142 items of closely related food clusters were identified at three food group levels. At the first sub-food group level, bread emerged as central to food combinations at lunch, but unprocessed meat appeared for this at dinner. The dinner meal was characterised by more varieties of vegetables and of foods in general. The definitions of food groups played a pivotal role in identifying food choice patterns at main meals. Given the large number of foods available, having an understanding of eating patterns in which key foods drive overall meal content can help translate and develop novel dietary strategies for weight loss at the individual level.

Key Area: Body Weight and Composition

Guasch-Ferré M, Li J, Hu FB, Salas-Salvadó J, Tobias DK. Effects of walnut consumption on blood lipids and other cardiovascular risk factors: an updated meta-analysis and systematic review of controlled trials.

Am J Clin Nutr. 2018, 108(1):174-187. doi: 10.1093/ajcn/nqy091

Abstract: BACKGROUND: Intervention studies suggest that incorporating walnuts into the diet may improve blood lipids without promoting weight gain. OBJECTIVE: We conducted a systematic review and meta-analysis of controlled trials evaluating the effects of walnut consumption on blood lipids and other cardiovascular risk factors. Design: We conducted a comprehensive search of PubMed and EMBASE databases (from database inception to January 2018) of clinical trials comparing walnut-enriched diets with control diets. We performed random-effects meta-analyses comparing walnut-enriched and control diets for changes in pre-post intervention in blood lipids (mmol/L), apolipoproteins (mg/dL), body weight (kg), and blood pressure (mm Hg). RESULTS: Twenty-six clinical trials with a total of 1059 participants were included. The following weighted mean differences (WMDs) in reductions were obtained for walnut-enriched diets compared with control groups: -6.99 mg/dL (95% CI: -9.39, -4.58 mg/dL; $P < 0.001$) (3.25% greater reduction) for total blood cholesterol

(TC) and -5.51 mg/dL (95% CI: -7.72, -3.29 mg/dL; $P < 0.001$) (3.73% greater reduction) for low-density lipoprotein (LDL) cholesterol. Triglyceride concentrations were also reduced in walnut-enriched diets compared with control [WMD = -4.69 (95% CI: -8.93, -0.45); $P = 0.03$; 5.52% greater reduction]. More pronounced reductions in blood lipids were observed when walnut interventions were compared with American and Western diets [WMD for TC = -12.30 (95% CI: -23.17, -1.43) and for LDL = -8.28 (95% CI: -13.04, -3.51); $P < 0.001$]. Apolipoprotein B (mg/dL) was also reduced significantly more on walnut-enriched diets compared with control groups [WMD = -3.74 (95% CI: -6.51, -0.97); $P = 0.008$] and a trend towards a reduction was observed for apolipoprotein A [WMD = -2.91 (95% CI: -5.98, 0.08); $P = 0.057$]. Walnut-enriched diets did not lead to significant differences in weight change (kg) compared with control diets [WMD = -0.12 (95% CI: -2.12, 1.88); $P = 0.90$], systolic blood pressure (mm Hg) [WMD = -0.72 (95% CI: -2.75, 1.30); $P = 0.48$], or diastolic blood pressure (mm Hg) [WMD = -0.10 (95% CI: -1.49, 1.30); $P = 0.88$]. Conclusions: Incorporating walnuts into the diet improved blood lipid profile without adversely affecting body weight or blood pressure.

Key area: Heart Health

Holscher HD, Guetterman HM, Swanson KS, An R, Matthan NR, Lichtenstein AH, Novotny JA, Baer DJ. Walnut consumption alters the gastrointestinal microbiota, microbially derived secondary bile acids, and health markers in healthy adults: a randomized controlled trial.

J Nutr. 2018 Jun 1;148(6):861-867. doi: 10.1093/jn/nxy004

Abstract: Background: Epidemiologic data suggest that diets rich in nuts have beneficial health effects, including reducing total and cause-specific mortality from cancer and heart disease. Although there is accumulating preclinical evidence that walnuts beneficially affect the gastrointestinal microbiota and gut and metabolic health, these relations have not been investigated in humans. Objective: We aimed to assess the impact of walnut consumption on the human gastrointestinal microbiota and metabolic markers of health. Methods: A controlled-feeding, randomized crossover study was undertaken in healthy men and women [$n = 18$; mean age = 53.1 y; body mass index (kg/m²): 28.8]. Study participants received isocaloric diets containing 0 or 42 g walnuts/d for two 3-wk periods, with a 1-wk washout between diet periods. Fecal and blood samples were collected at baseline and at the end of each period to assess secondary outcomes of the study, including effects of walnut consumption on fecal microbiota and bile acids and metabolic markers of health. Results: Compared with after the control period, walnut consumption resulted in a 49-160% higher relative abundance of *Faecalibacterium*, *Clostridium*, *Dialister*, and *Roseburia* and 16-38% lower relative abundances of *Ruminococcus*, *Dorea*, *Oscillospira*, and *Bifidobacterium* ($P < 0.05$). Fecal secondary bile acids, deoxycholic acid and lithocholic acid, were 25% and 45% lower, respectively, after the walnut treatment compared with the control treatment ($P < 0.05$). Serum LDL cholesterol and the noncholesterol sterol campesterol concentrations were 7% and 6% lower, respectively, after walnut consumption compared with after the control treatment ($P < 0.01$). Conclusions: Walnut consumption affected the composition and function of the human gastrointestinal microbiota, increasing the relative abundances of Firmicutes species in butyrate-producing *Clostridium* clusters XIVa and IV, including *Faecalibacterium* and *Roseburia*, and reducing microbially derived, proinflammatory secondary bile acids and LDL cholesterol. These results suggest that the gastrointestinal microbiota may contribute to the underlying mechanisms of the beneficial health effects of walnut consumption.

Key Area: Gut Health

Jacobs DR Jr, Petersen KS, Svendsen K, Ros E, Sloan CB, Steffen LM, Tapsell LC, Kris-Etherton PM. Considerations to facilitate a US study that replicates PREDIMED.

Metabolism. 2018 Aug;85:361-367. doi: 10.1016/j.metabol.2018.05.001. Epub 2018 May 5.

Abstract: The PREDIMED clinical trial provided strong evidence that a Mediterranean dietary pattern (MedDiet) could help prevent cardiovascular disease (CVD) events in high risk middle-aged/older

people. This report considers the feasibility of replicating PREDIMED in the U.S., including recommendations for dietary and behavioral principles. A 14-point Mediterranean diet Adherence Score (MEDAS) guided the PREDIMED MedDiet recommendations. At baseline MEDAS points were ~8.5. During intervention this score increased to nearly 11 in MedDiet vs. 9 in control. In the MedDiet groups, only about 0.5 points of the net 2 point MEDAS increase was attributable to the gratis supplements of olive oil or nuts. An issue in a U.S. replication is the large difference in typical U.S. versus Spanish diet and lifestyle. A typical U.S. diet would achieve a MEDAS of 1-2. A replication is scientifically feasible with an assumption such as that the MedDiet reflects a continuum of specific food choices and meal patterns. As such, a 2 point change in MEDAS at any point on the continuum would be hypothesized to reduce incident CVD. A conservative approach would aim for a randomized 4 point MEDAS difference, e.g. 5-6 points vs. an average U.S. diet group that achieved only 1-2 points.

Key Area: Research Methodology

Lee HJ, Han YM, An JM, Kang EA, Park YJ, Cha JY, Hahm KB. Role of omega-3 polyunsaturated fatty acids in preventing gastrointestinal cancers: current status and future perspectives.

Expert Rev Anticancer Ther. 2018 Dec;18(12):1189-1203. doi: 10.1080/14737140.2018.1524299. Epub 2018 Sep 24.

Abstract: Although inflammation is defensive and healing process that maintains organ homeostasis, unresolved inflammation can lead to diseases. Polyunsaturated fatty acids (PUFAs), especially n-6 PUFAs abundant in Western diet, are precursors of pro-inflammatory mediators, whereas n-3 PUFAs possess anti-inflammatory properties. Therefore, interest in the cancer-preventive effect of n-3 PUFAs is increasing. Areas covered: We have observed significant reductions of gastrointestinal tumorigenesis in the Fat-1 transgenic mouse as evidenced that the decrease in *Helicobacter pylori*-infected gastric tumorigenesis, colon, biliary, and pancreatic cancer was seen in Fat-1 mice producing n-3 PUFAs. However, despite many studies showing benefits, evidence-based medicine regarding molecular pathology, epidemiology, and clinical achievement of cancer prevention of n-3 PUFAs are still limited. Expert commentary: Primary deficiency of eicosapentaenoic acids and docosahexaenoic acids in Western diets can explain the risk of cancer development and the importance of n-3/n-6 PUFA ratio in reducing cancer risk. Alteration of cell membrane composition during carcinogenesis is particularly important, due to increased rate of lipid/cholesterol synthesis in cancerous tissues. Here, we discuss that direct incorporation of n-3 PUFAs in the cell membrane corrects abnormal cellular proliferation and decreases inflammation-associated carcinogenesis. This is exemplified by cancer-preventive effects of n-3 PUFAs as fat sources for gastrointestinal cancers.

Key Area: Cancer

McArthur BM, Mattes RD, Considine RV. Mastication of nuts under realistic eating conditions: implications for energy balance.

Nutrients. 2018 Jun 1;10(6). pii: E710. doi: 10.3390/nu10060710

Abstract: The low digestibility and high satiety effects of nuts have been partly attributed to mastication. This work examines chewing forces and the bolus particle size of nuts (walnuts, almonds, pistachios) varying in physical properties under different conditions (with and without water, juice, sweetened yogurt and plain yogurt) along with satiety sensations and gut hormone concentrations following walnut consumption (whole or butter). In a randomized, cross-over design with 50 adults (25 males, 25 females; Body Mass Index (BMI) 24.7 ± 3.4 kg/m²; age: 18–52 years old (y/o), the chewing forces and particle size distribution of chewed nuts were measured under different chewing conditions. Appetite sensations were measured at regular intervals for 3 h after nut intake, and plasma samples were collected for the measurement of glucose, insulin and Glucagon-like peptide-1 (GLP-1). The three nuts displayed different particle sizes at swallowing though no differences in chewing forces were observed.

Walnuts with yogurt yielded larger particle sizes than the other treatments. Particle size was not correlated with either food palatability or flavor. Fullness sensations were higher after whole nut than nut butter consumption though there were no significant changes in glucose, insulin, or GLP-1 concentrations under any condition. Changing the conditions at swallowing might influence the release of energy from nuts.

Key Area: Body Weight and Composition

Ndanuko RN, Tapsell LC, Charlton KE, Neale EP, Batterham MJ. Effect of individualised dietary advice for weight loss supplemented with walnuts on blood pressure: the HealthTrack study.

Eur J Clin Nutr. 2018 Jun;72(6):894-903. doi: 10.1038/s41430-018-0123-0. Epub 2018 Mar 20.

Abstract: BACKGROUND/OBJECTIVES: In addition to weight-loss, healthy dietary patterns and lower sodium intakes can help reduce blood pressure (BP), but individualised dietary advice may be necessary to achieve these effects. This study aimed to examine the impact of individualised dietary advice on BP in the intensive phase of a weight-loss trial. SUBJECTS/METHODS: Secondary analysis of baseline and 3-month data from the HealthTrack randomised controlled trial (n = 211). Participants were randomly assigned to one of three dietary advice groups: general advice (control), individualised advice (intervention group, I), or intervention group supplemented with 30 g walnuts/day (IW). Resting BP and 24-h urine sodium and potassium were measured. Dietary intake was evaluated through diet history interviews. RESULTS: Unadjusted SBP reduced significantly in all groups (IW and I groups $P < 0.001$; control group $P = 0.002$) and DBP in IW and I groups ($P < 0.001$). Compared to controls, the reductions in BP were 3-4 mmHg greater in the I and IW groups, but this only reached significance for DBP in the I group (-3.3 mmHg; $P = 0.041$). After controlling for age, sex, medication, weight-loss, physical activity and smoking, only the IW group showed a significant association between SBP reduction and increased urinary potassium ($\beta = -0.101$, $P = 0.044$), decreased sodium:potassium ratio ($\beta = 2.446$, $P = 0.037$) and increased consumption of seed and nut products and dishes ($\beta = -0.108$, $P = 0.034$). CONCLUSIONS: Dietary patterns with distinctive foods and lower sodium:potassium ratios may enhance the effects of weight-loss on BP. The patterns were best achieved with individualised dietary advice and food supplements.

Key Area: Body Weight and Composition

Pandareesh MD, Chauhan V, Chauhan A. Walnut supplementation in the diet reduces oxidative damage and improves antioxidant status in transgenic mouse model of Alzheimer's disease.

J Alzheimers Dis. 2018;64(4):1295-1305. doi: 10.3233/JAD-180361

Abstract: Our previous study has shown beneficial effects of walnuts on memory and learning skills in transgenic mouse model of Alzheimer's disease (AD-tg). To understand underlying mechanism, we studied here whether walnuts can reduce oxidative stress in AD. From 4 months of age, experimental AD-tg mice were fed diets containing 6% (T6) or 9% walnuts (T9) (equivalent to 1 or 1.5 oz, of walnuts per day in humans) for 5, 10, or 15 months. The control groups, i.e., AD-tg (T0) and wild-type (Wt) mice, were fed diets without walnuts. Free radicals, i.e., reactive oxygen species (ROS), lipid peroxidation, protein oxidation, and antioxidant enzymes were assessed in these mice at different ages. AD-tg mice on control diet (T0) showed significant age-dependent increase in ROS levels, lipid peroxidation, and protein oxidation coupled with impaired activities of antioxidant enzymes [superoxide dismutase, catalase, and glutathione peroxidase] compared to Wt mice. Oxidative stress was significantly reduced in AD-tg mice on diets with walnuts (T6, T9), as evidenced by decreased levels of ROS, lipid peroxidation, and protein oxidation, as well as by enhanced activities of antioxidant enzymes compared to T0 mice. Long-term supplementation with walnuts for 10 or 15 months was more effective in reducing oxidative stress in AD-tg mice. Our findings indicate that walnuts can reduce oxidative stress, not only by scavenging free radicals, but also by protecting antioxidant status, thus leading to

reduced oxidative damage to lipids and proteins in AD. Therefore, by reducing oxidative stress, a walnut-enriched diet may help reduce the risk or delay the onset and progression of AD.

Key Area: Cognitive Health

Song EK, Liu Y, Kim HS, Park H. Daily walnut consumption favourably changed lipid profiles among Korean subjects with higher waist circumference.

Acta Scientific Nutritional Health. 2.5 (2018): 21-26. Web link:

<https://www.actascientific.com/ASNH/pdf/ASNH-02-0076.pdf>.

Abstract: Even though many studies have shown that walnuts have beneficial effects on lipid profiles in various populations, there have been limited data on the effects of walnuts in Korean populations. We examined not only the effects of walnut intake on lipid profiles among Korean adults but also focused on the sub-classification by waist circumference (WC). 89 subjects out of 119 completed trial with daily consumption of 45 g of walnuts for 16 weeks. Blood lipid profiles including triglycerides (TG), non-HDL cholesterol (non-HDL-C), LDL cholesterol (LDL-C), total cholesterol (TC), and HDL cholesterol (HDL-C), apolipoprotein B, anthropometric measurements (WC, weight, body mass index (BMI) and blood pressure) and glucose metabolism parameters including fasting blood sugar and insulin levels were assessed. Those with WC greater than 85 cm for female and 90 cm for male were classified as higher WC group (n=48) and others were classified as normal WC group (n=41). Blood levels of non-HDL-C, LDL-C, TC and apolipoprotein B were improved after daily consumption of 45 g of walnuts (P=0.003, P=0.011, P=0.002, and P=0.012, respectively) compared to baseline levels. Systolic blood pressure, TG, non-HDL-C, LDL-C and TC were significantly decreased in the higher WC groups (P=0.048, P=0.002, P=0.002 and P=0.001, respectively) compared to normal WC group. The results suggest that consuming 45 g of walnuts daily for 16 weeks had beneficial effects on lipid profiles in general, and these results were even much stronger among the subjects with abdominal obesity as waist circumference compared to those with non-abdominal obesity.

Key Area: Diabetes

Tene L, Shelef I, Schwarzfuchs D, Gepner Y, Meir AY, Tsaban G, Zelicha H, Bilitzky A, Komy O, Cohen N, Bril N, Rein M, Serfaty D, Kenigsbuch S, Chassidim Y, Sarusy B, Ceglarek U, Stumvoll M, Blüher M, Thiery J, Stampfer MJ, Rudich A, Shai I. The effect of long-term weight-loss intervention strategies on the dynamics of pancreatic-fat and morphology: An MRI RCT study.

Clinical Nutrition ESPEN. 2018 Apr;24:82-89. doi: 10.1016/j.clnesp.2018.01.008. Epub 2018 Feb 6.

Abstract: BACKGROUND & AIMS: The ability to mobilize pancreatic-fat and the meaning of decreased fat in the pancreas remain controversial. We followed the dynamics of pancreatic-fat and its morphology during various long weight-loss induced lifestyle-interventions. METHODS: In isolated workplace with monitored/provided lunch, we randomly assigned healthy persons with abdominal obesity or dyslipidemia for one of two 18-month equal-caloric diets: low-fat (LF) or Mediterranean/low-carbohydrate (Med/LC, with provided 1oz walnuts/day), with or without added moderate exercise (supervised gym membership). We used magnetic-resonance-imaging to quantify pancreatic-fat and morphology. RESULTS: At baseline, 277 eligible participants (mean age = 48 years; 88% men; pancreatic-fat = $17.4 \pm 5.1\%$) had higher pancreatic-fat in men ($17.7 \pm 4.9\%$ vs $14.9 \pm 5.5\%$ in women; $p = 0.004$). Following 18-month intervention (adherence = 86.3%) and moderate weight-loss (mean = -3.0 ± 5.5 kg), pancreatic-fat decreased moderately but significantly ($-0.26 \pm 2.18\%$ units; $p = 0.049$). Med/LC diet induced a greater decrease in pancreatic-fat compared to LF ($p = 0.043$), and the combination of Med/LC diet + exercise exhibited the highest reduction (-0.69% units) as compared to LF diet without exercise ($+0.12\%$ units; $p = 0.027$ between groups). In multivariate regression models, after further adjusted for visceral adipose-tissue (Δ VAT), pancreatic-fat loss associated with both decreases in pancreatic-morphology ratio (perimeter divided by area; beta = 0.361; $p < 0.001$) and superficial-subcutaneous adipose-tissue loss (beta = 0.242; $p = 0.001$), but not with changes in

intrahepatic-fat ($\beta = -0.034$; $p = 0.638$). Pancreatic-fat loss associated with increased intake of polyunsaturated-fat ($\beta = -0.137$; $p = 0.032$), as with improved high-density lipoprotein-cholesterol (HDL; $\beta = -0.156$; $p = 0.023$) and triglycerides/HDL ratio ($\beta = 0.162$; $p = 0.015$), independently of Δ VAT, but not with glycemic-control parameters (e.g. HbA1c, HOMA-IR and HOMA-beta; $p > 0.2$ for all). **CONCLUSIONS:** Pancreatic-fat loss is mainly associated with improved lipid, rather than glycemic profiles. Med/LC diet, mostly with exercise, may benefit pancreatic-fat loss. Pancreatic-morphology could serve as a biomarker of pancreatic-fat state.

Key area: Body Weight & Composition

Thangthaeng N, Poulouse SM, Fisher DR, Shukitt-Hale B. Walnut extract modulates activation of microglia through alteration in intracellular calcium concentration.

Nutr Res. 2018 Jan;49:88-95. doi: 10.1016/j.nutres.2017.10.016, Epub 2017 Nov 4.

Abstract: Diets supplemented with walnuts have shown to protect brain against oxidative and inflammatory cytotoxicity and promote protective cellular and cognitive function. The current study was undertaken to test the hypothesis that whole walnut extract inhibits LPS-induced microglial activation by regulating calmodulin (CaM) expression through $[Ca^{2+}]_i$. To test this hypothesis, we used an in vitro model the highly aggressively proliferating immortalized (HAPI) cells, a rat microglial cell-line, treated with various concentrations of walnut extracts (WNE). Treatment with walnut extract (1.5, 3 or Volume 47. bioactive compounds in walnut are capable of modulating microglial activation through regulation of intracellular calcium and CaM expression. Nutritional interventions using walnuts may be effective in the amelioration of chronic inflammation and neurodegeneration.

Key area: Cognitive Health

Zelicha H, Schwarzfuchs D, Shelef I, Gepner Y, Tsaban G, Ten L, Yaskolka Meir A, Bilitzky A, Komy O, Cohen N, Bril N, Rein M, Serfaty D, Kenigsbuch S, Chassidim Y, Sarusi B, Thiery J, Ceglarek U, Stumvoll M, Blüher M, Haviv YS, Stampfer MJ, Rudich A, Shai I. Changes of renal sinus fat and renal parenchymal fat during an 18-month randomized weight loss trial.

Clin Nutr. 2018 Aug;37(4):1145-1153. doi: 10.1186/s40795-017-0157-z. Epub 2017 May 2.

Abstract: **BACKGROUND & AIMS:** Data regarding the role of kidney adiposity, its clinical implications, and its dynamics during weight-loss are sparse. We investigated the effect of long-term weight-loss induced intervention diets on dynamics of renal-sinus-fat, an ectopic fat depot, and %renal-parenchymal-fat, lipid accumulation within the renal parenchyma. **METHODS:** We randomized 278 participants with abdominal obesity/dyslipidemia to low-fat or Mediterranean/low-carbohydrate diets, with or without exercise. We quantified renal-sinus-fat and %renal-parenchymal-fat by whole body magnetic-resonance-imaging. **RESULTS:** Participants (age = 48 years; 89% men; body-mass-index = 31 kg/m²) had 86% retention to the trial after 18 months. Both increased renal-sinus-fat and %renal-parenchymal-fat were directly associated with hypertension, and with higher abdominal deep-subcutaneous-adipose-tissue and visceral-adipose-tissue (p of trend < 0.05 for all) after adjustment for body weight. Higher renal-sinus-fat was associated with lower estimated-glomerular-filtration-rate and with higher microalbuminuria and %HbA1C beyond body weight. After 18 months of intervention, overall renal-sinus-fat (-9%; $p < 0.05$ vs. baseline) but not %renal-parenchymal-fat (-1.7%; $p = 0.13$ vs. baseline) significantly decreased, and similarly across the intervention groups. Renal-sinus-fat and %renal-parenchymal-fat changes were correlated with weight-loss per-se ($p < 0.05$). In a model adjusted for age, sex, and visceral-adipose-tissue changes, 18 months reduction in renal-sinus-fat associated with decreased pancreatic, hepatic and cardiac fats ($p < 0.05$ for all) and with decreased cholesterol/high-density lipoprotein-cholesterol (HDL-c) ($\beta = 0.13$; $p = 0.05$), triglycerides/HDL-c ($\beta = 0.13$; $p = 0.05$), insulin ($\beta = 0.12$; $p = 0.05$) and gamma glutamyl transpeptidase ($\beta = 0.24$; $p = 0.001$), but not with improved renal function parameters or blood pressure. Decreased intake of sodium was associated with a reduction in %renal-parenchymal-fat, after adjustment for 18 months weight-loss ($\beta =$

0.15; $p = 0.026$) and hypertension ($\beta = 0.14$; $p = 0.04$). **CONCLUSIONS:** Renal-sinus-fat and renal-parenchymal-fat are fairly related to weight-loss. Decreased renal-sinus-fat is associated with improved hepatic parameters, independent of changes in weight or hepatic fat, rather than with improved renal function or blood pressure parameters.

Key area: Body Weight and Composition

2017

Bamberger C, Rossmeier A, Lechner K, Wu L, Waldmann E, Stark RG, Altenhofer J, Henze K, Parhofer KG. A walnut-enriched diet reduces lipids in healthy Caucasian subjects, independent of recommended macronutrient replacement and time point of consumption: a prospective, randomized, controlled trial.

Nutrients. 2017 Oct 6;9(10). pii: E1097. doi: 10.3390/nu9101097

Abstract: Studies indicate a positive association between walnut intake and improvements in plasma lipids. We evaluated the effect of an isocaloric replacement of macronutrients with walnuts and the time point of consumption on plasma lipids. We included 194 healthy subjects (134 females, age 63 ± 7 years, BMI 25.1 ± 4.0 kg/m²) in a randomized, controlled, prospective, cross-over study. Following a nut-free run-in period, subjects were randomized to two diet phases (8 weeks each). Ninety-six subjects first followed a walnut-enriched diet (43 g walnuts/day) and then switched to a nut-free diet.

Ninety-eight subjects followed the diets in reverse order. Subjects were also randomized to either reduce carbohydrates ($n = 62$), fat ($n = 65$), or both ($n = 67$) during the walnut diet, and instructed to consume walnuts either as a meal or as a snack. The walnut diet resulted in a significant reduction in fasting cholesterol (walnut vs control: -8.5 ± 37.2 vs. -1.1 ± 35.4 mg/dL; $p = 0.002$), non-HDL cholesterol (-10.3 ± 35.5 vs. -1.4 ± 33.1 mg/dL; $p \leq 0.001$), LDL-cholesterol (-7.4 ± 32.4 vs. -1.7 ± 29.7 mg/dL; $p = 0.029$), triglycerides (-5.0 ± 47.5 vs. 3.7 ± 48.5 mg/dL; $p = 0.015$) and apoB (-6.7 ± 22.4 vs. -0.5 ± 37.7 ; $p \leq 0.001$), while HDL-cholesterol and lipoprotein (a) did not change significantly. Neither macronutrient replacement nor time point of consumption significantly affected the effect of walnuts on lipids. Thus, 43 g walnuts/d improved the lipid profile independent of the recommended macronutrient replacement and the time point of consumption.

Key area: Heart Health

Bitok E, Jaceldo-Siegl K, Rajaram S, Serra-Mir M, Roth I, Feitas-Simoes T, Ros E, Sabaté, J. (2017). Favourable nutrient intake and displacement with long-term walnut supplementation among elderly: Results of a randomised trial.

Br J Nutr. 2017 Aug;118(3):201-209. doi: 10.1017/S0007114517001957

Abstract: Older adults tend to require fewer energy content and higher levels of nutrients to promote and maintain optimal health. Regrettably, dietary variety and quality are known to decline with advancing age. We conducted a 2-year prospective, randomised, dietary intervention trial where we asked free-living elderly subjects (63–79 years) on self-selected habitual diets to incorporate walnuts daily into their diet (15 % energy). We then compared their nutrient intake with that of a similar group of concurrent participants on self-selected habitual diets but abstaining from walnut consumption (control). No recipes or advice on use of nuts were provided. Dietary intake was assessed by multiple unannounced 24-h telephone dietary recalls. On average, walnut supplement consumption was 43 g/d or 1171.5 kJ (281 kcal). The mean daily energy intake was 954 kJ (228 kcal) higher in the walnut group than in the control group ($P < 0.001$). Compared with control, participants in the walnut group reported significantly higher intake of total protein, vegetable protein, total PUFA and n-3 and n-6 PUFA; and significantly lower intake of total carbohydrate, animal protein, SFA, and Na. An estimated 19 % of total energy and 25 % of total fat from other food sources was displaced. Displacement of MUFA and total

PUFA was 21 and 16 %, respectively. Thus adding a daily supplement of walnuts to an ad libitum diet of older adults can induce favourable modifications to the nutrient profile in a way that addresses declining nutrient intake associated with aging.

Key area: Body Weight and Composition

Byerley LO, Samuelson D, Blanchard E, Luo M, Lorenzen BN, Banks S, Ponder MA, Welsh DA, Taylor CM. Changes in the gut microbial communities following addition of walnuts to the diet.

J Nutr Biochem. 2017 Oct;48:94-102. doi: 10.1016/j.jnutbio.2017.07.001. Epub 2017 Jul 9.

Abstract: Walnuts are rich in omega-3 fatty acids, phytochemicals and antioxidants making them unique compared to other foods. Consuming walnuts has been associated with health benefits including a reduced risk of heart disease and cancer. Dysbiosis of the gut microbiome has been linked to several chronic diseases. One potential mechanism by which walnuts may exert their health benefit is through modifying the gut microbiome. This study identified the changes in the gut microbial communities that occur following the inclusion of walnuts in the diet. Male Fischer 344 rats (n=20) were randomly assigned to one of two diets for as long as 10 weeks: 1) walnut (W), and 2) replacement (R) in which the fat, fiber, and protein in walnuts were matched with corn oil, protein casein, and a cellulose fiber source. Intestinal samples were collected from the descending colon, the DNA isolated, and the V3-V4 hypervariable region of 16S rRNA gene deep sequenced on an Illumina MiSeq for characterization of the gut microbiota. Body weight and food intake did not differ significantly between the two diet groups. The diet groups had distinct microbial communities with animals consuming walnuts displaying significantly greater species diversity. Walnuts increased the abundance of Firmicutes and reduced the abundance of Bacteroidetes. Walnuts enriched the microbiota for probiotic-type bacteria including Lactobacillus, Ruminococcaceae, and Roseburia while significantly reducing Bacteroides and Anaerotruncus. The class Alphaproteobacteria was also reduced. Walnut consumption altered the gut microbial community suggesting a new mechanism by which walnuts may confer their beneficial health effects.

Key area: Gut Health

Coffua LS, Martin-Deleon PA. Effectiveness of a walnut-enriched diet on murine sperm: involvement of reduced peroxidative damage.

Heliyon. 2017 Feb 20;3(2):e00250. doi : 10.1016/j.heliyon.2017.e00250. eCollection 2017 Feb.

Abstract: A walnut supplement for a Western-style diet in men was shown to improve sperm motility, vitality, and morphology. To gain further insights into factors underlying this improvement, we administered a parallel walnut-enriched diet to mice [including those with a defect in sperm motility due to deletion of Plasma Membrane Ca²⁺-ATPase 4 (Pmca4^{-/-})] to determine if there is a similar improvement that is accompanied by reduced sperm membrane peroxidative damage. Although sperm vitality and acrosome reaction rate were unaffected, the diet led to a significant improvement in motility (P < 0.05) and morphology (P < 0.04) in wild-type sperm and in morphology (P < 0.01) in Pmca4^{-/-}, confirming the diet's efficacy, which appeared to be more modest in mice than in humans. In both strains of mice, the diet resulted in a significant decrease in sperm lipid peroxidation (oxidative stress) levels, but did not rescue the significantly increased apoptotic levels seen in the testis and epididymis of Pmca4 nulls. Our findings support the effectiveness of walnuts on sperm quality, associated with reduced peroxidative damage; and suggest that oxidative stress is involved in the mechanism(s) underlying male reproductive defects in Pmca4^{-/-}.

Key area: Reproductive Health

Fisher DR, Poulouse SM, Bielinski DF, Shukitt-Hale B. Serum metabolites from walnut-fed aged rats attenuate stress-induced neurotoxicity in BV-2 microglial cells.

Nutr Neurosci. 2017 Feb;20(2):103-109. doi: 10.1179/1476830514Y.0000000150. Epub 2016 Mar 2.

Abstract: The shift in equilibrium towards excess reactive oxygen or nitrogen species production from innate antioxidant defenses in brain is a critical factor in the declining neural function and cognitive deficit accompanying age. Previous studies from our laboratory have reported that walnuts, rich in polyphenols, antioxidants, and omega fatty acids such as alpha-linolenic acid and linoleic acid, improve the age-associated declines in cognition and neural function in rats. Possible mechanisms of action of these effects include enhancing protective signaling, altering membrane microstructures, decreasing inflammation, and preventing accumulation of polyubiquitinated protein aggregates in critical regions of the brain. In the current study, we investigated whether the serum collected from aged animals fed with walnut diets (0, 6, and 9%, w/w) would enhance protection on stressed BV-2 microglia in vitro. In the growth medium, fetal bovine serum was substituted with the serum collected from 22-month-old rats fed per protocol for 12 weeks. Walnut diet serum (6 and 9%) significantly attenuated lipopolysaccharide-induced nitrite release compared to untreated control cells and those treated with serum from rats fed 0% walnut diets. The results also indicated a significant reduction in pro-inflammatory tumor necrosis factor-alpha, cyclooxygenase-2, and inducible nitric oxide synthase. These results suggest antioxidant and anti-inflammatory protection or enhancement of membrane-associated functions in brain cells by walnut serum metabolites.

Key area: Cognitive Health

Gepner Y, Shelef I, Schwarzfuchs D, Cohen N, Bril N, Rein M, Tsaban G, Zelicha H, Yaskolka Meir A, Tene L, Sarusy B4, Rosen P, Hoffman JR, Stout JR, Thiery J, Ceglarek U, Stumvoll M, Blüher M, Stampfer MJ, Shai I. Intramyocellular triacylglycerol accumulation across weight loss strategies; Sub-study of the CENTRAL trial.

PLoS One. 2017 Nov 30;12(11):e0188431. doi: 10.1371/journal.pone.0188431. eCollection 2017.

Abstract: BACKGROUND: Intramyocellular triacylglycerol (IMTG) is utilized as metabolic fuel during exercise and is linked to insulin resistance, but the long-term effect of weight loss strategies on IMTG among participants with abdominal fat, remain unclear. METHODS: In an 18-month trial, sedentary participants with abdominal fat/dyslipidemia were randomized to either a low-fat (LF) or Mediterranean/low-carbohydrate (MED/LC) diet (including 28g·day⁻¹ of walnuts). After 6-months, the participants were re-randomized to moderate intense physical activity (PA+) or non-physical activity (PA-). Magnetic resonance imaging (MRI) was used to quantify changes of IMTG, abdominal sub-depots, hepatic and intermuscular fats. RESULTS: Across the 277 participants [86% men, age = 48 years, body-mass-index (BMI) = 31kg/m², visceral fat = 33%] 86% completed the 18-m trial. At baseline, women had higher IMTG than men (3.4% vs. 2.3%, p<0.001) and increased IMTG was associated with aging and higher BMI, visceral and intermuscular fats, HbA1c%, HDL-c and leptin (p<0.05), but not with intra-hepatic fat. After 18 month of intervention and a -3 kg mean weight loss, participants significantly increased IMTG by 25%, with a distinct effect in the MED/LCPA+ group as compared to the other intervention groups (57% vs. 9.5-18.5%, p<0.05). Changes in IMTG were associated with visceral and intermuscular fat, metabolic syndrome, insulin and leptin (p<0.05 for all), however, these associations did not remain after adjustment for visceral fat changes. CONCLUSIONS: Lifestyle strategies differentially affect IMTG accumulation; combination of exercise with decreased carbohydrate/increased unsaturated fat proportion intake greatly increase IMTG. Our findings suggest that increased IMTG during diet-induced moderate weight loss may not be directly related to cardiometabolic risk.

Key Area: Body Weight and Composition

Ndanuko RN, Tapsell LC, Charlton KE, Neale EP, Batterham MJ. Associations between dietary patterns and blood pressure in a clinical sample of overweight adults.

J Acad Nutr Diet. 2017 Feb;117(2):228-239. doi: 10.1016/j.jand.2016.07.019. Epub 2016 Sep 22.

Abstract: BACKGROUND: Dietary pattern analysis provides important evidence revealing diet-disease relationships. It may be especially useful in areas less well researched, such as diet and hypertension in clinical populations. OBJECTIVE: The aim of this study was to identify the association between dietary patterns and blood pressure (BP) in a sample of overweight adults volunteering for a clinical trial for weight loss. DESIGN: This cross-sectional analysis used baseline data from the HealthTrack study, a 12-month randomized controlled trial. Dietary intake was evaluated with 4-day food records. PARTICIPANTS/SETTING: Participants were 328 adults recruited from the Illawarra region of New South Wales, Australia, between May 2014 and April 2015. MAIN OUTCOME MEASURES: Resting BP and 24-hour urine sodium and potassium were measured. STATISTICAL ANALYSIS: Dietary patterns were derived by principal component analysis from 21 food groups. Multiple regression analysis was performed to assess the association between the extracted dietary patterns and BP. RESULTS: The participants' mean age was 43.6 ± 8.0 years, mean body mass index was 32.4 ± 4.2 , and mean systolic BP/diastolic BP was $124.9 \pm 14.5/73.3 \pm 9.9$ mm Hg. Six major dietary patterns were identified: "nuts, seeds, fruit, and fish," "milk and meat," "breads, cereals, and snacks," "cereal-based products, fats, and oils," "alcohol, eggs, and legumes," and "savory sauces, condiments, and meat." The "nuts, seeds, fruit, and fish" dietary pattern was significantly and inversely associated with systolic BP ($F [7,320]=15.248$; $P<0.0005$; adjusted $R^2=0.234$ and diastolic BP ($F [7,320]=17.351$; $P<0.0005$; adjusted $R^2=0.259$) and sodium-to-potassium ratio ($F [7,320]=6.210$; $P<0.0005$; adjusted $R^2=0.100$). CONCLUSIONS: A dietary pattern rich in nuts, seeds, fruit, and fish was inversely associated with blood pressure in this clinical sample. The findings suggest that current dietary guidelines are relevant to an overweight clinical population and support the value of dietary pattern analysis when exploring the diet-disease relationship.

Key area: Heart Health

Neale EP, Tapsell LC, Martin A, Batterham MJ, Wibisono C, Probst YC. Impact of providing walnut samples in a lifestyle intervention for weight loss: a secondary analysis of the HealthTrack trial.

Food Nutr Res. 2017 Jul 3;61(1):1344522. doi: 10.1080/16546628.2017.1344522. eCollection 2017.

Abstract: BACKGROUND: Being more specific about individual food choices may be advantageous for weight loss. Including a healthy food (e.g. walnuts) may help to expose effects. OBJECTIVE: To examine the impact of including walnuts in diets for weight loss. Design: Secondary analysis of the HealthTrack lifestyle intervention trial. Overweight and obese participants were randomized to: usual care (C), interdisciplinary intervention including individualized dietary advice (I), or interdisciplinary intervention including 30 g walnuts/day (IW). Changes in body weight, energy intake, intake of key foods, physical activity, and mental health over three and 12 months were explored. RESULTS: A total of 293 participants completed the intensive three-month study period, and 175 had data available at 12 months. The IW group achieved the greatest weight loss at three months. IW reported significant improvements in healthy food choices, and decreased intakes of discretionary foods/beverages, compared to C. Weight loss remained greatest in IW at 12 months. DISCUSSION: Significant effects were seen after three months, with the IW group achieving greater weight loss and more favorable changes in food choices. CONCLUSIONS: Including 30 grams walnuts/day in an individualized diet produced weight loss and positive changes in food choice.

Key Area: Body Weight and Composition

Rajaram S, Valls-Pedret C, Cofán M, Sabaté J, Serra-Mir M, Pérez-Heras Ana M., Arechiga A, Casaroli-Marano RP, Alforja S, Sala-Vila A, Doménech M, Roth I, Freitas-Simoes TM., Calvo C, López-Illamola A, Haddad E, Bitok E, Kazzzi N, Huey L, Fan J, Ros E. The Walnuts and Healthy Aging Study (WAHA): Protocol for a nutritional intervention trial with walnuts on brain aging. *Front Aging Neurosci.* 2017 Jan 10;8:333. doi: 10.3389/fnagi.2016.00333. eCollection 2016.

Abstract: INTRODUCTION: An unwanted consequence of population aging is the growing number of elderly at risk of neurodegenerative disorders, including dementia and macular degeneration. As nutritional and behavioral changes can delay disease progression, we designed the Walnuts and Healthy Aging (WAHA) study, a two-center, randomized, 2-year clinical trial conducted in free-living, cognitively healthy elderly men and women. Our interest in exploring the role of walnuts in maintaining cognitive and retinal health is based on extensive evidence supporting their cardio-protective and vascular health effects, which are linked to bioactive components such as n-3 fatty acids and polyphenols. METHODS: The primary aim of WAHA is to examine the effects of ingesting walnuts daily for 2 years on cognitive function and retinal health, assessed with a battery of neuropsychological tests and optical coherence tomography, respectively. All participants followed their habitual diet, adding walnuts at 15% of energy (~30-60 g/d) (walnut group) or abstaining from walnuts (control group). Secondary outcomes include changes in adiposity, blood pressure, and serum and urinary biomarkers in all participants and brain magnetic resonance imaging in a subset. Results: From May 2012 to May 2014, 708 participants (mean age 69 years, 68% women) were randomized. The study ended in May 2016 with a 90% retention rate. DISCUSSION: The results of WAHA might provide high-level evidence of the benefit of regular walnut consumption in delaying the onset of age-related cognitive impairment and retinal pathology. The findings should translate into public health policy and sound recommendations to the general population.

Key area: Cognitive Health

Rana BK, Flatt SW, Heath DD, Pakiz B, Quintana EL, Natarajan L, Rock CL. The IL-6 gene promoter SNP and plasma IL-6 in response to diet intervention.

Nutrients. 2017 May 27;9(6). pii: E552. doi: 10.3390/nu9060552

Abstract: We recently reported that interleukin-6 (IL-6), an inflammatory marker associated with breast pathology and the development of breast cancer, decreases with diet intervention and weight loss in both insulin-sensitive and insulin-resistant obese women. Here, we tested whether an individual's genotype at an IL6 SNP, rs1800795, which has previously been associated with circulating IL-6 levels, contributes to changes in IL-6 levels or modifies the effect of diet composition on IL-6 in these women. We genotyped rs1800795 in overweight/obese women (N = 242) who were randomly assigned to a lower fat (20% energy), higher carbohydrate (65% energy) diet; a lower carbohydrate (45% energy), higher fat (35% energy) diet; or a walnut-rich (18% energy), higher fat (35% energy), lower carbohydrate (45% energy) diet in a 1-year weight loss intervention study of obesity-related biomarkers for breast cancer incidence and mortality. Plasma IL-6 levels were measured at baseline, 6 and 12 months. At baseline, individuals with a CC genotype had significantly lower IL-6 levels than individuals with either a GC or GG genotype ($p < 0.03$; 2.72 pg/mL vs. 2.04 pg/mL), but this result was not significant when body mass index (BMI) was accounted for; the CC genotype group had lower BMI ($p = 0.03$; 32.5 kg/m² vs. 33.6 kg/m²). We did not observe a 2-way interaction of time*rs1800795 genotype or diet*rs1800795 genotype. Our findings provide evidence that rs1800795 is associated with IL-6 levels, but do not support a differential interaction effect of rs1800795 and diet composition or time on changes in circulating IL-6 levels. Diet intervention and weight loss are an important strategy for reducing plasma IL-6, a risk factor of breast cancer in women, regardless of their rs1800795 genotype.

Key area: Cancer

Rock CL, Flatt SW, Barkai HS, Pakiz B, Heath DD. Walnut consumption in a weight reduction intervention: effects on body weight, biological measures, blood pressure and satiety.

Nutr J. 2017 Dec 4;16(1):76. doi: 10.1186/s12937-017-0304-z

Abstract: BACKGROUND: Dietary strategies that help patients adhere to a weight reduction diet may increase the likelihood of weight loss maintenance and improved long-term health outcomes. Regular nut consumption has been associated with better weight management and less adiposity. The objective

of this study was to compare the effects of a walnut-enriched reduced-energy diet to a standard reduced-energy-density diet on weight, cardiovascular disease risk factors, and satiety. **METHODS:** Overweight and obese men and women ($n = 100$) were randomly assigned to a standard reduced-energy-density diet or a walnut-enriched (15% of energy) reduced-energy diet in the context of a behavioral weight loss intervention. Measurements were obtained at baseline and 3- and 6-month clinic visits. Participants rated hunger, fullness and anticipated prospective consumption at 3 time points during the intervention. Body measurements, blood pressure, physical activity, lipids, tocopherols and fatty acids were analyzed using repeated measures mixed models. **RESULTS:** Both study groups reduced body weight, body mass index and waist circumference (time effect $p < 0.001$ for each). Change in weight was $-9.4 (0.9)\%$ vs. $-8.9 (0.7)\%$ (mean [SE]), for the standard vs. walnut-enriched diet groups, respectively. Systolic blood pressure decreased in both groups at 3 months, but only the walnut-enriched diet group maintained a lower systolic blood pressure at 6 months. The walnut-enriched diet group, but not the standard reduced-energy-density diet group, reduced total cholesterol and low-density lipoprotein cholesterol (LDL-C) at 6 months, from 203 to 194 mg/dL and 121 to 112 mg/dL, respectively ($p < 0.05$). Self-reported satiety was similar in the groups. **CONCLUSIONS:** These findings provide further evidence that a walnut-enriched reduced-energy diet can promote weight loss that is comparable to a standard reduced-energy-density diet in the context of a behavioral weight loss intervention. Although weight loss in response to both dietary strategies was associated with improvements in cardiovascular disease risk factors, the walnut-enriched diet promoted more favorable effects on LDL-C and systolic blood pressure.

Key Area: Body Weight and Composition

Rock CL, Flatt SW, Nichols JF, Pakiz B, Barkai HS, Wing DR, Heath DD, Buehler AE. Changes in disinhibition, restraint and hunger and associated characteristics during a weight loss intervention.

Front. Aging Neurosci. 2017 Jan 10;8:333. doi: 10.3389/fnagi.2016.00333

Abstract: **BACKGROUND:** Understanding how diet composition and personal characteristics relate to eating behaviors of individuals in weight loss programs could better inform the development and expectations of prescribed weight loss regimens. The purpose of this study was to examine whether diet composition has a significant effect on eating behaviors of individuals participating in a weight loss intervention and what characteristics significantly correlate with changes in such behaviors.

METHODS: The Eating Inventory questionnaire was used to assess eating behaviors of restraint, disinhibition and hunger at baseline and 6 months among individuals participating in a weight loss intervention who were prescribed a standard reduced-energy-density diet or a walnut-enriched reduced-energy diet as one component of a behavioral weight loss intervention. **Results:** After 6 months of intervention, there were significant improvements in both study arms on all scales and most subscales, with overall restraint increasing, disinhibition decreasing and hunger decreasing. Other correlations were that as restraint increased, weight loss increased; as hunger decreased, disinhibition decreased; and as physical activity increased, disinhibition decreased. Among personal characteristics, restraint was significantly associated with sex, age and education at baseline, with women reporting higher restraint than men, younger participants reporting lower restraint than older individuals, and college graduates reporting lower restraint than noncollege graduates. Over the course of the weight loss program, there were significant correlations between increased restraint and being male as well as decreased hunger and being a college graduate. **CONCLUSION:** Our results highlight the significance of restraint among the three eating behaviors as it was the only behavior significantly correlated with weight loss and was also correlated with several personal characteristics. Disinhibition and hunger showed other significant correlations with one another that do not directly correlate with weight loss but may be important in other aspects of weight control such as weight loss maintenance.

Key Area: Body Weight and Composition

Rock CL, Flatt SW, Barkai HS, Pakiz B, Heath DD. A walnut-containing meal had similar effects on early satiety, CCK, and PYY, but attenuated the postprandial GLP-1 and insulin response compared to a nut-free control meal.

Appetite. 2017 Oct 1;117:51-57. doi: 10.1016/j.appet.2017.06.008. Epub 2017 Jun 10.

Abstract: Regular nut consumption is associated with lower adiposity and reduced weight gain in adulthood. Walnut feeding studies have observed minimal effect on body weight despite potential additional energy intake. Several mechanisms may explain why consuming nuts promotes weight control, including increased early phase satiety, possibly reflected in postprandial response of gastrointestinal and pancreatic peptides hypothesized to affect appetite. The purpose of this study was to compare postprandial insulin, glucagon and gastrointestinal peptide response and satiety following a meal with ~54% of energy from walnuts or cream cheese, using a within-subject crossover study design in overweight/obese adults (N = 28). Sixty minutes after the walnut-containing meal, glucagon-like peptide-1 was lower than after the reference meal ($p=0.0433$), and peptide YY, cholecystokinin and ghrelin did not differ after the two meals. Sixty and 120 min after the walnut-containing meal, pancreatic polypeptide ($p = 0.0014$ and $p = 0.0002$) and glucose-dependent insulintropic peptide ($p < 0.0001$ and $p = 0.0079$) were lower than after the reference meal, and 120 min after the walnut-containing meal, glucagon was higher ($p=0.0069$). Insulin and C-peptide increased at 60 min in response to both meals but were lower at 120 min after the walnut-containing meal ($p=0.0349$ and 0.0237 , respectively). Satiety measures were similar after both meals. These findings fail to support the hypothesis that acute postprandial gastrointestinal peptide response to a walnut-containing meal contributes to increased satiety. However, inclusion of walnuts attenuated the postprandial insulin response, which may contribute to the more favorable lipid profile observed in association with regular walnut consumption.

Key Area: Body Weight and Composition

Schlörmann W, Lamberty J, Ludwig D, Lorkowski S, Gleis M. In vitro-fermented raw and roasted walnuts induce expression of CAT and GSTT2 genes, growth inhibition, and apoptosis in LT97 colon adenoma cells.

Nutr Res. 2017 Nov;47:72-80. doi: 10.1016/j.nutres.2017.09.004. Epub 2017 Sep 18.

Abstract: Walnuts are rich in bioactive compounds such as polyunsaturated fatty acids, polyphenols, and dietary fiber. Therefore, the consumption of walnuts can contribute to a healthy diet and may reduce the risk for colon cancer. Heat treatment like roasting may change the chemical composition of walnuts and therefore their chemopreventive properties. Therefore, the hypothesis of the present study is that different roasting conditions (RCs) alter the chemopreventive effects of walnuts. Thus, the aim of the present study was to investigate whether different RCs (RC1 = 139.7°C/25 min, RC2 = 154.5°C/20 min, and RC3 = 185.5°C/25 min) alter the chemopreventive effects of walnuts. Raw and roasted walnuts were subjected to in vitro digestion and fermentation. After treatment of LT97 colon adenoma cells with fermentation supernatants (FSs), expression of CAT, SOD2, GPx1, GSTP1, and GSTT2 genes as well as cell growth and apoptosis was examined. In comparison to the fermentation blank control, walnut FS particularly increased mRNA levels of CAT 1.7-fold and GSTT2 3.1-fold, whereas GPx1 levels were significantly decreased 0.6-fold. Walnut FS decreased growth of adenoma cells in a time- and dose-dependent manner. In particular, higher concentrations of walnut FS (5%) significantly increased the number of early apoptotic cells 2.0-fold and induced caspase-3 activity 6.8-fold compared with the blank control. The roasting process had no direct impact on the observed effects. In sum, our results indicate that walnuts exhibit chemopreventive effects regarding the risk for colon cancer development by inducing expression of genes involved in detoxification (CAT, GSTT2) and by inducing growth inhibition and apoptosis in colon adenoma cells unaffected by moderate roasting.

Key Area: Cancer

Stevenson JL, Miller MK, Skillman HE, Paton CM, Cooper JA. A PUFA-rich diet improves fat oxidation following saturated fat-rich meal.

Eur J Nutr. 2017 Aug;56(5):1845-1857. doi: 10.1007/s00394-016-1226-9. Epub 2016 May 18.

Abstract: PURPOSE: To determine substrate oxidation responses to saturated fatty acid (SFA)-rich meals before and after a 7-day polyunsaturated fatty acid (PUFA)-rich diet versus control diet. METHODS: Twenty-six, normal-weight, adults were randomly assigned to either PUFA or control diet. Following a 3-day lead-in diet, participants completed the pre-diet visit where anthropometrics and resting metabolic rate (RMR) were measured, and two SFA-rich HF meals (breakfast and lunch) were consumed. Indirect calorimetry was used to determine fat oxidation (Fox) and energy expenditure (EE) for 4 h after each meal. Participants then consumed a PUFA-rich diet (50 % carbohydrate, 15 % protein, 35 % fat, of which 21 % of total energy was PUFA) or control diet (50 % carbohydrate, 15 % protein, 35 % fat, of which 7 % of total energy was PUFA) for the next 7 days. Following the 7-day diet, participants completed the post-diet visit. RESULTS: From pre- to post-PUFA-rich diet, there was no change in RMR (16.3 ± 0.8 vs. 16.4 ± 0.8 kcal/20 min) or in incremental area under the curve for EE (118.9 ± 20.6 - 126.9 ± 14.1 kcal/8h, ns). Fasting respiratory exchange ratio increased from pre- to post-PUFA-rich diet only (0.83 ± 0.1 - 0.86 ± 0.1 , $p < 0.05$). The postprandial change in Fox increased from pre- to post-visit in PUFA-rich diet (0.03 ± 0.1 - 0.23 ± 0.1 g/15 min for cumulative Fox; $p < 0.05$), whereas controls showed no change. CONCLUSIONS: Adopting a PUFA-rich diet initiates greater fat oxidation after eating occasional high SFA meals compared to a control diet, an effect achieved in 7 days.

Key area: Heart Health

Stevenson, JL, Paton CM, Cooper JA. Hunger and satiety responses to high-fat meals after a high-polyunsaturated fat diet: A randomized trial.

Nutrition. 2017 Sep;41:14-23. doi: 10.1016/j.nut.2017.03.008. Epub 2017 Mar 29.

Abstract: OBJECTIVE: Previous studies have shown that polyunsaturated fats (PUFAs) elicit a greater response in satiety after a single-meal challenge compared with other types of fats. The long-term effects of PUFAs on satiety, however, remain unknown. The aim of this study was to determine subjective and physiological hunger and satiety responses to high-fat (HF) meals before and after a 7-d PUFA-rich diet. METHODS: Twenty-six, healthy weight (body mass index 18–24.9 kg/m²), sedentary adults were randomly assigned to either a 7-d PUFA-rich diet ($n = 8$ men and $n = 8$ women) or a 7-d control diet ($n = 5$ men and $n = 5$ women). After a 3-d lead-in diet, participants reported for the baseline visit where anthropometrics, fasting visual analog scale (VAS) measurements, and a fasting blood sample were collected. Then, two HF meals (breakfast and lunch) were consumed. Postprandial blood draws and VAS measures were collected approximately every 30 min for 4 h after each meal, for a total of 8 h. Results From pre- to post-PUFA-rich diet, there was a decrease in fasting ghrelin ($P < 0.05$) and an increase in fasting peptide YY (PYY; $P < 0.05$); however, there were no changes in fasting insulin or leptin concentrations. The postprandial response for PYY was higher after the PUFA-rich diet visit compared to baseline ($P < 0.01$). However, there were no differences in the postprandial response for ghrelin, insulin, leptin, or VAS measures from pre- to post-diet in either the PUFA-rich diet or control (ns). CONCLUSION: A PUFA-rich diet consumed for 7 d favorably altered fasting and postprandial physiological markers of hunger and satiety; yet, did not alter subjective ratings of hunger or fullness.

Key Area: Body Weight and Composition

Tapsell LC, Lonergan M, Batterham MJ, Neale EP, Martin A, Thorne R, Deane F, Peoples G. Effect of interdisciplinary care on weight loss: a randomised controlled trial.

BMJ Open 2017;7:e014533. doi:10.1136/bmjopen-2016-014533

Abstract: OBJECTIVE: To determine the effectiveness of a novel interdisciplinary treatment compared with usual care on weight loss in overweight and obese adult volunteers. Design Single blinded

controlled trial. Participants randomly assigned to usual care (C, general guideline based diet and exercise advice), intervention (I, interdisciplinary protocol) or intervention + a healthy food supplement (30 g walnuts/day) (IW). Setting Community based study, Illawarra region, south of Sydney, Australia. PARTICIPANTS: Generally well volunteer adult residents, 25-54 years, body mass index (BMI) 25-40kg/m² were eligible. At baseline 439 were assessed, 377 were randomised, 298 completed the 3-month intensive phase and 178 completed the 12-month follow-up. Interventions Treatment was provided at clinic visits intensively (0 months, 1 month, 2 months, 3 months) then quarterly to 12 months. Support phone calls were quarterly. All participants underwent blinded assessments for diet, exercise and psychological status. PRIMARY AND SECONDARY MEASURES: The primary outcome was difference in weight loss between baseline and 12 months (clinically relevant target 5% loss). Secondary outcomes were changes in blood pressure, fasting blood glucose and lipids, and changes in diet, exercise and psychological parameters. RESULTS: At 12 months, differences in weight loss were identified ($p < 0.001$). The I group lost more than controls at 3 months (91.11 (92.23,90.00), $p < 0.05$) and the IW more than controls at 3 months (91.25 (92.35,90.15), $p < 0.05$) and 6 months (92.20 (93.90,90.49), $p < 0.01$). The proportion achieving 5% weight loss was significantly different at 3 months, 6 months and 9 months ($p = 0.04$, $p = 0.03$, $p = 0.03$), due to fewer controls on target at 3 months, 6 months and 9 months and more IW participants at 6 months. Reductions in secondary outcomes (systolic blood pressure, blood glucose/lipid parameters and lifestyle measures) followed the pattern of weight loss. CONCLUSIONS: An interdisciplinary intervention produced greater and more clinically significant and sustained weight loss compared with usual care. The intensive phase was sufficient to reach clinically relevant targets, but long-term management plans may be required.

Key Area: Body Weight and Composition

Tsaban G, Wolak A, Avni-Hassid H, Gepner Y, Shelef I, Henkin Y, Schwarzfuchs D, Cohen N, Bril N, Rein M, Serfaty D, Kenigsbuch S, Tene L, Zelicha H, Yaskolka-Meir A, Komy O, Bilitzky A, Chassidim Y, Ceglarek U, Stumvoll M, Blüher M, Thiery J, Dicker D, Rudich A, Stampfer MJ, Shai I. Dynamics of intrapericardial and extrapericardial fat tissues during long-term, dietary-induced, moderate weight loss.

Am J Clin Nutr. 2017 Oct;106(4):984-995. doi:10.3945/ajcn.117.157115. Epub 2017 Aug 16.

Abstract: BACKGROUND: In view of evidence linking pericardial fat accumulation with increased cardiovascular disease risk, strategies to reduce its burden are needed. Data comparing the effects of specific long-term dietary interventions on pericardial fat tissue mobilization are sparse. OBJECTIVE: We sought to evaluate intrapericardial-fat (IPF) and extrapericardial-fat (EPF) changes during weight-loss interventions by different dietary regimens. DESIGN: During 18 mo of a randomized controlled trial, we compared a Mediterranean/low-carbohydrate (MED/LC) diet plus 28 g walnuts/d with a calorically equal low-fat (LF) diet among randomly assigned participants with moderate abdominal obesity. We performed whole-body MRI and volumetrically quantified IPF and EPF among 80 participants to follow the 18-mo changes. RESULTS: The participants [mean age: 48.6 y; mean body mass index (BMI; in kg/m²); 31.7; 90% men] had baseline IPF and EPF (mean \pm SD) volumes of 172.4 \pm 53.3 mL and 194.9 \pm 71.5 mL, respectively. The 18-mo moderate weight loss of 3.7 kg was similar in both groups, but the reduction in waist circumference was higher in the MED/LC group (-6.9 \pm 6.6 cm) than in the LF diet group (-2.3 \pm 6.5 cm; $P = 0.01$). After 18 mo, the IPF volume had reduced twice as much in the MED/LC group compared with the LF group [-37 \pm 26.2 mL (-22% \pm 15%) compared with -15.5 \pm 26.2 mL (-8% \pm 15%), respectively; $P < 0.05$, after adjustment for changes in weight or visceral adipose tissue]. The EPF volume had reduced similarly in both groups [-41.6 \pm 30.2 mL (-23% \pm 16%) in the MED/LC group compared with -37.9 \pm 28.3 mL (-19% \pm 14%) in the LF group; $P > 0.1$]. After controlling for weight loss, IPF and EPF volume reduction paralleled changes in lipid profile but not with improved glycemic profile variables: the IPF relative reduction was associated with a decrease in triglycerides (TGs) ($\beta = 0.090$; 95% CI: 0.026, 0.154; $P = 0.007$) and the ratio of TGs to high-density

lipoprotein (HDL) cholesterol ($\beta = 2.689$; 95% CI: 0.373, 5.003; $P = 0.024$), and the EPF relative reduction was associated with an increase in HDL cholesterol ($\beta = -0.452$; 95% CI: -0.880, -0.023; $P = 0.039$) and a decrease in total cholesterol and HDL cholesterol ($\beta = 3.766$; 95% CI: 1.092, 6.440; $P = 0.007$). **CONCLUSIONS:** Moderate but persistent dietary-induced weight loss substantially decreased both IPF and EPF volumes. Reduction of pericardial adipose tissues is independently associated with an improved lipid profile. The Mediterranean diet, rich in unsaturated fats and restricted carbohydrates, is superior to an LF diet in terms of the IPF burden reduction.

Key Area: Heart Health

Vitolins MZ, Blackwell CS, Williamson JD, Foy CG, Wilmoth S, Sink KM, Reynolds LM, Byington RP, Reboussin DM. The feasibility of walnut and extra virgin olive oil supplementation in older adults.

J Food Nutr Sci. 2017; 4(1):49-54. doi: 10.15436/2377-0619.17.1342

Abstract: Researchers in Spain provided randomized, controlled trial evidence that adding extra virgin olive oil (EVOO) and nuts to diets of older adults lowered cardiovascular disease risk. Supplementing these foods may represent a simple and straightforward approach to favourable dietary change with potential for dissemination to the broader public. This was an 8-week feasibility trial in which all participants were asked to supplement their ad libitum diets with both walnuts and EVOO to determine their interest in participating and to assess retention and adherence once enrolled. Inclusion criteria were broad: Adults ≥ 55 years old treated for hypertension with medication; exclusions included walnuts/EVOO allergies, homebound or diagnosis of dementia. Recruitment was assessed as number of weeks to accrue 25 participants. Adherence was assessed by participant self-report using a daily diary. Blood pressure (BP), body weight, and HDL cholesterol were measured to estimate the variability of outcomes. Results: Twenty-seven participants were recruited in 2 ½ weeks; 26 of the 27 participants remained in the study for a retention rate of 96% (95% CI: 78% - 100%). Of 216 possible diaries, 185 were returned (86%). On average, weight increased over 8 weeks by 0.8 pounds. Mean systolic BP dropped by 0.25 mmHg while mean diastolic BP decreased by 1.0 mmHg. Mean HDL increased by 1.96 mg/dL. A full scale walnut/EVOO trial in older adults with hypertension seems realistic given our high rates of recruitment, retention, and adherence, coupled with minimal weight gain and favorable trends in BP and HDL.

Key area: Research Methodology

Wibisono C, Probst Y, Neale E, Tapsell L. Changes in diet quality during a 12 month weight loss randomised controlled trial.

BMC Nutrition. 2017;3:38 doi: 10.1186/s40795-017-0157-z

Abstract: **BACKGROUND:** Reductions in energy intake are seen in weight loss trials, but whether this occurs with improvements to diet quality (DQ) is less established. The aim of this study was to evaluate changes in diet quality in a sample of volunteers in a weight loss trial. **METHODS:** This was a secondary analysis of dietary data from a lifestyle intervention trial (the HealthTrack study) which advised on dietary guidelines. The trial ran for 12 months with three treatment groups: control (general advice C), intervention (individualised advice, I), and intervention plus a supplement of walnuts (IW). Both the published a priori diet quality score (APDQS, maximum score 164) and a study specific Diet Quality Tracker (DQT, maximum score 44) indicated compliance to dietary advice. DQ scores calculated at 0, 3months and 12months were evaluated using two-way RMANOVA, one-way ANOVA and one-way RMANOVA. Changes in intakes of food groups and nutrients were analysed using Kruskal-Wallis and Friedman's tests. Results There were no differences between groups at baseline, but at 3months IW recorded higher DQ scores (APDQS: 96 ± 10 ; DQT: 22 ± 5 , $P < 1 \times 10^{-3}$ for both) compared to I (APDQS: 91 ± 13 , $P < 1 \times 10^{-3}$; DQT: 21 ± 4 , $P < 1 \times 10^{-2}$) and C (APDQS: 87 ± 12 , $P < 5 \times 10^{-2}$; DQT: 19 ± 4 , $P > 5 \times 10^{-2}$), and a higher consumption of nuts at 3 months ($P < 1 \times 10^{-3}$),

and 12 months ($P < 1 \times 10^{-2}$). All groups reported decreased intakes of discretionary foods/beverages assessed by the DQT ($P < 1 \times 10^{-3}$ for IW and I; $P < 1 \times 10^{-2}$ for C). The APDQS showed this as reduced intakes of grain based desserts ($P < 1 \times 10^{-3}$ at 3 and 12 months), and salty snacks at 12 months ($P < 1 \times 10^{-3}$ for IW and I; $P < 5 \times 10^{-2}$ for C). Intakes of monounsaturated and saturated fatty acids were lowest, and polyunsaturated fatty acids highest for IW ($P < 1 \times 10^{-3}$), resulting in a higher dietary polyunsaturated:saturated fat ratio ($P < 1 \times 10^{-3}$). **CONCLUSIONS:** Lifestyle intervention addressing dietary guidelines can lead to significant reductions in consumption of discretionary foods and saturated fat, but individualised advice may have a greater impact on improving overall DQ regardless of DQI used. Providing a healthy food supplement may help assure higher DQ and where the food is walnuts, produce commensurate differences in dietary fatty acid profiles.

Key area: Body Weight and Composition

2016

Al Wattar BH, Dodds J, Placzek A, Spyrelli E, Moore A, Hooper R, Beresford L, Roseboom TJ, Bes-Rastrollo M, Hitman G, Khan KS, Thangaratinam S; ESTEEM study group. Effect of simple, targeted diet in pregnant women with metabolic risk factors on maternal and fetal outcomes (ESTEEM): study protocol for a pragmatic multicentre randomised trial.

BMJ Open. 2016 Oct 21;6(10):e013495. doi: 10.1136/bmjopen-2016-013495

Abstract: **INTRODUCTION:** Women with metabolic risk factors are at higher risk of adverse pregnancy outcomes. Mediterranean-based dietary interventions have the potential to minimise these risks. We aim to evaluate the effectiveness of a simple, targeted intervention modelled on Mediterranean diet in preventing maternal and fetal complications in pregnant women with metabolic risk factors. **METHODS AND ANALYSIS:** Pregnant women with a singleton pregnancy <18 weeks gestation, and without pre-existing diabetes, chronic renal disease and autoimmune diseases will be recruited. Women with metabolic risk factors will be randomised to receive a dietary intervention based on a Mediterranean pattern, supplemented with extra virgin olive oil and mixed nuts until delivery. The intervention will be delivered through a series of one to one and group sessions. The primary outcome is a composite maternal outcome of pre-eclampsia or gestational diabetes and a composite fetal outcome of stillbirth, small for gestational age fetus or admission to the neonatal intensive care unit. Secondary outcomes include maternal, fetal, dietary and laboratory outcomes. We aim to randomise 1230 eligible women with metabolic risk factors. We will also compare the outcomes in women with and without these risk factors. The sample size will provide us with 80% power at 5% significance, assuming a 20% loss to follow-up to detect a 30% reduction in maternal and fetal complications. **ETHICS AND DISSEMINATION:** The ESTEEM trial is designed to provide a definitive estimate of the effects of Mediterranean dietary pattern in pregnancy on maternal and fetal outcomes. The pragmatic nature of ESTEEM ensures the applicability of its findings into clinical practice. The findings of the study will be published in peer-reviewed journals and presented at national and international scientific meetings and congresses.

Key area: Reproductive Health

Baer D, Gebauer S, Novotny J. Walnuts consumed by healthy adults provide less available energy than predicted by the Atwater Factors

J Nutr. 2016 Jan;146(1):9-13. doi: 10.3945/jn.115.217372. doi: 10.3945/jn.115.217372

Abstract: **BACKGROUND:** Previous studies have shown that the metabolizable energy (ME) content (energy available to the body) of certain nuts is less than predicted by the Atwater factors. However, very few nuts have been investigated to date, and no information is available regarding the ME of walnuts. **OBJECTIVE:** A study was conducted to determine the ME of walnuts when consumed as part

of a typical American diet. **METHODS:** Healthy adults (n = 18; mean age = 53.1 y; body mass index = 28.8 kg/m²) participated in a randomized crossover study with 2 treatment periods (3 wk each). The study was a fully controlled dietary feeding intervention in which the same base diet was consumed during each treatment period; the base diet was unsupplemented during one feeding period and supplemented with 42 g/d walnuts during the other feeding period. Base diet foods were reduced in equal proportions during the walnut period to achieve isocaloric food intake during the 2 periods. After a 9 d diet acclimation period, subjects collected all urine and feces for 1 wk (as marked by a Brilliant Blue fecal collection marker) for analysis of energy content. Administered diets, walnuts, and fecal and urine samples were subjected to bomb calorimetry, and the resulting data were used to calculate the ME of the walnuts. **RESULTS:** One 28-g serving of walnuts contained 146 kcal (5.22 kcal/g), 39 kcal/serving less than the value of 185 kcal/ serving (6.61 kcal/g) currently used for food labeling. The ME of the walnuts was 21% less than that predicted by the Atwater factors (P < 0.0001). **CONCLUSION:** Consistent with other tree nuts, Atwater factors overestimate the metabolizable energy value of walnuts. These results could help explain the observations that consumers of nuts do not gain excessive weight, and improve the accuracy for food labeling.

Key area: Nutrient & Bioactive Composition

Chung J, Kim YS, Lee J, Le JH, Choi SW, Kim Y. Compositional analysis of walnut lipid extracts and properties as an anti-cancer stem cell regulator via suppression of the self-renewal capacity.

Food Sci. Biotechnol. 25(2): 623-629 2016. doi: 10.1007/s10068-016-0087-6

Abstract: Colon cancer is a leading cause of cancer-related deaths worldwide. Effects of walnut (*Juglans regia* L.) lipid extracts (WLEs) on the self-renewal capacity of cancer stem cells (CSCs) in colon cancer were investigated. The dominant component of WLEs was α -linoleic acid (64.6%), followed by α -linolenic acid (14.6%), and oleic acid (12.6%). A higher concentration of γ -tocopherol (37.1%) was also present than of α -tocopherol (0.6%). CD133+CD44+CSCs treated with WLEs showed inhibition of colony formation and sphere formation, indicating a decrease in the self-renewal capacity. Treatment with WLEs also resulted in down-regulation of protein levels, including Notch1, phospho-GSK3 β (p-GSK3 β), and β -catenin, which are associated with CSCs and the self-renewing capacity. WLEs rich in essential fatty acids and γ -tocopherol can exert therapeutic actions on colon cancer via targeting of CSCs.

Key area: Cancer

Djoussé L, Lu B, Gaziano MJ. Effects of walnut consumption on endothelial function in people with type 2 diabetes: a randomized pilot trial.

Curr Nutr Rep. 2016 Jan 25; 1-8. doi: 10.1007/s13668-016-0149-7

Abstract: The aim of this study was to obtain preliminary data to test the hypothesis that (1) a 12-week intervention with 28 g/day of walnuts improves endothelial function in people with type 2 diabetes mellitus (DM) and (2) intake of walnuts improves plasma adipokines after 12 weeks of intervention. In this pilot randomized, single-blinded, controlled trial of 26 adult subjects with prevalent DM, each subject was randomized to a usual diet with 28 g of walnuts per day or usual diet without walnuts (control group). Reactive hyperemia index (RHI), a measure of endothelial function, was measured non-invasively at baseline and after 12 weeks using Endo-PAT2000. We used linear regression to examine the effects of the intervention on RHI. The mean age at baseline was 64.8 \pm 11.6 years; 61.5 % of participants were female, and 15.4 % had coronary artery disease. The standard error of RHI was 0.19. The difference in change in RHI during the intervention between the two groups was -0.029 (95 % confidence interval (CI) -0.52, 0.46, p = 0.23). Walnut intervention led to a suggestive increase in adiponectin, albeit non-statistically significant (difference 0.50 μ g/ml (95 % CI -0.10, 1.09), p = 0.65). We demonstrated the feasibility of the proposed randomized trial and obtained needed standard deviations to calculate the required sample size to test proposed hypotheses in an efficacy trial.

Key area: Heart Health

Donnan MS, Heath DD, Flatt SW, PakizB, Quintana EL, Rana BK, Natarajan L, Rock CL. Factors associated with tocopherol status in obese women: effects of diet composition and weight loss.

Vitam Miner. 2016 5:147. doi: 10.4172/2376-1318.1000147

Abstract: OBJECTIVE: The objective of this study was to assess factors associated with plasma α -, β -, γ -, and δ -tocopherol in obese women and to examine change in tocopherol levels after a 1-year weight loss intervention across three dietary approaches. Factors examined were dietary factors (alcohol consumption, diet composition, and supplement use) and non-dietary factors (body mass index, physical activity, plasma cholesterol levels, waist circumference, and age). METHODS: Overweight/obese, nondiabetic women were randomly assigned to one of three diets: lower carbohydrate (45% energy), higher fat (35% energy), lower fat (20% energy), higher carbohydrate (65% energy), or walnut-rich (18% energy), higher fat (35% energy), lower carbohydrate (45% energy). Data and blood samples were obtained at baseline, 6- and 12-month clinic visits (n=245, 213, and 194 respectively). RESULTS: At baseline, age was directly related to plasma α -tocopherol and inversely related to γ - and δ -tocopherol ($P<0.05$ for each); body mass index was inversely associated with plasma α -tocopherol and positively associated with β -, γ - and δ -tocopherol ($P<0.05$ for each). Physical activity was directly associated with α -tocopherol at baseline ($P<0.05$) and inversely associated with β -tocopherol at 12 months ($P=0.03$). Dietary supplement use was positively associated with α -tocopherol at baseline ($P<0.05$) and 12 months ($P=0.007$), and negatively associated with 12-month γ -tocopherol ($P=0.02$). Plasma cholesterol was positively associated with 12-month α - ($P<0.001$), β - ($P=0.003$), and γ -tocopherol ($P=0.007$). The walnut-rich diet group had higher plasma γ -tocopherol concentration than other diet groups at 12 months ($P=0.002$). CONCLUSIONS: Plasma tocopherol levels generally declined in association with weight loss in obese women, although age, adiposity, physical activity, plasma cholesterol, and dietary supplement use influenced these levels. Responses were similar to lower carbohydrate and lower fat diets, and walnut prescription minimized the reduction in plasma γ -tocopherol.

Key area: Body Weight and Composition

Gepner Y, Bril N, Shelef I, Schwarzfuchs D, Serfaty D, Rein M, Cohen N, Shemesh E, Tangi-Rosental O, Sarusi B, Goshen E, Kenigsbuch S, Chassidim Y, Golan R, Witkow S, Henkin Y, Stampfer MJ, Rudich A, Shai I. Higher visceral adiposity is associated with an enhanced early thermogenic response to carbohydrate-rich food.

Clin Nutr. 2016 Apr;35(2):422-427. doi: 10.1016/j.clnu.2015.03.004

Abstract: BACKGROUND: Studies examining the dynamics of the thermic effect of feeding (TEF) of specific food items and the relationship of TEF to visceral adiposity are limited. METHODS: We measured resting energy expenditure (REE) and early-TEF (40-min postprandial, e-TEF) after 8-h fast by indirect calorimetry in 40 obese men, and imaged abdominal fat tissues by magnetic resonance imaging. Each participant was examined on two occasions, 3-weeks apart. At each examination we measured fasting REE and then postprandial REE following the isocaloric [~ 380 kcal] consumption of either 56 gr walnuts [(8% carbohydrates; 84% fat, of which 72% polyunsaturated fat)], or 5-slices (150gr) of whole-grain bread (48% carbohydrates; 32% fat). e-TEF was calculated as the area under the curve between the fasting and postprandial tests. RESULTS: Participants had a mean age of 45 ± 8 years, body-mass-index (BMI) = 31.1 ± 3.8 kg/m², total abdominal fat area = 901.4 ± 240 cm², visceral fat area (VAT) = 260 ± 102.9 cm², fasting REE = 1854 ± 205 kcal, REE/kg = 19.39 ± 1.73 kcal/kg, and respiratory quotient (RQ, CO₂ eliminated/O₂ consumed) = 0.82 ± 0.04 . Individuals who exhibited increased e-TEF (top Δ AUC median) to bread had higher VAT (299 cm² vs. 223 cm²; $p = 0.024$) and higher BMI (32.4 kg/m² vs. 30.0 kg/m²; $p = 0.013$), compared to their peers with the lower e-TEF response (Δ AUC below median). As expected, postprandial e-TEF was higher after whole-grain bread

consumption [$\Delta\text{AUC} = +14 \text{ kcal}/40\text{min}$] compared to walnuts [$\Delta\text{AUC} = -2 \text{ kcal}/40 \text{ min}$; $p < 0.001$].
CONCLUSIONS: Higher early thermic effect of high-carbohydrate food, likely reflecting digestion, early absorption and/or sympathetic tone (rather than metabolic utilization (oxidation)), associates with visceral adiposity. Future studies are required to determine if this association represents an added causality between early carbohydrate processing and visceral fat accumulation.

Key area: Body Weight and Composition

Hagan KA, Chiuve SE, Stampfer MJ, Katz JN, Grodstein F. Greater adherence to the Alternative Healthy Eating Index is associated with lower incidence of physical function impairment in the Nurses' Health Study.

J Nutr. 2016 Jul;146(7):1341-7. doi: 10.3945/jn.115.227900

Abstract: **BACKGROUND:** Physical function is integral to healthy aging, in particular as a core component of mobility and independent living in older adults, and is a strong predictor of mortality. Limited research has examined the role of diet, which may be an important strategy to prevent or delay a decline in physical function with aging. **OBJECTIVE:** We prospectively examined the association between the Alternative Healthy Eating Index-2010 (AHEI-2010), a measure of diet quality, with incident impairment in physical function among 54,762 women from the Nurses' Health Study. **METHODS:** Physical function was measured by the Medical Outcomes Short Form-36 (SF-36) physical function scale and was administered every 4 y from 1992 to 2008. Cumulative average diet was assessed using food frequency questionnaires, administered approximately every 4 y. We used multivariable Cox proportional hazards models to estimate the HRs of incident impairment of physical function. **RESULTS:** Participants in higher quintiles of the AHEI-2010, indicating a healthier diet, were less likely to have incident physical impairment than were participants in lower quintiles ($P\text{-trend} < 0.001$). The multivariable-adjusted HR of physical impairment for those in the top compared with those in the bottom quintile of the AHEI-2010 was 0.87 (95% CI: 0.84, 0.90). For individual AHEI-2010 components, higher intake of vegetables ($P\text{-trend} = 0.003$) and fruits ($P\text{-trend} = 0.02$); lower intake of sugar-sweetened beverages ($P\text{-trend} < 0.001$), trans fats ($P\text{-trend} = 0.03$), and sodium ($P\text{-trend} < 0.001$); and moderate alcohol intake ($P\text{-trend} < 0.001$) were each significantly associated with reduced rates of incident physical impairment. Among top contributors to the food components of the AHEI-2010, the strongest relations were found for increased intake of oranges, orange juice, apples and pears, romaine or leaf lettuce, and walnuts. However, associations with each component and with specific foods were generally weaker than the overall score, indicating that overall diet pattern is more important than individual parts. **CONCLUSIONS:** In this large cohort of older women, a healthier diet was associated with a lower risk of developing impairments in physical function.

Key area: Cognitive Health

Lee J, Kim YS, Lee J, Heo SC, Lee KL, Choi SW, Kim Y. Walnut phenolic extract and its bioactive compounds suppress colon cancer cell growth by regulating colon cancer stemness.

Nutrients. 2016 Jul 21;8(7). doi: 10.3390/nu8070439

Abstract: Walnut has been known for its health benefits, including anti-cardiovascular disease and anti-oxidative properties. However, there is limited evidence elucidating its effects on cancer stem cells (CSCs) which represent a small subset of cancer cells that provide resistance against chemotherapy. This study aimed to evaluate the anti-CSCs potential of walnut phenolic extract (WPE) and its bioactive compounds, including (+)-catechin, chlorogenic acid, ellagic acid, and gallic acid. In the present study, CD133+CD44+ cells were isolated from HCT116 cells using fluorescence-activated cell sorting (FACS) and then treated with WPE. As a result, survival of the CD133+CD44+ HCT116 cells was inhibited and cell differentiation was induced by WPE. In addition, WPE down-regulated the CSC markers, CD133, CD44, DLK1, and Notch1, as well as the β -catenin/p-GSK3 γ signaling pathway. WPE suppressed the self-renewal capacity of CSCs. Furthermore, the WPE exhibited stronger anti-CSC effects than its

individual bioactive compounds. Finally, the WPE inhibited specific CSC markers in primary colon cancer cells isolated from primary colon tumor. These results suggest that WPE can suppress colon cancer by regulating the characteristics of colon CSCs.

Key area: Cancer

Luo T, Miranda-Garcia O, Adamson A, Hamilton-Reeve J, Sullivan DK, Kinchen JM, Shay NF. Consumption of walnuts in combination with other whole foods produces physiologic, metabolic, and gene expression changes in obese C57BL/6J high-fat-fed male mice.

J Nutr. 2016 Sep;146(9):1641-50. doi: 10.3945/jn.116.234419

Abstract: BACKGROUND: Although a reductionist approach has sought to understand the roles of individual nutrients and biochemicals in foods, it has become apparent that there can be differences when studying food components in isolation or within the natural matrix of a whole food. OBJECTIVE: The objective of this study was to determine the ability of whole-food intake to modulate the development of obesity and other metabolic dysfunction in mice fed a high-fat, Western-style obesogenic diet. To test the hypothesis that an n-3 (ω -3) polyunsaturated fatty acid-rich food could synergize with other, largely polyphenol-rich foods by producing greater reductions in metabolic disease conditions, the intake of English walnuts was evaluated in combination with 9 other whole foods. METHODS: Eight-week-old male C57BL/6J mice were fed low-fat (LF; 10% fat) and high-fat (HF) control diets, along with an HF diet with 8.6% (wt:wt) added walnuts for 9 wk. The HF control diet contained 46% fat with added sucrose (10.9%, wt:wt) and cholesterol (1%, wt:wt); the added sucrose and cholesterol were not present in the LF diet. Other groups were provided the walnut diet with a second whole food—raspberries, apples, cranberries, tart cherries, broccoli sprouts, olive oil, soy protein, or green tea. All of the energy-containing whole foods were added at an energy level equivalent to 1.5 servings/d. Body weights, food intake, and glucose tolerance were determined. Postmortem, serum lipids and inflammatory markers, hepatic fat, gene expression, and the relative concentrations of 594 biochemicals were measured. RESULTS: The addition of walnuts with either raspberries, apples, or green tea reduced glucose area under the curve compared with the HF diet alone (−93%, −64%, and −54%, respectively, $P < 0.05$). Compared with HF-fed mice, mice fed walnuts with either broccoli sprouts or green tea (−49% and −61%, respectively, $P < 0.05$) had reduced hepatic fat concentrations. There were differences in global gene expression patterns related to whole-food content, with many examples of differences in LF- and HF-fed mice, HF- and walnut-fed mice, and mice fed walnuts and walnuts plus other foods. The mean \pm SEM increase in relative hepatic concentrations of the n-3 fatty acids α -linolenic acid, eicosapentanoic acid, and docosapentanoic acid in all walnut-fed groups was $124\% \pm 13\%$, $159\% \pm 11\%$, and $114\% \pm 10\%$, respectively ($P < 0.0001$), compared with LF- and HF-fed mice not consuming walnuts. CONCLUSIONS: In obese male mice, walnut consumption with a high-fat Western-style diet caused changes in hepatic fat concentrations, gene expression patterns, and fatty acid concentrations. The addition of a second whole food in combination with walnuts produced other changes in metabolite concentrations and gene expression patterns and other physiologic markers. Importantly, these substantial changes occurred in mice fed typical amounts of intake, representing only 1.5 servings each food/d.

Key area: Body Weight and Composition

Nakanishi M, Chen Y, Qendro V, Miyamoto S, Weinstock E, Weinstock GM, Rosenberg DW. Effects of walnut consumption on colon carcinogenesis and microbial community structure.

Cancer Prev Res (Phila). 2016 Aug;9(8):692-703. doi: 10.1158/1940-6207.CAPR-16-0026

Abstract: Walnuts are comprised of a complex array of biologically active constituents with individual cancer-protective properties. Here, we assessed the potential benefit of whole walnut consumption in a mouse tumor bioassay using azoxymethane (AOM). In study 1, a modest reduction (1.3-fold) in tumor numbers was observed in mice fed a standard diet (AIN-76A) containing 9.4% walnuts (15% of total

fat). In Study 2, the effects of walnut supplementation were tested in the Total Western Diet (TWD). There was a significant reduction (2.3-fold; $p < 0.02$) in tumor numbers in male mice fed TWD containing 7% walnuts (10.5% of total fat). Higher concentrations of walnuts lacked inhibitory effects, particularly in female mice, indicating there may be optimal levels of dietary walnut intake for cancer prevention. Since components of the Mediterranean diet have been shown to affect the gut microbiome, the effects of walnuts were therefore tested in fecal samples using 16S rRNA gene sequencing. Carcinogen treatment reduced the diversity and richness of the gut microbiome, especially in male mice, which exhibited lower variability and greater sensitivity to environmental changes. Analysis of individual operational taxonomic units (OTUs) identified specific groups of bacteria associated with carcinogen exposure, walnut consumption and/or both variables. Correlation analysis also identified specific OTU-clades that were strongly associated with the presence and number of tumors. Taken together, our results indicate that walnuts afford partial protection to the colon against a potent carcinogenic insult, and this may be due in part to walnut-induced changes to the gut microbiome.

Key Area: Cancer

Njike VY, Yarandi N, Petraro P, Ayettey RG, Treu JA, Katz DL. Inclusion of walnut in the diets of adults at risk for type 2 diabetes and their dietary pattern changes: a randomized, controlled, cross-over trial.

BMJ Open Diabetes Res Care. 2016 Oct 19;4(1):e000293. doi: 10.1136/bmjdr-2016-000293

Abstract: BACKGROUND: In our recently published study, including walnuts in the diets of adults with prediabetes led to overall improvement in diet quality. This report adds to those study findings by examining the food groups displaced during walnut inclusion in the diets of those adults with prediabetes. METHODS: Randomized, controlled, modified Latin square parallel design with 2 treatment arms. The 112 participants (31 men, 81 women) were randomly assigned to a diet with or without dietary counseling to regulate calorie intake in a 1:1 ratio. Within each treatment arm, participants were further randomized to 1 of 2 sequence permutations to receive a walnut included diet with 56 g (366 kcal) of walnuts per day and a walnut-excluded diet. Participants in the calorie regulated arm received advice from a dietitian to preserve an isocaloric condition while including walnuts. We analyzed the 12 components of the 2010 Healthy Eating Index to examine dietary pattern changes of study participants. RESULTS: Seafood and plant protein foods intake significantly increased with walnut inclusion, compared with their exclusion (2.14 ± 2.06 vs -0.49 ± 2.33 ; $p = 0.003$). The ingestion of healthful fatty acids also significantly increased with walnut inclusion, compared with their exclusion (1.43 ± 4.53 vs -1.76 ± 4.80 ; $p = 0.02$). Dairy ingestion increased with walnut inclusion in the calorie-regulated phase, compared with walnut inclusion without calorie regulation (1.06 ± 4.42 vs -2.15 ± 3.64 ; $p = 0.02$). CONCLUSIONS: Our data suggest that walnut inclusion in the diets of adults at risk for diabetes led to an increase in intake of other healthful foods.

Key area: Diabetes

Pribis P. Effects of walnut consumption on mood in young adults-a randomized controlled trial.

Nutrients. 2016 Oct 25;8(11). pii: E668. doi: 10.3390/nu8110668

Abstract: Walnuts contain a number of potentially neuroprotective compounds like vitamin E, folate, melatonin, several antioxidative polyphenols and significant amounts of ω -3 fatty acids. The present study sought to determine the effect of walnuts on mood in healthy volunteers. Sixty-four college students were randomly assigned to two treatment sequences in a crossover fashion: walnut-placebo or placebo-walnut. At baseline mood was assessed using Profiles of Mood States (POMS). Data was collected again after eight weeks of intervention. After six-weeks of washout, the intervention groups followed the diets in reverse order. Data was collected once more at the end of the eight-week intervention period. No significant changes in mood were observed in the analyses with both genders combined and in females. However, we have observed a significant medium effect size improvement in

the Total Mood Disturbance score (-27.49%, $p = 0.043$, Cohen's $d = 0.708$) in males. In non-depressed healthy young males, walnuts seem to have the ability to improve mood.

Key area: Cognitive Health

Rock CL, Flatt SW, Pakiz B, Quintana EL, Heath DD, Rana BK, Natarajan L. Effects of diet composition on weight loss, metabolic factors and biomarkers in a 1-year weight loss intervention in obese women examined by baseline insulin resistance status.

Metabolism. 2016 Nov;65(11):1605-1613. doi: 10.1016/j.metabol.2016.07.008

Abstract: BACKGROUND: Obesity is a risk factor for postmenopausal breast cancer incidence and pre- and postmenopausal breast cancer mortality, which may be explained by several metabolic and hormonal factors (sex hormones, insulin resistance, and inflammation) that are biologically related. Differential effects of dietary composition on weight loss and these metabolic factors may occur in insulin-sensitive vs. insulin-resistant obese women. OBJECTIVE: To examine the effect of diet composition on weight loss and metabolic, hormonal and inflammatory factors in overweight/obese women stratified by insulin resistance status in a 1-year weight loss intervention. METHODS AND RESULTS: Nondiabetic women who were overweight/obese ($n = 245$) were randomly assigned to a lower fat (20% energy), higher carbohydrate (65% energy) diet; a lower carbohydrate (45% energy), higher fat (35% energy) diet; or a walnut-rich (18% energy), higher fat (35% energy), lower carbohydrate (45% energy) diet. All groups lost weight at follow-up ($P < 0.0001$), with mean (SEM) percent loss of 9.2 (1.1)% in lower fat, 6.5 (0.9)% in lower carbohydrate, and 8.2 (1.0)% in walnut-rich groups at 12 months. The diet \times time \times insulin resistance status interaction was not statistically significant in the model for overall weight loss, although insulin sensitive women at 12 months lost more weight in the lower fat vs. lower carbohydrate group (7.5 kg vs 4.3 kg, $P = 0.06$), and in the walnut-rich vs. lower carbohydrate group (8.1 kg vs 4.3 kg, $P = 0.04$). Sex hormone binding globulin increased within each group except in the lower carbohydrate group at 12 months ($P < 0.01$). C-reactive protein and interleukin-6 decreased at follow-up in all groups ($P < 0.01$). CONCLUSIONS: Findings provide some support for differential effects of diet composition on weight loss depending on insulin resistance status. Prescribing walnuts is associated with weight loss comparable to a standard lower fat diet in a behavioral weight loss intervention. Weight loss itself may be the most critical factor for reducing the chronic inflammation associated with increased breast cancer risk and progression.

Key area: Body Weight and Composition

Sala-Vila A, Guasch-Ferré M, Hu FB, Sánchez-Tainta A, Bulló M, Serra-Mir M, López-Sabater C, Sorlí JV, Arós F, Fiol M, Muñoz MA, Serra-Majem L, Martínez JA, Corella D, Fitó M, Salas-Salvadó J, Martínez-González MA, Estruch R, Ros E; and PREDIMED Investigators. Dietary α -linolenic acid, marine ω -3 fatty acids, and mortality in a population with high fish consumption: findings from the PREvención con DIeta MEDiterránea (PREDIMED) study.

J Am Heart Assoc. 2016 Jan 26;5(1). doi: 10.1161/JAHA.115.002543

Abstract: BACKGROUND: Epidemiological evidence suggests a cardioprotective role of α -linolenic acid (ALA), a plant-derived ω -3 fatty acid. It is unclear whether ALA is beneficial in a background of high marine ω -3 fatty acids (long-chain n-3 polyunsaturated fatty acids) intake. In persons at high cardiovascular risk from Spain, a country in which fish consumption is customarily high, we investigated whether meeting the International Society for the Study of Fatty Acids and Lipids recommendation for dietary ALA (0.7% of total energy) at baseline was related to all-cause and cardiovascular disease mortality. We also examined the effect of meeting the society's recommendation for long-chain n-3 polyunsaturated fatty acids (≥ 500 mg/day). METHODS AND RESULTS: We longitudinally evaluated 7202 participants in the PREvención con DIeta MEDiterránea (PREDIMED) trial. Multivariable-adjusted Cox regression models were fitted to estimate hazard ratios. ALA intake

correlated to walnut consumption ($r=0.94$). During a 5.9-y follow-up, 431 deaths occurred (104 cardiovascular disease, 55 coronary heart disease, 32 sudden cardiac death, 25 stroke). The hazard ratios for meeting ALA recommendation ($n=1615$, 22.4%) were 0.72 (95% CI 0.56-0.92) for all-cause mortality and 0.95 (95% CI 0.58-1.57) for fatal cardiovascular disease. The hazard ratios for meeting the recommendation for long-chain n-3 polyunsaturated fatty acids ($n=5452$, 75.7%) were 0.84 (95% CI 0.67-1.05) for all-cause mortality, 0.61 (95% CI 0.39-0.96) for fatal cardiovascular disease, 0.54 (95% CI 0.29-0.99) for fatal coronary heart disease, and 0.49 (95% CI 0.22-1.01) for sudden cardiac death. The highest reduction in all-cause mortality occurred in participants meeting both recommendations (hazard ratio 0.63 [95% CI 0.45-0.87]). **CONCLUSIONS:** In participants without prior cardiovascular disease and high fish consumption, dietary ALA, supplied mainly by walnuts and olive oil, relates inversely to all-cause mortality, whereas protection from cardiac mortality is limited to fish-derived long-chain n-3 polyunsaturated fatty acids.

Key area: Heart Health

Sánchez-González C1, Ciudad CJ, Izquierdo-Pulido M, Sánchez-González C1, Ciudad CJ, Izquierdo-Pulido M, Noé V. Urolithin A causes p21 up-regulation in prostate cancer cells.

Eur J Nutr. 2016 Apr;55(3):1099-112. doi: 10.1007/s00394-015-0924-z

Abstract: **PURPOSE:** Walnuts contain several bioactive compounds, including pedunculagin, a polyphenol metabolized by microbiota to form urolithins, namely urolithin A (UA). The aim of this study was to determine gene expression changes in prostate cancer cells after incubation with UA. **METHODS:** We performed a genomic analysis to study the effect of UA on LNCaP prostate cells. Cells were incubated with 40 μ M UA for 24 h, and RNA was extracted and hybridized to Affymetrix Human Genome U219 array. Microarray results were analyzed using GeneSpring v13 software. Differentially expressed genes ($p < 0.05$, fold change > 2) were used to perform biological association networks. Cell cycle was analyzed by flow cytometry and apoptosis measured by the rhodamine method and by caspases 3 and 7 activation. Cell viability was determined by MTT assay. **RESULTS:** We identified two nodes, FN-1 and CDKN1A, among the differentially expressed genes upon UA treatment. CDKN1A was validated, its mRNA and protein levels were significantly up-regulated, and the promoter activation measured by luciferase. Cell cycle analysis showed an increase in G1-phase, and we also observed an induction of apoptosis and caspases 3 and 7 activation upon UA treatment. **CONCLUSION:** Our results indicate a potential role of UA as a chemopreventive agent for prostate cancer.

Key area: Cancer

Yaskolka Meir A, Shelef I, Schwarzfuchs D, Gepner Y, Tene L, Zelicha H, Tsaban G, Bilitzky A, Komy O, Cohen N, Bril N, Rein M, Serfaty D, Kenigsbuch S, Chassidim Y, Zeller L, Ceglarek U, Stumvoll M, Blüher M, Thiery J, Stampfer MJ, Rudich A, Shai I. Intermuscular adipose tissue and thigh muscle area dynamics during an 18-month randomized weight loss trial.

J Appl Physiol. 2016;121:518-527. doi: 10.1152/jappphysiol.00309.2016

Abstract: It remains unclear whether intermuscular adipose tissue (IMAT) has any metabolic influence or whether it is merely a marker of abnormalities, as well as what are the effects of specific lifestyle strategies for weight loss on the dynamics of both IMAT and thigh muscle area (TMA). We followed the trajectory of IMAT and TMA during 18-mo lifestyle intervention among 278 sedentary participants with abdominal obesity, using magnetic resonance imaging. We measured the resting metabolic rate (RMR) by an indirect calorimeter. Among 273 eligible participants (47.8 ± 9.3 yr of age), the mean IMAT was 9.6 ± 4.6 cm(2). Baseline IMAT levels were directly correlated with waist circumference, abdominal subdepots, C-reactive protein, and leptin and inversely correlated with baseline TMA and creatinine ($P < 0.05$ for all). After 18 mo (86.3% adherence), both IMAT (-1.6%) and TMA (-3.3%) significantly decreased ($P < 0.01$ vs. baseline). The changes in both IMAT and TMA were similar across the lifestyle intervention groups and directly corresponded with moderate weight loss ($P < 0.001$). IMAT change did

not remain independently associated with decreased abdominal subdepots or improved cardiometabolic parameters after adjustments for age, sex, and 18-mo weight loss. In similar models, 18-mo TMA loss remained associated with decreased RMR, decreased activity, and with increased fasting glucose levels and IMAT ($P < 0.05$ for all). Unlike other fat depots, IMAT may not represent a unique or specific adipose tissue, instead largely reflecting body weight change per se. Moderate weight loss induced a significant decrease in thigh muscle area, suggesting the importance of resistance training to accompany weight loss programs.

Key area: Body Weight and Composition

2015

Arab L, Ang A. A cross sectional study of the association between walnut consumption and cognitive function among adult us populations represented in NHANES.

J Nutr Health Aging. 2015 Mar;19(3):284-90. doi:10.1007/s12603-014-0569-2

Abstract: **OBJECTIVE:** To examine the association between walnut consumption and measures of cognitive function in the US population. **Design:** Nationally representative cross sectional study using 24 hour dietary recalls of intakes to assess walnut and other nut consumption as compared to the group reporting no nut consumption. **Setting:** 1988–1994 and 1999–2002 rounds of the National Health and Nutrition Examination Survey (NHANES). **Population** Representative weighted sample of US adults 20 to 90 years of age. **Main Outcome Measure:** The Neurobehavioral Evaluation System 2 (NES2), consisting of simple reaction time (SRTT), symbol digit substitution (SDST), the single digit learning (SDLT), Story Recall (SRT) and digit-symbol substitution (DSST) tests. **RESULTS:** Adults 20–59 years old reporting walnut consumption of an average of 10.3 g/d required 16.4ms less time to respond on the SRTT, $P=0.03$, and 0.39s less for the SDST, $P=0.01$. SDLT scores were also significantly lower by 2.38s ($P=0.05$). Similar results were obtained when tertiles of walnut consumption were examined in trend analyses. Significantly better outcomes were noted in all cognitive test scores among those with higher walnut consumption ($P < 0.01$). Among adults 60 years and older, walnut consumers averaged 13.1 g/d, scored 7.1 percentile points higher, $P=0.03$ on the SRT and 7.3 percentile points higher on the DSST, $P=0.05$. Here also trend analyses indicate significant improvements in all cognitive test scores ($P < 0.01$) except for SRTT ($P = 0.06$) in the fully adjusted models. **CONCLUSION:** These significant, positive associations between walnut consumption and cognitive functions among all adults, regardless of age, gender or ethnicity suggest that daily walnut intake may be a simple beneficial dietary behavior.

Key area: Cognitive Health

Austel A, Ranke C, Wagner N, Gorge J, Ellrott T. Weight loss with a modified Mediterranean-type diet using fat modification: a randomized controlled trial.

Eur J Clin Nutr. 2015 Aug;69(8):878-84. doi: 10.1038/ejcn.2015.11

Abstract: **BACKGROUND/OBJECTIVE:** There is evidence that Mediterranean diets with a high proportion of olive oil and nuts can be effective for weight management and prevention of cardiovascular disease. It might be difficult for populations with other eating habits to follow such diets. Therefore, a modified Mediterranean-type diet using fat modification through neutral and butter-flavored canola oil, walnuts and walnut oil with two portion-controlled sweet daily snacks was tested in Germany. **Subjects/Methods:** Randomized waiting-list control study with overweight/grade 1 obese subjects: 12-week self-help modified Mediterranean-type diet, 6 weeks of diet plans and 6 weeks of weight loss maintenance training. Trial duration was 12 months. Intervention group (IG) included 100 participants (average age of 52.4 years, weight 85.1 kg and body mass index (BMI) 30.1 kg/m²), waiting-list control group (CG) included 112 participants (52.6 years, 84.1 kg and 30.1 kg/m²). **RESULTS:** Per-protocol weight loss after 12 weeks was 5.2 kg in IG vs 0.4 kg in CG ($P \leq 0.0001$), BMI

-1.8 vs -0.1 kg/m² ($P \leq 0.0001$), waist circumference -4.7 vs -0.9 cm ($P \leq 0.0001$). Triglycerides, total cholesterol and LDL cholesterol improved significantly in IG but not in CG. One-year dropouts: 44% in IG and 53% in CG. Weight loss after 12 months: 4.2 kg (pooled data). **CONCLUSIONS:** A five-meal modified Mediterranean-type diet with two daily portion-controlled sweet snacks was effective for weight management in a self-help setting for overweight and grade 1 obese subjects. Fat modification through canola oil, walnuts and walnut oil improved blood lipids even at 12 months.

Key area: Body Weight and Composition

Holt RR, Yim SJ, Shearer GC, Hackman RM, Djurica D, Newman JW, Shindel AW, Keen CL. Effects of short-term walnut consumption on human microvascular function and its relationship to plasma epoxide content.

J Nutr Biochem. 2015 Dec;26(12):1458-66. doi: 10.1016/j.jnutbio.2015.07.012

Abstract: Improved vascular function after the incorporation of walnuts into controlled or high fat diets has been reported, however the mechanism(s) underlying this effect of walnuts are poorly defined. The objective of the current study was to evaluate the acute and short-term effects of walnut intake on changes in microvascular function and the relationship of these effects to plasma epoxides, the cytochrome P450 derived metabolites of fatty acids. Thirty-eight hypercholesterolemic postmenopausal women were randomized to 4 weeks of 5 g or 40 g of daily walnut intake. All outcomes were measured after an overnight fast and 4 hours after walnut intake. Microvascular function, assessed as the reactive hyperemia index (RHI) was the primary outcome measure, with serum lipids and plasma epoxides as secondary measures. Compared to 5 g of daily walnut intake, consuming 40 g/d of walnuts for 4 weeks increased the RHI and Framingham RHI. Total cholesterol and low and high density-cholesterol did not significantly change after walnut intake. The change in RHI after 4 weeks of walnut intake was associated with the change in the sum of plasma epoxides ($r=0.65$, $p = 0.002$), but not with the change in the sum of plasma hydroxyeicosatetraenoic acids (HETE). Of the individual plasma epoxides, arachidonic acid derived 14(15)-epoxyeicosatrienoic acid (EpETrE) was most strongly associated with the change in microvascular function ($r=0.72$, $p < 0.001$). These data support the concept that the intake of walnut-derived fatty acids can favorably affect plasma epoxide production, resulting in improved microvascular function.

Key area: Heart Health

Njike VY, Ayettey R, Petraro P, Treu, Katz DL. Walnut ingestion in adults at risk for diabetes: effects on body composition, diet quality, and cardiac risk measures.

BMJ Open Diabetes Research and Care. 2015;3:e000115. doi: 10.1136/bmjdr-2015-000115

Abstract: **BACKGROUND:** Despite their energy density, walnuts can be included in the diet without adverse effects on weight or body composition. The effect of habitual walnut intake on total calorie intake is not well studied. Effects on overall diet quality have not been reported. **METHODS:** Randomized, controlled, modified Latin square parallel design study with 2 treatment arms. The 112 participants were randomly assigned to a diet with or without dietary counseling to adjust calorie intake. Within each treatment arm, participants were further randomized to 1 of the 2 possible sequence permutations to receive a walnut-included diet with 56 g (providing 366 kcal) of walnuts per day and a walnut-excluded diet. Participants were assessed for diet quality, body composition, and cardiac risk measures. **RESULTS:** When compared with a walnut-excluded diet, a walnut-included diet for 6 months, with or without dietary counseling to adjust caloric intake, significantly improved diet quality as measured by the Healthy Eating Index 2010 (9.14 ± 17.71 vs 0.40 ± 15.13 ; $p=0.02$ and 7.02 ± 15.89 vs -5.92 ± 21.84 ; $p=0.001$, respectively). Endothelial function, total and low density lipoprotein (LDL) cholesterol improved significantly from baseline in the walnut-included diet. Body mass index, percent body fat, visceral fat, fasting glucose, glycated hemoglobin, and blood pressure did not change significantly. **CONCLUSION:** The inclusion of walnuts in an ad libitum diet for 6 months, with or

without dietary counseling to adjust calorie intake, significantly improved diet quality, endothelial function, total and LDL cholesterol, but had no effects on anthropometric measures, blood glucose level, and blood pressure.

Key area: Diabetes

Ros E. Nuts and CVD.

Br J Nutr. 2015 Apr;113 Suppl 2:S111-20. doi: 10.1017/S0007114514003924

Abstract: Nuts are nutrient-dense foods with complex matrices rich in unsaturated fatty acids and other bioactive compounds, such as l-arginine, fibre, healthful minerals, vitamin E, phytosterols and polyphenols. By virtue of their unique composition, nuts are likely to beneficially affect cardiovascular health. Epidemiological studies have associated nut consumption with a reduced incidence of CHD in both sexes and of diabetes in women, but not in men. Feeding trials have clearly demonstrated that consumption of all kinds of nuts has a cholesterol-lowering effect, even in the context of healthy diets. There is increasing evidence that nut consumption has a beneficial effect on oxidative stress, inflammation and vascular reactivity. Blood pressure, visceral adiposity and the metabolic syndrome also appear to be positively influenced by nut consumption. Contrary to expectations, epidemiological studies and clinical trials suggest that regular nut consumption is not associated with undue weight gain. Recently, the PREvención con DIeta MEDiterránea randomised clinical trial of long-term nutrition intervention in subjects at high cardiovascular risk provided first-class evidence that regular nut consumption is associated with a 50 % reduction in incident diabetes and, more importantly, a 30 % reduction in CVD. Of note, incident stroke was reduced by nearly 50 % in participants allocated to a Mediterranean diet enriched with a daily serving of mixed nuts (15 g walnuts, 7.5 g almonds and 7.5 g hazelnuts). Thus, it is clear that frequent nut consumption has a beneficial effect on CVD risk that is likely to be mediated by salutary effects on intermediate risk factors.

Key area: Heart Health

Sánchez-González C, Izquierdo-Pulido M. Health benefits of walnut polyphenols: an exploration beyond their lipid profile.

Crit Rev Food Sci Nutr. 2015 Dec 29;0. doi: 10.1080/10408398.2015.1126218

Abstract: Walnuts are commonly found in our diet and have been recognized for their nutritious properties for a long time. Traditionally, walnuts have been known for their lipid profile which has been linked to a wide array of biological properties and health-promoting effects. In addition to essential fatty acids, walnuts contain a variety of other bioactive compounds such as, vitamin E and polyphenols. Among common foods and beverages, walnuts represent one of the most important sources of polyphenols, hence, their effect over human health warrants attention. The main polyphenol in walnuts is pedunculagin, an ellagitannin. After consumption, ellagitannins are hydrolyzed to release ellagic acid, which is converted by gut microflora to urolithin A and other derivatives, such as urolithins B, C and D. Ellagitannins possess well known antioxidant and anti-inflammatory bioactivity and several studies have assessed the potential role of ETs against disease initiation and progression, including cancer, cardiovascular and neurodegenerative diseases. The purpose of this review is to summarize current available information relating to the potential effect of walnut polyphenols in health maintenance and disease prevention.

Key area: Nutrient & Bioactive Composition

Schlörmann W, Birringer M, Böhme V, Löber K, Jahreis G, Lorkowski S, Müller AK, Schöne F, Gleis M. Influence of roasting conditions on health-related compounds in different nuts.

Food Chem. 180 (2015) 77–85. doi: 10.1016/j.foodchem.2015.02.017

Abstract: Due to their health-beneficial ingredients the consumption of nuts can contribute to a healthy diet. The composition of hazelnuts, almonds, macadamia nuts, pistachios and walnuts regarding

health-promoting and potentially harmful compounds was examined before and after roasting under different time and temperature conditions. Fatty acid compositions were not affected by roasting. Malondialdehyde increased with higher roasting temperatures (17-fold in walnuts). Levels of tocopherol isomers were reduced after roasting (a-T: 38%, b-T: 40%, c-T: 70%) and hydrophilic antioxidant capacity decreased significantly in hazelnuts (1.4-fold), macadamia nuts (1.7-fold) and walnuts (3.7-fold). Increasing roasting temperatures supported the formation of significant amounts of acrylamide only in almonds (1220 lg kg⁻¹). In general, nuts roasted at low/middle temperatures (120–160 °C) exhibited best sensory properties. Therefore, desired sensory quality along with a favourable healthy nut composition may be achieved by roasting over a low to medium temperature range.

Key area: Nutrient & Bioactive Composition

Tsoukas MA, Ko BJ, Witte TR, Dincer F, Hardman WE, Mantzoros CS. Dietary walnut suppression of colorectal cancer in mice: Mediation by miRNA patterns and fatty acid incorporation.

J Nutr Biochem. 2015 Jul;26(7):776-83. doi: 10.1016/j.jnutbio.2015.02.009

Abstract: Colorectal cancer, unlike many other malignancies, may be preventable. Recent studies have demonstrated an inverse association between nut consumption and incidence of colon cancer; however, the underlying mechanisms are not fully understood. An emerging concept suggests that microribonucleic acids (miRNAs) may help explain the relationship between walnut consumption and decreased colorectal neoplasia risk. Seven days after HT-29 colon cancer cell injection, mice were randomized to either control or walnut diets for 25 days of diet treatment. Thirty samples of tumor and of omental adipose were analyzed to determine changes in lipid composition in each dietary group. In the tumors of the walnut-containing diet, we found significant increases in α -linolenic, eicosapentaenoic, docosahexaenoic and total omega-3 acids, and a decrease in arachidonic acid, as compared to the control diet. Final tumor size measured at sacrifice was negatively associated with percentage of total omega-3 fatty acid composition ($r=-0.641$, $P=.001$). MicroRNA expression analysis of colorectal tumor tissue revealed decreased expression of miRNAs 1903, 467c and 3068 ($P<.05$) and increased expression of miRNA 297a* ($P=.0059$) in the walnut-treated group as compared to control diet. Our results indicate that changes in the miRNA expression profiles likely affect target gene transcripts involved in pathways of anti-inflammation, antivascularization, antiproliferation and apoptosis. We also demonstrate the incorporation of protective fatty acids into colonic epithelium of walnut-fed mice, which may independently alter miRNA expression profiles itself. Future studies of the mechanism of widespread miRNA regulation by walnut consumption are needed to offer potential prognostic and therapeutic targets.

Key area: Cancer

2014

Burns-Whitmore B, Haddad E, Sabaté J, Rajaram S. Effects of supplementing n-3 fatty acid enriched eggs and walnuts on cardiovascular disease risk markers in healthy free-living lacto-ovo-vegetarians: a randomized, crossover, free-living intervention study.

Nutr J. 2014 Mar 27;13(1):29. doi: 10.1186/1475-2891-13-29

Abstract: BACKGROUND: Plant and marine n-3 fatty acids (FA) may favorably modify select markers of cardiovascular disease risk. Whether supplementing the habitual diet of lacto-ovo-vegetarians (LOV) with walnuts (containing α -linolenic acid, ALA) and n-3 FA enriched eggs (containing primarily

docosahexaenoic acid, DHA and ALA) would have equivalent effects on CVD risk factors is explored in this study. **METHODS:** In this study, 20 healthy free-living LOVs following their habitual diet were randomly assigned in a crossover design to receive one of three supplements: n-3 FA enriched egg (6/week), walnuts (28.4 g, 6/week) or a standard egg, 6/week (control) for 8 weeks each with 4-wk washout between treatments. Erythrocyte membrane fatty acids, serum lipids and inflammatory markers were measured at the end of each treatment. **RESULTS:** Dietary compliance was observed by an expected increase in erythrocyte membrane ALA following the walnut treatment and in DHA following the n-3 FA enriched egg treatment. Walnut treatment lowered serum triacylglycerol, total cholesterol and Apo B ($p < 0.05$) compared to the standard egg but not the n-3 FA enriched egg treatment. However, walnut treatment significantly reduced total: HDL cholesterol ratio compared to both egg treatments. There were no differences between treatments for any of the inflammatory markers. **CONCLUSIONS:** For LOV, a direct source of DHA such as n-3 FA enriched eggs seems necessary to increase membrane levels of DHA. However for producing an overall favorable blood lipid profile, daily consumption of a handful of walnuts rich in ALA may be a preferred option for lacto-ovo vegetarians.

Key area: Heart Health

Fleming JA, Kris-Etherton PM. The evidence for α -linolenic acid and cardiovascular disease benefits: comparisons with eicosapentaenoic acid and docosahexaenoic acid.

Adv Nutr. 2014 Nov 14;5(6):863S-76S. doi: 10.3945/an.114.005850

Abstract: Our understanding of the cardiovascular disease (CVD) benefits of α -linolenic acid (ALA, 18:3n-3) has advanced markedly during the past decade. It is now evident that ALA benefits CVD risk. The expansion of the ALA evidence base has occurred in parallel with ongoing research on eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) and CVD. The available evidence enables comparisons to be made for ALA vs. EPA + DHA for CVD risk reduction. The epidemiologic evidence suggests comparable benefits of plant-based and marine derived n-3 (omega-3) PUFAs. The clinical trial evidence for ALA is not as extensive; however, there have been CVD event benefits reported. Those that have been reported for EPA + DHA are stronger because only EPA + DHA differed between the treatment and control groups, whereas in the ALA studies there were diet differences beyond ALA between the treatment and control groups. Despite this, the evidence suggests many comparable CVD benefits of ALA vs. EPA + DHA. Thus, we believe that it is time to revisit what the contemporary dietary recommendation should be for ALA to decrease the risk of CVD. Our perspective is that increasing dietary ALA will decrease CVD risk; however, randomized controlled clinical trials are necessary to confirm this and to determine what the recommendation should be. With a stronger evidence base, the nutrition community will be better positioned to revise the dietary recommendation for ALA for CVD risk reduction.

Key area: Heart Health

Grace MH, Warlick CW, Neff SA, Lila MA. Efficient preparative isolation and identification of walnut bioactive components using high-speed counter-current chromatography and LC-ESI-IT-TOF-MS.

Food Chem. 2014 Sep 1;158:229-38. doi: 10.1016/j.foodchem.2014.02.117

Abstract: Preparative isolation of complex mixtures of compounds from walnut polar extracts was established by a combination of high-speed counter-current chromatography (HSCCC) and electrospray ionization-ion trap-time of flight mass spectrometry (ESI-IT-TOF-MS). Compounds were isolated after a solvent optimization selection based on solute distribution in a biphasic solvent system. Isolation was achieved through one or two successive HSCCC runs, and final purification on Sephadex LH-20. Isolated compounds included ellagitannins, gallic acid, dicarboxylic acid glucosides, hydrojuglone glucoside, catechin, procyanidin B2, and megasterone glucosides. Praecoxin D was isolated for the first time from walnut, while praecoxin A methyl ester (5) and glansreginin A n-butyl ester (14) are newly

identified compounds. The purity and identity of isolated compounds were confirmed by NMR and HPLC-ESI-MS/MS. These results provided a foundation for in depth characterization of walnut compounds and offered an efficient strategy for isolation of potentially health-relevant phytochemicals from walnuts.

Key area: Nutrient & Bioactive Composition

Haddad EH, Gaban-Ghong N, Oda K, Sabate J. Effect of a walnut meal on postprandial oxidative stress and antioxidants in healthy individuals.

Nutr J. 2014 Jan 10;13(1):4. doi: 10.1186/1475-2891-13-4

Abstract: BACKGROUND: In vitro studies rank walnuts (*Juglans regia*) among the plant foods high in antioxidant capacity, but whether the active constituents of walnuts are bioavailable to humans remains to be determined. The intention of this study was to examine the acute effects of consuming walnuts compared to refined fat on meal induced oxidative stress. At issue is whether the ellagitannins and tocopherols in walnuts are bioavailable and provide postprandial antioxidant protection. METHODS: A randomized, crossover, and controlled-feeding study was conducted to evaluate a walnut test meal compared to one composed of refined ingredients on postprandial serum antioxidants and biomarkers of oxidative status in healthy adults (n = 16) with at least 1 week between testing sessions. Following consumption of a low phenolic diet for one day and an overnight fast, blood was sampled prior to the test meals and at intervals up to 24 hours post ingestion and analyzed for total phenols, malondialdehyde (MDA), oxidized LDL, ferric reducing antioxidant power (FRAP), hydrophilic and lipophilic oxygen radical absorbance capacity (ORAC), uric acid, catechins and urinary excretion of phenylacetate metabolites and of urolithin A. RESULTS: Mixed linear models demonstrated a diet effect ($P < 0.001$) for plasma γ -tocopherol but not for α -tocopherol with the walnut meal. Following the walnut test meal, the incremental 5 hour area under the curve (AUC0-5h) was reduced 7.4% for MDA, increased 7.5% for hydrophilic and 8.5% for lipophilic ORAC and comparable for total phenols, FRAP and uric acid. Oxidized LDL was reduced at 2 hours after the walnut meal. Plasma concentrations of gallicocatechin gallate (GCG), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) increased significantly at 1 hour after the walnut test meal. Quantities of urolithin-A excreted in the urine were significantly higher following the walnut meal. CONCLUSIONS: Compared to the refined control meal, the walnut meal acutely increased postprandial γ -tocopherol and catechins and attenuated some measures of oxidative stress.

Key area: Heart Health

Hardman WE. Diet components can suppress inflammation and reduce cancer risk.

Nutr Res Pract. 2014 Jun;8(3):233-40. doi: 10.4162/nrp.2014.8.3.233

Abstract: Epidemiology studies indicate that diet or specific dietary components can reduce the risk for cancer, cardiovascular disease and diabetes. An underlying cause of these diseases is chronic inflammation. Dietary components that are beneficial against disease seem to have multiple mechanisms of action and many also have a common mechanism of reducing inflammation, often via the NF κ B pathway. Thus, a plant based diet can contain many components that reduce inflammation and can reduce the risk for developing all three of these chronic diseases. We summarize dietary components that have been shown to reduce cancer risk and two studies that show that dietary walnut can reduce cancer growth and development. Part of the mechanism for the anticancer benefit of walnut was by suppressing the activation of NF κ B. In this brief review, we focus on reduction of cancer risk by dietary components and the relationship to suppression of inflammation. However, it should be remembered that most dietary components have multiple beneficial mechanisms of action that can be additive and that suppression of chronic inflammation should reduce the risk for all three chronic diseases.

Key area: Cancer

Hardman WE. Walnuts have potential for cancer prevention and treatment in mice.

J Nutr. 2014 Apr;144 (4 Suppl):555S-560S. doi: 10.3945/jn.113.188466

Abstract: Cancer may not be completely the result of novel or inherited genetic mutations but may in fact be a largely preventable disease. Researchers have identified biochemicals, including n-3 (ω -3) fatty acids, tocopherols, β -sitosterol, and pedunculagin, that are found in walnuts and that have cancer-prevention properties. Mouse studies in which walnuts were added to the diet have shown the following compared with the control diet: 1) the walnut-containing diet inhibited the growth rate of human breast cancers implanted in nude mice by ~80%; 2) the walnut-containing diet reduced the number of mammary gland tumors by ~60% in a transgenic mouse model; 3) the reduction in mammary gland tumors was greater with whole walnuts than with a diet containing the same amount of n-3 fatty acids, supporting the idea that multiple components in walnuts additively or synergistically contribute to cancer suppression; and 4) walnuts slowed the growth of prostate, colon, and renal cancers by antiproliferative and antiangiogenic mechanisms. Cell studies have aided in the identification of the active components in walnuts and of their mechanisms of action. This review summarizes these studies and presents the notion that walnuts may be included as a cancer-preventive choice in a healthy diet.

Key area: Cancer

Jacobs DR Jr. What comes first: the food or the nutrient? Executive summary of a symposium.

J Nutr. 2014 Apr;144(4 Suppl):543S-546S. doi: 10.3945/jn.113.182840

Abstract: This article summarizes background materials and presentations at a symposium that considered the issue of the role of foods and dietary patterns vs. nutrients in relation to chronic disease risk. A model of food synergy is presented as a basis for studying whole foods and dietary patterns. Findings from a series of studies of walnuts were presented and support the concept that walnuts are a healthy food, with specific benefits in a mouse model of breast cancer growth, reductions in cardiovascular disease risk factors in humans, and motor performance and in vitro, in vivo, and ex vivo cellular response to challenge in an aged-rat model.

Key area: Research Methodology

Katz DL. Diet and diabetes: lines and dots.

J Nutr. 2014 Apr;144(4 Suppl):567S-570S. doi: 10.3945/jn.113.182923

Abstract: Diabetes, particularly type 2 diabetes, is epidemic in the United States among adults and children alike, and increasingly prevalent around the world. On its current trajectory, the increasing incidence of diabetes has the potential to ravage both public health and economies. There has, however, been evidence for decades that lifestyle has enormous potential to prevent chronic disease, diabetes included. Studies suggest that the combination of tobacco avoidance, routine physical activity, optimal dietary pattern, and weight control could eliminate as much as 80% of all chronic disease, and 90% of cases of diabetes specifically. None of these factors is necessarily easily achieved, but most are simple. Diet, on the other hand, is complex, and arguments abound for competing diets and related health benefits. From an expansive review of relevant literature, the case emerges that the overall theme of optimal eating for human beings is very well established, whereas the case for any given variation on that theme is substantially less so. Once the theme of healthful eating is acknowledged, the challenge shifts to getting there from here. Although much effort focuses on the wholesale conversion of dietary patterns, the introduction or removal of highly nutritious foods can have direct health effects, and potentially reverberate through the diet as well, shifting the quality of the diet and related health effects. Studies demonstrating favorable effects of daily walnut ingestion in diabetes and insulin resistance are profiled as an illustration, and an ongoing study examining the implications of daily walnut ingestion on diet quality and various biometric variables is described. The line between dietary pattern and the epidemiology of diabetes is indelibly established; we must work to connect the dots between here and there.

Key area: Diabetes

Kim H, Yokoyama W, Davis PA. TRAMP prostate tumor growth is slowed by walnut diets through altered IGF-1 levels, energy pathways, and cholesterol metabolism.

J Med Food. 2014 Dec;17(12):1281-6. doi: 10.1089/jmf.2014.0061

Abstract: Dietary changes could potentially reduce prostate cancer morbidity and mortality. Transgenic adenocarcinoma of the mouse prostate (TRAMP) prostate tumor responses to a 100 g of fat/kg diet (whole walnuts, walnut oil, and other oils; balanced for macronutrients, tocopherols [α - and γ]) for 18 weeks ad libitum were assessed. TRAMP mice (n=17 per group) were fed diets with 100 g fat from either whole walnuts (diet group WW), walnut-like fat (diet group WLF, oils blended to match walnut's fatty acid profile), or as walnut oil (diet group WO, pressed from the same walnuts as WW). Fasted plasma glucose was from tail vein blood, blood was obtained by cardiac puncture, and plasma stored frozen until analysis. Prostate (genitourinary intact [GUI]) was weighed and stored frozen at -80°C . Plasma triglyceride, lipoprotein cholesterol, plasma multianalyte levels (Myriad RBM Rat Metabolic MAP), prostate (GUI), tissue metabolites (Metabolon, Inc., Durham, NC, USA), and mRNA (by Illumina NGS) were determined. The prostate tumor size, plasma insulin-like growth factor-1 (IGF-1), high density lipoprotein, and total cholesterol all decreased significantly ($P<.05$) in both WW and WO compared to WLF. Both WW and WO versus WLF showed increased insulin sensitivity (Homeostasis Model Assessment [HOMA]), and tissue metabolomics found reduced glucose-6-phosphate, succinylcarnitine, and 4-hydroxybutyrate in these groups suggesting effects on cellular energy status. Tissue mRNA levels also showed changes suggestive of altered glucose metabolism with WW and WO diet groups having increased PCK1 and CIDEA mRNA expression, known for their roles in gluconeogenesis and increased insulin sensitivity, respectively. WW and WO group tissues also had increased MSMB mRNA a tumor suppressor and decreased COX-2 mRNA, both reported to inhibit prostate tumor growth. Walnuts reduced prostate tumor growth by affecting energy metabolism along with decreased plasma IGF-1 and cholesterol. These effects are not due to the walnut's N-3 fatty acids, but due to component(s) found in the walnut's fat component.

Key area: Cancer

Kranz S, Hill AM, Fleming JA, Hartman TJ, West SG, Kris-Etherton PM. Nutrient displacement associated with walnut supplementation in men.

J Hum Nutr Diet. 2014 Apr;27 Suppl 2:247-54. doi: 10.1111/jhn.12146

Abstract: BACKGROUND: Dietary guidance issued by various global government agencies recommends nut consumption within the context of a healthy-eating pattern. Nuts are nutrient dense and may promote nutrient adequacy. As an energy-dense food, nuts must replace other foods in the diet to prevent an excess of calories. METHODS: We evaluated how recommending the inclusion of walnuts (75 g day⁻¹) in the diet affected energy and nutrient intake in men (45-75 years; mean body mass index = 27.6 kg m⁻²; n = 19) at risk for developing prostate cancer. Guidance was provided about incorporating walnuts isocalorically in a healthy diet. Three-day food records and body weight were collected at baseline and after two 8-week diet periods (usual versus walnut supplement diets). RESULTS: Energy intake on the walnut supplement diet exceeded the usual diet, although body weight was maintained. Energy intake was lower on the actual walnut supplement diet than the calculated walnut diet [10 865 kJ (2595 kcal) versus 11 325 kJ (2705 kcal) per day, respectively] and contributed 23% less energy than 75 g of walnuts. Approximately, 86% and 85% of the total fat and saturated fatty acids from walnuts were not displaced, whereas the increase in fibre from the usual diet to the actual walnut supplement diet represented less than one-half (39%) of the fibre provided by 75 g of walnuts. Walnuts were substituted, in part, for other foods, and the nutrient profile of the diet was improved, however, the beneficial effect of walnuts on the diet quality was not optimized. CONCLUSIONS:

Individuals do not optimally implement food-based guidance. Consequently, nutrition professionals play a key role in teaching the implementation of food-based recommendations.

Key area: Body Weight and Composition

Kris-Etherton PM. Walnuts decrease risk of cardiovascular disease: a summary of efficacy and biologic mechanisms.

J Nutr. 2014 Apr;144(4 Suppl):547S-554S. doi: 10.3945/jn.113.182907

Abstract: Given the pressing need to reduce cardiovascular disease (CVD) morbidity and mortality, there has been a focus on optimizing dietary patterns to reduce the many contributing risk factors. Over the past 2 decades, many studies have been conducted that have evaluated the effects of walnut consumption on CVD risk factors. Walnuts have been shown to decrease low density lipoprotein cholesterol (by ~9-16%) and blood pressure (diastolic blood pressure by ~2-3 mm Hg), 2 major risk factors for CVD. In addition, walnuts improve endothelial function, decrease both oxidative stress and some markers of inflammation, and increase cholesterol efflux. The effect of walnuts on multiple CVD targets over relatively short periods of time supports recommendations for their inclusion in a heart-healthy diet.

Key area: Heart Health

Le V, Esposito D, Grace MH, Ha D, Pham A, Bortolazzo A, Bevens Z, Kim J, Okuda R, Komarnytsky S, Lila MA, White JB. Cytotoxic effects of ellagitannins isolated from walnuts in human cancer cells.

Nutr Cancer. 2014 Sep 29;1-11. doi: 10.1080/01635581.2014.956246

Abstract: Walnuts contain many bioactive components that may slow cancer growth. A previous report showed that a diet supplemented with walnuts decreased the tumor size formed by MDA-MB-231 human cancer cells injected into nude mice. However, the mechanism of action was never determined. We characterized the effects of a methanol extract prepared from walnuts on human MDA-MB-231, MCF7, and HeLa cells. The extract was cytotoxic to all cancer cells. We identified compounds from the methanol extract that induced this cytotoxicity. The predominant compounds were Tellimagrandin I and Tellimagrandin II, members of the ellagitannin family. We also show a walnut extract decreases the intracellular pH, depolarizes the mitochondrial membrane with release of cytochrome c and phosphatidylserine flipping. The antimitogenic effects of walnut extract were associated with a twofold reduction of mitochondria respiration. These results suggest impairment of mitochondrial function and apoptosis as relevant mechanism of anticancer effects of the walnut extract.

Key area: Cancer

Muthaiyah B, Essa MM, Lee M, Chauhan V, Kaur K, Chauhan A. Dietary supplementation of walnuts improves memory deficits and learning skills in transgenic mouse model of Alzheimer's Disease.

J Alzheimers Dis. 2014 Jan 1;42(4):1397-405. doi: 10.3233/JAD-140675

Abstract: Previous in vitro studies have shown that walnut extract can inhibit amyloid- β (A β) fibrillization, can solubilize its fibrils, and has a protective effect against A β -induced oxidative stress and cellular death. In this study, we analyzed the effect of dietary supplementation with walnuts on learning skills, memory, anxiety, locomotor activity, and motor coordination in the Tg2576 transgenic (tg) mouse model of AD (AD-tg). From the age of 4 months, the experimental groups of AD-tg mice were fed custom-mixed diets containing 6% walnuts (T6) or 9% walnuts (T9), i.e., equivalent to 1 or 1.5 oz, respectively, of walnuts per day in humans. The control groups, i.e., AD-tg and wild-type mice, were fed a diet without walnuts (T0, Wt). These experimental and control mice were examined at the ages of 13-14 months by Morris water maze (for spatial memory and learning ability), T maze (for position discrimination learning ability), rotarod (for psychomotor coordination), and elevated plus maze (for

anxiety-related behavior). AD-tg mice on the control diet (T0) showed memory deficit, anxiety-related behavior, and severe impairment in spatial learning ability, position discrimination learning ability, and motor coordination compared to the Wt mice on the same diet. The AD-tg mice receiving the diets with 6% or 9% walnuts (T6 and T9) showed a significant improvement in memory, learning ability, anxiety, and motor development compared to the AD-tg mice on the control diet (T0). There was no statistically significant difference in behavioral performance between the T6/T9 mice on walnuts-enriched diets and the Wt group on the control diet. These findings suggest that dietary supplementation with walnuts may have a beneficial effect in reducing the risk, delaying the onset, or slowing the progression of, or preventing AD.

Key area: Cognitive Health

O'Brien J, Okereke O, Devore E, Rosner B, Breteler M, Grodstein F. Long-term intake of nuts in relation to cognitive function in older women.

J Nutr Health Aging. 2014;18(5):496-502. doi: 10.1007/s12603-014-0014-6

Abstract: **OBJECTIVE:** Nuts contain nutrients that may benefit brain health; thus, we examined long-term intake of nuts in relation to cognition in older women. **Design:** Population-based prospective cohort study. **Setting** Academic research using data from the Nurses' Health Study. **Participants:** Nut intake was assessed in a food frequency questionnaire beginning in 1980, and approximately every four years thereafter. Between 1995–2001, 16,010 women age 70 or older (mean age = 74 years) without a history of stroke were administered 4 repeated telephone-based cognitive interviews over 6 years. Our final sample included 15,467 women who completed an initial cognitive interview and had complete information on nut intake. **Main Outcome Measures:** The Telephone Interview for Cognitive Status (TICS), a global score averaging the results of all tests (TICS, immediate and delayed verbal recall, category fluency, and attention), and a verbal memory score averaging the results of tests of verbal recall. **RESULTS:** In multivariable-adjusted linear regression models, higher long-term total nut intake was associated with better average cognitive status for all cognitive outcomes. For the global composite score combining all tests, women consuming at least 5 servings of nuts/week had higher scores than non-consumers (mean difference=0.08 standard units, 95% confidence interval 0.00-0.15; p-trend=0.003). This mean difference of 0.08 is equivalent to the mean difference we find between women 2 years apart in age. Long-term intake of nuts was not associated with rates of cognitive decline. **CONCLUSIONS:** Higher nut intake may be related to better overall cognition at older ages, and could be an easily-modifiable public health intervention.

Key area: Cognitive Health

Poulose SM, Miller MG, Shukitt-Hale B. Role of walnuts in maintaining brain health with age.

J Nutr. 2014 Apr;144(4 Suppl):561S-566S. doi: 10.3945/jn.113.184838

Abstract: Because of the combination of population growth and population aging, increases in the incidence of chronic neurodegenerative disorders have become a societal concern, both in terms of decreased quality of life and increased financial burden. Clinical manifestation of many of these disorders takes years, with the initiation of mild cognitive symptoms leading to behavioral problems, dementia and loss of motor functions, the need for assisted living, and eventual death. Lifestyle factors greatly affect the progression of cognitive decline, with high-risk behaviors including unhealthy diet, lack of exercise, smoking, and exposure to environmental toxins leading to enhanced oxidative stress and inflammation. Although there exists an urgent need to develop effective treatments for age-related cognitive decline and neurodegenerative disease, prevention strategies have been underdeveloped. Primary prevention in many of these neurodegenerative diseases could be achieved earlier in life by

consuming a healthy diet, rich in antioxidant and anti-inflammatory phytochemicals, which offers one of the most effective and least expensive ways to address the crisis. English walnuts (*Juglans regia* L.) are rich in numerous phytochemicals, including high amounts of polyunsaturated fatty acids, and offer potential benefits to brain health. Polyphenolic compounds found in walnuts not only reduce the oxidant and inflammatory load on brain cells but also improve interneuronal signaling, increase neurogenesis, and enhance sequestration of insoluble toxic protein aggregates. Evidence for the beneficial effects of consuming a walnut-rich diet is reviewed in this article.

Key area: Cognitive Health

Regueiroa J, Sánchez-González C, Vallverdú-Queralt A, Simal-Gándara J, Lamuela-Raventós R, Izquierdo-Pulido M. Comprehensive identification of walnut polyphenols by liquid chromatography coupled to linear ion trap-Orbitrap mass spectrometry.

Food Chem. 2014;152:340-8. doi: 10.1016/j.foodchem.2013.11.158

Abstract: Epidemiologic studies and clinical trials have demonstrated consistent benefits of walnut consumption on coronary heart disease risk and other chronic diseases. Walnuts (*Juglans regia* L.) have been described previously as a rich source of polyphenols with a broad array of different structures. However, an accurate screening of its complete phenolic profile is still lacking. In the present work, liquid chromatography coupled with electrospray ionization hybrid linear trap quadrupole-Orbitrap mass spectrometry (LC–LTQ-Orbitrap) was applied for a comprehensive identification of phenolic compounds in walnuts. A total of 120 compounds, including hydrolysable and condensed tannins, flavonoids and phenolic acids were identified or tentatively identified on the base of their retention times, accurate mass measurements and subsequent mass fragmentation data, or by comparing with reference substances and literature. The peak area of each signal in mass chromatograms was used to provide semi-quantitative information for comparison purposes. The most abundant ions were observed for ellagitannins, ellagic acid and its derivatives. Furthermore, the high-resolution MS analysis revealed the presence of eight polyphenols that have never been reported in walnuts: stenophyllanin C, malabathrin A, eucalbanin A, cornusiin B, heterophyllin E, pterocaritin B, reginin A and alienanin B.

Key area: Nutrient & Bioactive Composition

Sánchez-González C, Ciudad CJ, Noé V, Izquierdo-Pulido M. Walnut polyphenol metabolites, urolithins A and B, inhibit the expression of the prostate-specific antigen and the androgen receptor in prostate cancer cells.

Food Funct. 2014 Oct 22;5(11):2922-30. doi: 10.1039/C4FO00542B

Abstract: Walnuts have been gathering attention for their health-promoting properties. They are rich in polyphenols, mainly ellagitannins (ETs) that after consumption are hydrolyzed to release ellagic acid (EA). EA is further metabolized by microbiota to form urolithins, such as A and B, which are absorbed. ETs, EA and urolithins have shown to slow the proliferation and growth of different types of cancer cells but the mechanisms remain unclear. We investigate the role of urolithins in the regulatory mechanisms in prostate cancer, specifically those related to the androgen receptor (AR), which have been linked to the development of this type of cancer. In our study, urolithins down-regulated the mRNA and protein levels of both prostate specific antigen (PSA) and AR in LNCaP cells. The luciferase assay performed with a construct containing three androgen response elements (AREs) showed that urolithins inhibit AR-mediated PSA expression at the transcriptional level. Electrophoretic mobility shift assays revealed that urolithins decreased AR binding to its consensus response element. Additionally, urolithins induced apoptosis in LNCaP cells, and this effect correlated with a decrease in Bcl-2 protein levels. In summary, urolithins attenuate the function of the AR by repressing its expression, causing a down-regulation of PSA levels and inducing apoptosis. Our results suggest that a diet rich in ET-containing foods, such as walnuts, could contribute to the prevention of prostate cancer.

Key area: Cancer

Toner, CD. Communicating clinical research to reduce cancer risk through diet: Walnuts as a case example.

Nutr Res Pract. 2014 Aug;8(4):347-5. doi: 10.4162/nrp.2014.8.4.347

Abstract: Inflammation is one mechanism through which cancer is initiated and progresses, and is implicated in the etiology of other conditions that affect cancer risk and prognosis, such as type 2 diabetes, cardiovascular disease, and visceral obesity. Emerging human evidence, primarily epidemiological, suggests that walnuts impact risk of these chronic diseases via inflammation. The published literature documents associations between walnut consumption and reduced risk of cancer, and mortality from cancer, diabetes, and cardiovascular disease, particularly within the context of the Mediterranean Diet. While encouraging, follow-up in human intervention trials is needed to better elucidate any potential cancer prevention effect of walnuts, per se. In humans, the far-reaching positive effects of a plant-based diet that includes walnuts may be the most critical message for the public. Indeed, appropriate translation of nutrition research is essential for facilitating healthful consumer dietary behavior. This paper will explore the translation and application of human evidence regarding connections with cancer and biomarkers of inflammation to the development of dietary guidance for the public and individualized dietary advice. Strategies for encouraging dietary patterns that may reduce cancer risk will be explored.

Key area: Cancer

Wu L, Piotrowski K, Rau T, Waldmann E, Broedl UC, Demmelmair H, Koletzko B, Stark RG, Nagel JM, Mantzoros CS, Parhofer KG. Walnut-enriched diet reduces fasting non-HDL-cholesterol and apolipoprotein B in healthy Caucasian subjects: a randomized controlled cross-over clinical trial. *Metabolism.* 2014 Mar;63(3):382-91.

doi:10.1016/j.metabol.2013.11.005

Abstract: BACKGROUND: Walnut consumption is associated with reduced risk of coronary heart disease (CHD). OBJECTIVE: We assessed the effect of walnuts on lipid and glucose metabolism, adipokines, inflammation and endothelial function in healthy Caucasian men and postmenopausal women ≥ 50 years old. Design: Forty subjects (mean \pm SEM: age 60 ± 1 years, BMI 24.9 ± 0.6 kg/m²; 30 females) were included in a controlled, cross-over study and randomized to receive first a walnut-enriched (43g/d) and then a Western-type (control) diet or vice-versa, with each lasting 8 weeks and separated by a 2-week wash-out. At the beginning and end of each diet phase, measurements of fasting values, a mixed meal test and an assessment of postprandial endothelial function (determination of microcirculation by peripheral artery tonometry) were conducted. Area under the curve (AUC), incremental AUC (iAUC) and treatment x time interaction (shape of the curve) were evaluated for postprandial triglycerides, VLDL-triglycerides, chylomicron-triglycerides, glucose and insulin. RESULTS: Compared with the control diet, the walnut diet significantly reduced non-HDL-cholesterol (walnut vs. control: -10 ± 3 vs. -3 ± 2 mg/dL; $p=0.025$) and apolipoprotein-B (-5.0 ± 1.3 vs. -0.2 ± 1.1 mg/dL; $p=0.009$) after adjusting for age, gender, BMI and diet sequence. Total cholesterol showed a trend toward reduction ($p=0.073$). Fasting VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and glucose, insulin, HOMA-IR, and HbA1c did not change significantly. Similarly, fasting adipokines, C-reactive protein, biomarkers of endothelial dysfunction, postprandial lipid and glucose metabolism and endothelial function were unaffected. CONCLUSION: Daily consumption of 43g of walnuts for 8 weeks significantly reduced non-HDL-cholesterol and apolipoprotein-B, which may explain in part the epidemiological observation that regular walnut consumption decreases CHD risk.

Key area: Heart Health

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 Jun;143(6):788-94. doi: 10.3945/jn.112.170993

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.

Key area: Heart Health

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.

Nutr Neurosci. 2013 Jan;16(1):13-20. doi: 10.1179/1476830512Y.0000000023

Abstract: Previous research from our lab has demonstrated that dietary walnut supplementation protects against age related 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. **OBJECTIVE:** 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.

Key area: Cognitive Health

Moon HS, Liu X, Nagel JM, Chamberland JP, Diakopoulos KN, Brinkoetter MT, Hatzia Apostolou 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. 2013 Apr;62(4):561-70. doi: 10.1136/gutjnl-2012-302092

Abstract: 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.

Key area: Cancer

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. 2013 Apr;143(4):512-8. 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.

Key area: Diabetes

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. 2013 May;24(5):912-9. doi: 10.1016/j.jnutbio.2012.06.009

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.

Key area: Cognitive Health

Reiter RJ, Tan DX, Manchester LC, Korkmaz A, Fuentes-Broto L, Hardman WE, Rosales-Corral SA, Qi W. A walnut-enriched diet reduces the growth of LNCaP human prostate cancer xenografts in nude mice.

Cancer Invest. 2013 Jul;31(6):365-73. doi: 10.3109/07357907.2013.800095

Abstract: It was investigated whether a standard mouse diet (AIN-76A) supplemented with walnuts reduced the establishment and growth of LNCaP human prostate cancer cells in nude (nu/nu) mice. The walnut-enriched diet reduced the number of tumors and the growth of the LNCaP xenografts; 3 of 16 (18.7%) of the walnut-fed mice developed tumors; conversely, 14 of 32 mice (44.0%) of the control diet-fed animals developed tumors. Similarly, the xenografts in the walnut-fed animals grew more slowly than those in the control diet mice. The final average tumor size in the walnut-diet animals was roughly one-fourth the average size of the prostate tumors in the mice that ate the control diet.

Key area: Cancer

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). 2013 Jan;22(1):48-57. doi: 10.1089/jwh.2012.3707

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/m²), 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 ($p \leq 0.002$) 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.

Key area: Body Weight and Composition

2012

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. doi: 10.1016/j.metabol.2011.09.008

Abstract: 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/m²) 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.

Key area: Metabolic Syndrome

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

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-1 β (IL-1 β), 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.

Key area: Heart Health

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. doi: 10.1210/er.2011-1015

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.

Key area: Cancer

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.

Key area: Cancer

Hudthagosol C, Haddad E, Jongsuwat R. Antioxidant activity comparison of walnuts and fatty fish.

J Med Assoc Thai. 2012 Jun;95 Suppl 6:S179-88.

Abstract: BACKGROUND: Walnuts and fatty fish contain high amounts of polyunsaturated fatty acids, which have been shown to decrease the incidence of cardiovascular disease. Walnuts and fatty fish also contain other nutrients, such as antioxidants, that contribute to the reduction of cardiovascular disease. OBJECTIVE: The purpose of the present study was to compare the effects of dietary walnuts and fatty fish on the plasma and urine oxygen radical absorbance capacity (ORAC) values. MATERIAL AND METHOD: Twenty-five subjects participated in this randomized 3 x 3 crossover study, which was performed under controlled metabolic feeding conditions. Subjects consumed 3 isoenergetic diets and each diet was consumed for 4 weeks: a control diet (no nuts or fish), a walnut diet (1.5 oz/day of walnuts, 6 times/week) and a fish diet (8 oz/week of salmon). Blood specimens were collected at baseline and at the end of each diet period. RESULTS: The results showed that the plasma hydrophilic ORAC was significantly higher in the walnut diet compared with the control diet and the fish diet ($p < 0.0001$). In addition, the urine ORAC was significantly higher in the walnut diet and the fish diet compared with the control diet ($p < 0.0001$). Moreover the hydrophilic/lipophilic ORAC for the food itself was significantly higher in the walnut diet compared with the control diet and the fish diet ($p < 0.0001$). CONCLUSION: The present results suggest that walnuts have a large antioxidant capacity; therefore, including walnuts in the daily diet may be beneficial to maintain an antioxidant status in the body.

Key area: Heart Health

Katz DL, Davidhi A, Ma Y, Kavak Y, Bifulco L, Njike VY. Effects of walnuts on endothelial function in overweight adults with visceral obesity: a randomized, controlled, crossover trial.

J Am Coll Nutr. 2012 Dec;31(6):415-23. doi: 10.1080/07315724.2012.10720468

Abstract: OBJECTIVES: Metabolic syndrome is a precursor of diabetes and cardiovascular disease (CVD). Walnut ingestion has been shown to reduce CVD risk indices in diabetes. This randomized controlled crossover trial was performed to investigate the effects of daily walnut consumption on endothelial function and other biomarkers of cardiac risk in a population of overweight individuals with visceral adiposity. METHODS: Forty-six overweight adults (average age, 57.4 years; 28 women, 18 men) with elevated waist circumference and 1 or more additional signs of metabolic syndrome were randomly assigned to two 8-week sequences of walnut-enriched ad libitum diet and ad libitum diet without walnuts, which were separated by a 4-week washout period. The primary outcome measure was the change in flow-mediated vasodilation (FMD) of the brachial artery. Secondary measures included serum lipid panel, fasting glucose and insulin, Homeostasis Model Assessment-Insulin Resistance values, blood pressure, and anthropometric measures. RESULTS: FMD improved significantly from baseline when subjects consumed a walnut-enriched diet as compared with the control diet ($1.4\% \pm 2.4\%$ versus $0.3\% \pm 1.5\%$; $p = 0.019$). Beneficial trends in systolic blood pressure reduction were seen, and maintenance of the baseline anthropometric values was also observed. Other measures were unaltered. CONCLUSION: Daily ingestion of 56 g of walnuts improves endothelial function in overweight adults with visceral adiposity. The addition of walnuts to the diet does not lead to weight gain. Further study of the potential role of walnut intake in diabetes and CVD prevention is warranted.

Key area: Heart Health

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. doi: 10.1016/j.nut.2011.03.004

Abstract: 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.

Key area: 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 Dec;96(6):1262-73. doi: 10.3945/ajcn.112.044040

Abstract: 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.

Key area: Heart Health

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 2012;107(9):1393–401.

Abstract: 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.

Key area: Cognitive Health

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 Oct 25;87(4):101. doi: 10.1095/biolreprod.112.101634

Abstract: **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.

Key area: Reproductive Health

Vanden 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;64(7):1078-86. doi: 10.1080/01635581.2012.717679. PMID: 23061909.

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.

Key area: Cancer

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. doi: 10.1016/j.jnutbio.2011.01.004

Abstract: 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.

Key area: Heart Health

2011

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. doi: 10.1080/01635581.2011.589959

Abstract: 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.

Key area: 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. doi: 10.1007/s11064-011-0533-z

Abstract: Amyloid beta-protein (A β) is the major component of senile plaques and cerebrovascular amyloid deposits in individuals with Alzheimer's disease. A β 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 A β , and solubilization of preformed fibrillar A β by walnut extract was previously reported. The present study was designed to investigate whether walnut extract can protect against A β -induced oxidative damage and cytotoxicity. The effect of walnut extract on A β -induced cellular damage, ROS generation and apoptosis in PC12 pheochromocytoma cells was studied. Walnut extract reduced A β -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 A β -induced oxidative stress and associated cell death.

Key area: Cognitive Health

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. doi: 10.1016/j.cct.2011.03.007

Abstract: 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.

Key area: Research Methodology

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. doi: 10.1186/1743-7075-8-61

Abstract: 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.

Key area: Heart Health

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. doi: 10.1038/oby.2009.409

Abstract: 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.

Key area: Metabolic Syndrome

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. doi: <https://doi.org/10.2337/dc09-1156>

Abstract: 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. $1.2 \pm 1.6\%$; $P = 0.04$). 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.

Key area: Diabetes

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. doi: 10.1186/1475-2891-9-21

Abstract: 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 ≥ 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.

Key area: Heart Health

Ros E, Tapsell LC, Sabaté J. Nuts and berries for heart health.

Curr Atheroscler Rep. 2010 Nov;12(6):397-406. doi: 10.1007/s11883-010-0132-5

Abstract: 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.

Key area: Heart Health

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. doi: 10.1038/ejcn.2009.152

Abstract: 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.

Key area: Heart Health

West SG, Krick AL, Klein LC, Zhao G, Wojtowicz TF, McGuinness 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. doi: 10.1080/07315724.2010.10719898

Abstract: 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 low-density 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 = 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.

Key area: Heart Health

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. doi: 10.1007/s10753-010-9189-0

Abstract: 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.

Key area: Cognitive Health

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. Lifestyle counseling and supplementation with flaxseed or walnuts influence the management of metabolic syndrome.

J Nutr. 2010 Nov;140(11):1937-42. doi: 10.3945/jn.110.126300

Abstract: 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.

Key area: Metabolic Syndrome

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. doi: 10.3945/ajcn.2009.27457

Abstract: 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/m²): 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.

Key area: Heart Health

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. doi: 10.3945/jn.109.108266

Abstract: 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.

Key area: Cognitive Health

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. doi: 10.3945/ajcn.2009.26736S

Abstract: 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.

Key area: Heart Health

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. doi: 10.1080/07315724.2009.10719793

Abstract: 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.

Key area: Body Weight and Composition

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. doi: 10.1038/ejcn.2009.19

Abstract: **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 ($P<0.004$), but 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.

Key area: Diabetes

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. doi: 10.1111/j.1365-277X.2008.00923.x

Abstract: **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.

Key area: Heart Health

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. doi: 10.1016/j.jacl.2009.06.002

Abstract: 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 1.67 μ M), whereas ALA did not. Likewise, LA and EPA decreased sterol response element binding protein-1c mRNA (EC(50) of 0.78 and 1.78 μ M), 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.

Key area: Heart Health

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. doi: 10.1017/S0007114508059369

Abstract: 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 α -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.

Key area: Cognitive Health

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. doi: 10.3945/ajcn.2009.26736J

Abstract: 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.

Key area: Cognitive Health

Willis, L, Shukitt-Hale, B, Joseph, JA. Dietary polyunsaturated fatty acids improve cholinergic transmission in the aged brain.

Genes Nutr. 2009 Dec;4(4):309-14. doi: 10.1007/s12263-009-0141-6

Abstract: 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.

Key area: Cognitive Health

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. doi: 10.1080/01635580802065302.

Abstract: 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.

Key area: Cancer

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. doi: 10.1080/01635580802065294

Abstract: 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 \geq 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.

Key area: Research Methodology

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. doi: 10.1186/1475-2891-7-13

Abstract: 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 $+0.83 \pm 0.52$ 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.

Key area: Cancer

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. doi: 10.1186/1475-2891-6-2

Abstract: 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.

Key area: Bone Health

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. doi: 10.1080/07315724.2007.10719617

Abstract: 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.

Key area: Cancer

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. doi: 10.1093/ajcn/85.2.385

Abstract: 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.

Key area: Heart Health

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. doi: 10.1016/j.jacc.2006.06.057

Abstract: **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.

Key area: Heart Health

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 Nutr. 2006; 136: 428-32. doi: 10.1093/jn/136.2.428

Abstract: 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.

Key area: Heart Health

2005

Gillen LJ, Tapsell LC, Patch CS, Owen A, Batterham 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. doi: 10.1016/j.jada.2005.04.007

Abstract: 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 (≥ 2.22 g alpha-linolenic acid, ≥ 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.

Key area: Diabetes

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. doi: 10.1016/j.nut.2005.02.005

Abstract: 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 \pm standard error melatonin concentrations were 3.5 \pm 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.

Key area: Nutrient & Bioactive Composition

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. doi: 10.1079/BJN20051567

Abstract: 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/m²), 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/m²). 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.

Key area: Body Weight and Composition

Zhao G, Etherton TD, Martin KR, Vanden Heuvel 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. doi: 10.1016/j.bbrc.2005.08.204

Abstract: 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.

Key area: Heart Health

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. doi: 10.1161/01.CIR.0000124477.91474.FF

Abstract: 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 ($-4.4 \pm 7.4\%$) and LDL cholesterol ($-6.4 \pm 10.0\%$) ($P < 0.05$ 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.

Key area: Heart Health

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. doi: 10.2337/diacare.27.12.2777

Abstract: 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.

Key area: 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. doi: 10.1093/jn/134.11.2991

Abstract: 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.

Key area: Heart Health

2002

Feldman EB. The scientific evidence for a beneficial health relationship between walnuts and coronary heart disease.

J Nutr. 2002; 132:1062S-1101S. doi: 10.1093/jn/132.5.1062S

Abstract: 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.

Key area: Heart Health

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. doi: 10.1038/sj.ejcn.1601400

Abstract: 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 ($P \leq 0.05$). 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.

Key area: Heart Health

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. doi: 10.1093/ajcn/74.1.72

Abstract: 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 (\pm SD age 60 \pm 8 y), walnut supplementation did not increase body weight despite increased energy intake and the LFD caused weight loss (1.3 \pm 0.5 kg; $P < 0.01$). When comparing the HD with the HD+W, linoleic acid concentrations increased from 29.94 \pm 1.14% to 36.85 \pm 1.13% and alpha-linolenic acid concentrations increased from 0.78 \pm 0.04% to 1.56 \pm 0.11%. During the LFD+W, plasma total cholesterol concentrations decreased by 0.58 \pm 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.

Key area: Heart Health

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. doi: 10.1093/jn/131.11.2837

Abstract: 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.

Key area: Heart Health

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. Link: <http://www.jlr.org/content/42/12/2069.long>

Abstract: 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.

Key area: Heart Health

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. doi: 10.7326/0003-4819-132-7-200004040-00005

Abstract: 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.

Key area: Heart Health

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. doi: 10.1056/NEJM199303043280902

Abstract: 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 (\pm SD) serum values for total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol were, respectively, 182 \pm 23, 112 \pm 16, and 47 \pm 11 mg per deciliter (4.71 \pm 0.59, 2.90 \pm 0.41, and 1.22 \pm 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.

Key area: Heart Health



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