

Final Report

The Effects of Macadamia Nuts on Cardiometabolic Risk Factors and Adiposity: A Randomized Intervention Study

Project leader:

Dr. Joan Sabate

Delivery partner:

Loma Linda University

Project code:

MC17005

Project:

The Effects of Macadamia Nuts on Cardiometabolic Risk Factors and Adiposity: A Randomized Intervention Study (MC17005)

Disclaimer:

Horticulture Innovation Australia Limited (Hort Innovation) makes no representations and expressly disclaims all warranties (to the extent permitted by law) about the accuracy, completeness, or currency of information in this Final Report.

Users of this Final Report should take independent action to confirm any information in this Final Report before relying on that information in any way.

Reliance on any information provided by Hort Innovation is entirely at your own risk. Hort Innovation is not responsible for, and will not be liable for, any loss, damage, claim, expense, cost (including legal costs) or other liability arising in any way (including from Hort Innovation or any other person's negligence or otherwise) from your use or non-use of the Final Report or from reliance on information contained in the Final Report or that Hort Innovation provides to you by any other means.

Funding statement:

This project has been funded by Hort Innovation, using the macadamia research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

ISBN 978-0-7341-4732-5

Published and distributed by: Hort Innovation

Level 7

141 Walker Street

North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

© Copyright 2021 Horticulture Innovation Australia

Study (MC17005)

Content

Content	3
Summary	4
Public summary	Erro
r! Bookmark not defined.	
Keywords	5
Introduction	6
Methodology	7
Outputs	9
Outcomes	10
Monitoring and evaluation	Erro
r! Bookmark not defined.	
Recommendations	12
Refereed scientific publications	13
References	14
Intellectual property, commercialisation and confidentiality	Erro
r! Bookmark not defined.	
Acknowledgements	Erro
r! Bookmark not defined.	
Appendices	16

Summary

The MAC Study (Macadamia Nut Effects on Adiposity and Cardiovascular Risk Factors) was a randomized 2x2 crossover trial in which we sought to assess the effect of daily consumption of macadamia nuts on body weight, waist circumference (WC), body composition, blood lipids, and glycemic indices in overweight and obese adults with elevated cardiometabolic risk. Thirty-five subjects (28 females) consumed macadamia nuts daily as 15% of energy for 8 weeks (intervention) and their usual diet for 8 weeks (control), with a 2-week washout. Subjects were adults aged 40–75 y with a BMI of 25–39 kg/m², a waist circumference (WC) of >102 cm for men and >89 cm for women, and one additional cardiometabolic risk factor. Dietary intake was assessed with 24-hour dietary recalls. Statistical analyses utilized a mixed model with treatment, sequence, period, and subgroups as fixed-effect terms and subjects as a random-effects term.

We found that Macadamia nuts were associated with higher intake of total and monounsaturated fat (MUFA), as well as with higher plasma MUFA and 16:1 n-7 palmitoleic acid, as expected, no change in intake of saturated fat (SFA), and a reduction in the dietary polyunsaturated fat (PUFA): SFA ratio and plasma n-6 PUFA. Compared to control, consumption of macadamia nuts led to nonsignificant reductions in mean weight of 350 g and in mean BMI of -0.14 kg/m², no change in WC, body fat mass or % body fat, and small but significant reductions in skeletal muscle mass, lean body mass, and total body water that were greater in obese versus overweight subjects. Compared to the control diet, macadamia nuts led to nonsignificant reductions in total cholesterol (TC) of 2.1% and low-density lipoprotein (LDL) of 4%. In women only, the cholesterol lowering effect of macadamia nuts was greater among those who were overweight compared to obese, had a WC < 100 cm, and % body fat less than the median of 44% ($p < 0.05$ for all). Apo B (Apolipoprotein B, the identifying protein of Very-Low-Density Lipoprotein (VLDL), Intermediate-Density Lipoprotein (IDL), and Low-Density Lipoprotein/LDL) was also lower in women with a WC < 100 cm. Triacylglycerol, HDL, small dense LDL, oxidized LDL, and Apo A1 (the identifying protein of High-Density Lipoprotein (HDL)) were not significantly altered. Glucose, insulin, and insulin resistance (HOMA-IR) were not changed with macadamia nuts in the overall cohort, but insulin and HOMA-IR were significantly higher after the MAC treatment among those with a baseline WC less than the median value of 108 cm.

Among overweight/obese adults with elevated cardiometabolic risk in free-living conditions, adding macadamia nuts to their habitual diet without altering saturated fat intake lowered TC and LDL, although not significantly, without gains in body weight or body fat. Adiposity appeared to modify the effects, as insulin and HOMA-IR increased slightly in subjects with a lower WC, while cholesterol lowering was greatest among women with lower (although still elevated) adiposity measures.

Study (MC17005)

Keywords

Macadamia nuts, body weight, body composition, adiposity, overweight, obesity, cholesterol, LDL, Apo B, palmitoleic acid.

Introduction

Nuts are now routinely considered part of a heart-healthy diet, having been associated with a reduced risk of cardiovascular disease (CVD) and death in several large epidemiologic studies (1-4). One serving (28 g) of nuts per day is thought to reduce coronary heart disease by 29% and CVD by 21% (5). The benefits of nut intake are at least partially related to their plasma lipid lowering effects. A meta-analysis of the effects of tree nuts (6) found a significant reduction in total (TC) (-4.7 mg/dL), LDL (-4.8 mg/dL), Apo B (-3.7 mg/dL), and triacylglycerol (TAG) (-2.2 mg/dL). Importantly, the health benefits of mixed nuts, and some individual nuts including walnuts, almonds, and pistachios occurred without an increase in body weight, Body Mass Index (BMI), or waist circumference, despite their high fat content (7-11). Whether macadamia nuts, the nut with the highest amount of fat, would have similar effects on adiposity and abdominal obesity remains unclear.

Macadamia nuts have a higher total fat content (75%) than other nuts, of which 82% is monounsaturated fat (MUFA), with only 0.2-2.4% polyunsaturated fat (PUFA), and 12.1-17.8% saturated fat (SFA) (12, 13). The MUFA content is also unique, being 60-65% oleic acid and 17-19% palmitoleic acid, a fatty acid found in few other foods. Data is emerging that adipose-derived palmitoleate may function as a beneficial lipokine, reducing hepatic de novo lipogenesis (DNL), insulin resistance, and adiposity (14-16). This would be expected to improve metabolic dyslipidemia, resulting in lower TAG, small dense LDL (sdLDL) and higher HDL, particularly in the setting of visceral adiposity (and greater metabolic dyslipidemia). In addition, high MUFA intake has been shown in a meta-analysis of feeding trials to lower fasting glucose and insulin resistance (HOMA-IR) when replacing carbohydrate (17).

To date, five small studies have found that macadamia containing diets lower TC and LDL and have variable effects on TAG and HDL (18-22). However, in the four studies that reported macronutrient intakes, all macadamia diets reduced SFA by 5-6% of energy compared to the reference diet (control or baseline) (18, 19-22). The three studies with documented macronutrient intakes and a control arm were all carefully controlled, with most of the food provided by the study center (18, 21, 22). The effects of macadamia nuts on lipids in free-living conditions, without a reduction in SFA, are unknown. Additionally, none of the studies examined apolipoproteins, the atherogenic lipoproteins sdLDL and oxidized LDL (oxLDL), or glycemic indices, all were of short duration (3-5 weeks), and four of the five studies focused on normal and overweight individuals. One study allowed subjects with a BMI of 22-35 kg/m² (18), but none considered effect modification by adiposity. There is evidence that the effectiveness of TC and LDL lowering with nuts may vary by BMI. In our pooled analysis of nut consumption in 25 intervention trials (23), the cholesterol-lowering effects of nuts were greatest in subjects with a normal weight (BMI < 25 kg/m²), and progressively lower, though still significant, in overweight and obese subjects. Only one study has examined the effect of macadamia nuts on inflammatory markers and found they significantly reduced leukotriene B₄ (24). Finally, only two studies examined body weight, finding small but significant reductions with macadamia nut interventions (19, 20); no studies have examined macadamia nut effects on body composition.

We aimed to determine the impact of daily consumption of macadamia nuts in overweight and obese adults at elevated cardiometabolic risk in a free-living environment in four distinct areas: 1) body weight and composition, 2) traditional and atherogenic plasma lipids and lipoproteins, 3) the glycemic variables glucose, insulin, and insulin resistance, and 4) inflammation and endothelial function. We hypothesized that macadamia nuts would reduce TC, LDL-C and the components of metabolic dyslipidemia (pre-specified primary outcomes), and that adiposity would be an effect modifier of the cholesterol-lowering effects (pre-specified secondary outcome). Additionally, we hypothesized that macadamia nuts would lower body weight, body fat, glycemic indices, and inflammatory markers (also pre-specified primary outcomes).

Methodology

Study Design

We conducted a randomized controlled 2x2 cross-over study in free-living adults consuming their habitual diet, with and without macadamia nuts as 15% of daily energy. Each diet was consumed for eight weeks with a two-week washout period.

Participants

Eligible participants were men and postmenopausal women aged 40 to 75 with a BMI of 25-39 kg/m², a waist circumference (WC) of ≥ 88.9 cm (35 in) for women and ≥ 101.6 cm (40 in) for men, and $\geq$ one additional cardiometabolic risk factor (plasma TC ≥ 200 mg/dL, LDL ≥ 100 mg/dL, TAG ≥ 150 mg/dL, fasting glucose ≥ 100 mg/dL, or hypertension defined as blood pressure $\geq 130/85$ mmHg or current use of antihypertensive medication). Exclusion criteria included nut allergies; medications affecting lipid or glucose metabolism or immune modulators; high doses of lipid-lowering dietary supplements in the last 6 months; the presence of significant chronic disease including diabetes; cancer in the last 10 years (excluding non-melanoma skin cancer); a weight change of $>10\%$ of body weight in the prior 3 months; high alcohol intake (>1 drink/d for women, >2 drinks/d for men); and tobacco use.

We calculated the sample size to provide 80% power to detect a 10 mg/dL change in LDL, with an α of 0.05, and a correlation of 0.9, allowing for a 15% dropout rate. Randomization was done utilizing a random numbers table by study authors JJ and CH. The study was approved by the Institutional Review Board at Loma Linda University. Written informed consent was obtained from all participants prior to beginning the study.

Dietary Intervention

Macadamia nuts were provided as 15% of estimated Total Energy Expenditure (TEE) in pre-measured daily portions during the intervention period. Daily energy requirements were estimated using the Mifflin St-Jeor equation recommended for use in overweight and obese subjects (25) adjusted by a factor for physical activity assessed with the Rapid Assessment of Physical Activity (RAPA) form (26). The estimated TEE was then used to assign each participant to a level of macadamia nut intake. Potential energy intakes were bundled into groups spanning 200-300 kcal, and the mean of each was used to calculate 15% of kcal to be provided as macadamia nuts. This resulted in 4 groups of nut intake: 1.25 oz (35g), 1.5oz (42g), 1.7oz (48g), and 2.1oz (59g).

All subjects received nutrition counseling on a biweekly basis. Subjects were asked to consume their habitual diet, with macadamia nuts replacing calories approximately equal to their allotment of macadamia nuts during the intervention period, and to avoid all other nuts for the duration of the study. Participants were counseled to replace high-calorie foods, particularly foods high in sugar or saturated fat, with the macadamia nuts. During the control phase, participants were asked to continue their habitual diet but abstain from all nuts. Data from 24-hour recalls was used to personalize the nutrition counseling.

Data Collection

All variables were measured at baseline and at the end of each diet period. Weight and body composition were measured with an InBody device (InBody570, Seoul, Korea). Fasting blood samples were immediately processed and aliquoted plasma was stored at -80°C . Laboratory analyses of plasma lipids, lipoproteins, glycemic and inflammatory variables, and fatty acids were performed at the Jean Mayer

Study (MC17005)

USDA Human Nutrition Research Center on Aging at Tufts university. Insulin resistance was calculated using the HOMA2 calculator from the University of Oxford (27).

Dietary intake was assessed with 24-hr recalls using the multiple-pass method with Nutrition Data System for software version 2018 (University of Minnesota, Minneapolis, MN). A total of six recalls were obtained per subject: two at baseline and two during each diet period. Recalls with unrealistic intakes <500 kcal or >4,100 kcal were excluded from analysis. Energy and nutrient intakes were averaged for each phase for analysis. Intake of macadamia nuts was documented daily in a diary provided to the subjects.

Compliance was monitored at bi-weekly visits by review of subject diaries, allowing us to rapidly address any issues. Compliance was objectively assessed with the 24-hr recalls and by differences in plasma palmitoleic acid between phases.

Data Analysis

Statistical analyses were performed with IBM SPSS Statistics version 27. Baseline data was evaluated with and without dropouts. Endpoint data was evaluated using a mixed effects regression model, with treatment, sequence, and period as fixed-effect terms and subjects as a random-effects term. To examine whether macadamia nut effects on TC, LDL, and the glycemic variables were modified by adiposity, we performed subgroup analyses with dichotomized variables for BMI (25-29.9 vs ≥ 30 kg/m²), WC (<108 cm vs ≥ 108 cm, the cohort median), and % body fat (<43% or $\geq 43\%$, the cohort median). The dichotomized variables were added to the above model as fixed effects and outcomes were evaluated by treatment (macadamia nuts vs. control). Additional subgroup analyses were done with stratification by gender and baseline TC and LDL (TC <200 vs ≥ 200 mg/dL; LDL <130 vs ≥ 130 mg/dL). We identified significant interactions between treatment, gender, and WC (< 100 cm for women, < 116 cm for men) for both TC and LDL. We therefore also performed the above analyses in women only (n=28).

Data analysis was performed 'per protocol'. Three subjects dropped out before any endpoint data was collected and were not included in the analyses. Independent sample t-tests comparing 'completers' to 'non-completers' showed no differences in weight, BMI, WC, TC, or LDL, though the non-completers were younger and appeared to have more severe metabolic syndrome with higher TAG and sdLDL, and lower HDL. The non-completers also consumed less energy, total fat, SFA, MUFA, and PUFA at baseline.

All variables were normally distributed except VLDL, TAG, insulin, and HOMA2-IR. The base 10 logarithm of all variables were normally distributed. Analyses of the logarithm of VLDL and TAG did not differ from that of the primary variables, and the primary variables were utilized. The base 10 logarithm was used for insulin and HOMA2-IR.

We recently received the laboratory analyses of inflammatory markers, including IL-6, TNF, ICAM-1, VCAM-1, 8-iso-PGF2, and MDA. Our data analysis of these variables is pending.

Study (MC17005)

Outputs

3 abstracts based on the MAC study were presented at the American Society for Nutrition annual meeting (Nutrition Live 2020) and subsequently published (references are here, full abstracts in appendices).

[The Perceived Impact of Macadamia Nut Consumption on Feelings of Satisfaction and Bowel Function.](#)

Celine Heskey, Sujatha Rajaram, Abigail Clarke, Julie Jones, Rawiwan Sirirat, Joan Sabaté. Curr Dev Nutr. 2020 Jun; 4(Suppl 2): 1127. Published online 2020 May 29. doi: 10.1093/cdn/nzaa055_012

[Effect of Daily Macadamia Nut Consumption on Anthropometric Indices in Overweight and Obese Men and Women.](#)

Julie Jones, Sujatha Rajaram, Celine Heskey, Rawiwan Sirirat, Abigail Clarke, Keiji Oda, Joan Sabaté. Curr Dev Nutr. 2020 Jun; 4(Suppl 2): 589. Published online 2020 May 29. doi: 10.1093/cdn/nzaa047_009

[Daily Macadamia Nut Intake and Its Effect on Macronutrient Intake and Nutrient Displacement in Overweight and Obese Adults.](#)

Abigail Clarke, Julie Jones, Rawiwan Sirirat, Kristie LeBeau, Celine Heskey, Keiji Oda, Sujatha Rajaram, Joan Sabaté. Curr Dev Nutr. 2020 Jun; 4(Suppl 2): 1622. Published online 2020 May 29. doi: 10.1093/cdn/nzaa063_020

Outcomes

Results

Of the 38 subjects enrolled in the study, 35 completed the study. Three subjects dropped out before completion of the first phase, 2 in the control group for family emergencies and one in the macadamia group due to a gallbladder disorder. There were no differences between randomized groups in baseline anthropometrics, nutrient intake, or plasma fatty acids. Twenty percent of the subjects were male. Approximately half ($n=18$) were overweight (BMI 25-29.9 kg/m²), and half ($n=17$) were classified as obese (BMI ≥ 30 kg/m²).

Mean daily energy intake was slightly higher during the macadamia nut phase (~97 kcal/d). Macronutrient intakes between the macadamia and control diet phases differed primarily in total fat and MUFA intake. Total fat was an average of 26 g/d higher with macadamia nuts, with most of the difference attributable to MUFA, which was 24.7 g/d higher with macadamia nuts, as expected. Mean SFA intake was slightly higher, while mean PUFA, protein, and carbohydrate intakes were all slightly lower during the macadamia nut phase. The PUFA:SFA ratio (P:S ratio) was nearly 20% lower with macadamia nut consumption. Plasma fatty acid levels confirmed the higher intake of MUFA and palmitoleic acid during the macadamia nut phase, while plasma n-6 PUFA and n-3 PUFA both made up a lower percentage of plasma fatty acids with nut consumption (plasma n-6 PUFA was significantly lower).

Compared to control, consumption of macadamia nuts led to a small mean weight reduction of 350 g (NS). BMI and waist circumference were essentially unchanged. Body composition analysis demonstrated minimal differences in body fat mass (BFM in kg) and % body fat with macadamia nuts, but significant small reductions in skeletal muscle mass (SMM), lean body mass (LBM), and total body water (TBW) following consumption of macadamia nuts. (Note that LBM is the sum of dry lean mass (DLM) and TBW; LBM plus BFM is equal to body weight; SMM and TBW may be considered overlapping components of lean body mass). In a stratified analysis by BMI (25-29.9 kg/m² vs. ≥ 30 kg/m²), obese subjects lost more weight, SMM, LBM, DLM, and TBW than did overweight subjects.

Given the significant increases in fat and MUFA intake, we explored associations between dietary total fat, SFA, MUFA and PUFA with the altered body compartments. SMM, LBM, and TBW were all positively and significantly associated with MUFA intake in the MAC phase. However, there were no interactions between MUFA and the body composition measures. Instead, dietary intake of PUFA interacted with both LBM and TBW (positively for the MAC phase and negatively for the control phase). There were no associations or interactions between plasma fatty acids and the same body composition measures (SMM, LBM, and TBW).

Compared to control, consumption of macadamia nuts also led to nonsignificant reductions of TC, LDL, and Apo B. TC was reduced by 2.1% and LDL by 4% compared to control. Subgroup analysis by baseline hypercholesterolemia showed no differences. Analyses by BMI (25-29.99 versus ≥ 30 kg/m²), median WC (<108cm, ≥ 108 cm) and median % body fat (< 43%, ≥ 43 %) showed nonsignificant trends towards greater lowering of TC and LDL among subjects with lower adiposity measures.

A stratified analysis in women only found no significant differences in lipid outcomes with macadamia nuts, though TC was reduced by 2.6% and LDL by 5.3% compared to control. In subgroup analyses in women by adiposity measures, both TC and LDL were significantly lower in those with lower adiposity as measured by BMI, WC, and % body fat. Apo B was significantly lower in women with a WC < 100 cm. The

Study (MC17005)

components of metabolic dyslipidemia (HDL, TAG, sdLDL) and oxLDL were not changed with macadamia nut consumption.

No significant differences were found between macadamia nut and control groups for glucose, insulin, or HOMA2-IR levels. Subgroup analyses showed no differences based on baseline BMI or % body fat. However, Insulin and HOMA-IR were significantly higher after the mac treatment among those with a lower WC at baseline.

Compliance with macadamia nut consumption was excellent, as measured by both 24-hour recalls and plasma palmitoleic acid. Macadamia nut inclusion in 24-hour dietary recalls was 94%. Plasma palmitoleate levels increased 42% from baseline during the MAC phase and 5% in the control phase.

Study (MC17005)

Recommendations

Macadamia nuts may be consumed as part of a healthy diet without weight gain or increased adiposity in overweight and obese individuals, and may therefore be recommended as a nutrient-dense food even among the overweight/obese population.

Refereed scientific publications

Two journal articles previously submitted are currently being combined into a single manuscript for submission in the next several months. Part of this time will be spent in further analysis of variables of inflammation.

Submitted manuscripts:

1. Jones, J., Heskey, C.E., Sirirat, R., Clarke, A., Miles, F., Sabaté, J., Rajaram, S. Macadamia Nuts Do Not Adversely Affect Body Weight or Adiposity in Free-Living Overweight and Obese Adults.
2. Jones, J., Sabaté, J., Heskey, C.E., Oda, K., A., Miles, F., Rajaram, S. Cholesterol-Lowering Effect of Macadamia Nuts is Modified by Adiposity Measures: A randomized trial.

References

>

- 1) Guasch-Ferré M, Liu X, Malik VS, Sun Q, Willett WC, Manson JE, Rexrode KM, Li Y, Hu FB, Bhupathiraju SN. Nut Consumption and Risk of Cardiovascular Disease. *J Am Coll Card* 2017;70(20):2519-2532.
- 2) Liu G, Guasch-Ferré M, Hu, Li Y, Hu FB, Rimm EB, Manson JE, Rexrode KM, Sun Q. Nut Consumption in Relation to Cardiovascular Disease Incidence and Mortality Among Patients With Diabetes Mellitus. *Circ Res* 2019;124:920-929.
- 3) Fraser GE, Sabaté J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary heart disease. The Adventist Health Study. *Arch Intern Med* 1992 Jul;152(7):1416-24.
- 4) Ros E. Nuts and CVD. *Br J Nutr*. 2015 Apr;113 Suppl 2: S111-20.
- 5) Aune D, Keum N, Giovannucci E, Fadnes LT, Boffetta P, Greenwood DC, Tonstad S, Vatten LJ, Riboli E, Norat T. Nut consumption and risk of cardiovascular disease, total cancer, all-cause and cause- specific mortality: a systematic review and dose-response meta-analysis of prospective studies. *BMC Medicine* 2016; 14:207.
- 6) Del Gobbo LC, Falk MC, Feldman R, Lewis K, Mozaffarian D. Effects of tree nuts on blood lipids, apolipoproteins, and blood pressure: systematic review, meta-analysis, and dose-response of 61 controlled intervention trials. *Am J Clin Nutr*. 2015;102(6):1347-1356. doi:10.3945/ajcn.115.110965.
- 7) Bes-Rastrollo M, Sabaté J, Gomez-Gracia E, et al. Nut consumption and weight gain in a Mediterranean cohort: the SUN Study. *Obesity* 2007; 15:107-116.
- 8) Bes-Rastrollo M, Wedick NM, Martinez-Gonzalez MA, et al. Prospective study of nut consumption, long-term weight change, and obesity risk in women. *Am J Clin Nutr* 2009;89:1913-1919.
- 9) Mozaffarian D, Hao T, Rimm EB, et al. Changes in diet and lifestyle and long-term weight gain in women and men. *N Engl J Med* 2001; 364:2392-2404.
- 10) Flores-Mateo G, Rojas-Rueda D, Basora J, Ros E, Salas-Salvadó J. Nut intake and adiposity: meta-analysis of clinical trials. *Am J Clin Nutr*. 2013;97(6):1346–1355. doi:10.3945/ajcn.111.031484
- 11) Babio N, Toledo E, Estruch R, et al. Mediterranean diets, and metabolic syndrome status in the PREDIMED randomized trial. *CMAJ*. 2014;186(17): E649-E657. doi:10.1503/cmaj.140764
- 12) USDA National Nutrient Database for Standard Reference Legacy Release, April 2018. <https://ndb.nal.usda.gov/ndb/search/list?home=true>.
- 13) Maguire LS, O’Sullivan SM, Galvin K, O’Connor TP, O’Brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *International Journal of Food Sciences and Nutrition* 2004;55(3):171-178.

Study (MC17005)

- 14) Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell*. 2008;134(6):933–944. doi: 10.1016/j.cell.2008.07.048.
- 15) Mozaffarian D, Cao H, King IB, et al. Circulating palmitoleic acid and risk of metabolic abnormalities and new-onset diabetes. *Am J Clin Nutr*. 2010;92(6):1350–1358. doi:10.3945/ajcn.110.003970.
- 16) Frigolet ME, Gutiérrez-Aguilar R. The Role of the Novel Lipokine Palmitoleic Acid in Health and Disease. *Adv Nutr*. 2017 Jan 17;8(1):173S-181S.
- 17) Imamura F, Micha R, Wu JH, de Oliveira Otto MC, Otite FO, Abioye AI, Mozaffarian D. Effects of Saturated Fat, Polyunsaturated Fat, Monounsaturated Fat, and Carbohydrate on Glucose-Insulin Homeostasis: A Systematic Review and Meta-analysis of Randomised Controlled Feeding Trials. *PLoS Med*. 2016 Jul 19;13(7):e1002087. doi: 10.1371/journal.pmed.1002087. PMID: 27434027; PMCID: PMC4951141.
- 18) Griel AE, Cao Y, Bagshaw DD, Cifelli AM, Holub B, Kris-Etherton PM. A Macadamia Nut-Rich Diet Reduces Total and LDL-Cholesterol in Mildly Hypercholesterolemic Men and Women. *J. Nutr* 2008; 138:761–767.
- 19) Hiraoka-Yamamoto J, Ikeda K, Negishi H, Mori M, Hirose A, Sawada S, Kazuya K, Onobayashi Y, Kitano S, Tashiro M, et al. Serum lipid effects of a monounsaturated (palmitoleic) fatty acid-rich diet based on macadamia nuts in healthy, young Japanese women. *Clinical and Experimental Pharmacology and Physiology* 2004;31: S37–S38.
- 20) Garg ML, Blake RJ, Wills RBH. Macadamia Nut Consumption Lowers Plasma Total and LDL Cholesterol Levels in Hypercholesterolemic Men. *J Nutr* 2003;133(4):1060-3.
- 21) Curb JD, Wergowske G, Dobbs JC, Abbott RD, Huang B. Serum Lipid Effects of a High–Monounsaturated Fat Diet Based on Macadamia Nuts. *Arch Intern Med* 2000; 160:1154-58.
- 22) Colquhoun DM, Humphries JA, Moores D, Somerset SM. Effects of a macadamia nut enriched diet on serum lipids and lipoproteins compared to a low-fat diet. *FoodAustralia* 1996;48(5):216-221.
- 23) Sabaté J, Oda K, Ros E. Nut consumption and blood lipid levels: a pooled analysis of 25 intervention trials. *Arch Intern Med*. 2010 May 10;170(9):821-7.
- 24) Garg ML, Blake RJ, Wills RB, Clayton EH. Macadamia nut consumption modulates favourably risk factors for coronary artery disease in hypercholesterolemic subjects. *Lipids*. 2007 Jun;42(6):583-7. doi: 10.1007/s11745-007-3042-8. Epub 2007 Apr 17. PMID: 17437143.
- 25) Academy of Nutrition and Dietetics Evidence Analysis Library “In overweight or obese adults, which predictive equation for estimating resting metabolic rate should be used?” Published 2014, accessed 9 December 2019 at: https://www.andeal.org/template.cfm?template=guide_summary&key=4341.
- 26) Rapid Assessment of Physical Activity (RAPA). University of Washington Health Promotion Research Center. <https://depts.washington.edu/hprc/resources/products-tools/rapa/>
- 27) The HOMA2 Calculator ©The University of Oxford 2004-2021. <http://www.dtu.ox.ac.uk/homacalculator/download.php>

Appendices

<

Appendix 1.

Authors: Jones JL, Rajaram S, Heskey C, Sirirat R, Clarke A, Oda K, MPH, Sabaté J

Abstract Title: Effect of Daily Macadamia Nut Consumption on Anthropometric Indices in Overweight and Obese Men and Women

Objectives: We sought to assess the effect of daily consumption of macadamia nuts as 15% of calories on body weight, BMI, waist circumference, percent body fat and skeletal muscle mass in overweight/obese men and women with elevated cardiometabolic risk.

Methods: Utilizing a randomized crossover design, we randomized 38 subjects to consume macadamia nuts daily as 15% of calories for 8 weeks (intervention) and their usual diet for 8 weeks (control), with a 2-week washout. Three subjects dropped out early; n = 35 for analysis. Subjects were healthy men and postmenopausal women with a BMI of 25-39, a waist circumference of >101.6 cm for men and >88.9 cm for women, and one additional cardiovascular risk factor (fasting plasma glucose >100 mg/dL, triglycerides ≥150 mg/dL, total cholesterol >200 mg/dL, LDL-C >100 mg/dL, blood pressure ≥130/85 mmHg or taking anti-hypertensive medication). Macadamia nuts were provided in pre-weighed daily portions as 15% of calories calculated using the Mifflin-St. Jeor equation. Percent body fat and skeletal muscle mass (kg) were determined by bioelectrical impedance analysis. A mixed model analysis was performed with treatment, sequence, phase, and baseline values as fixed-effect terms and subjects as a random-effects term.

Results: Compared to control, consumption of macadamia nuts led to a mean weight change of -348 g (84.13 vs. 83.78 kg; p = 0.15) a mean BMI change of -0.15 kg/m² (30.61 vs. 30.47 kg/m²; p = 0.12), and a mean waist circumference change of 0.17 cm (107.41 vs. 107.58 cm; p = 0.61). Percent body fat increased by an average of 0.26% after eating nuts (42.70 vs. 42.96%; p = 0.16). Skeletal muscle mass was slightly but significantly lower after eating nuts with a mean change of -0.237 kg (26.33 vs. 26.09 kg; p = 0.017).

Conclusions: Daily consumption of high-fat macadamia nuts for eight weeks in overweight and obese individuals did not change anthropometrics including body weight, BMI, waist circumference, and percent body fat. Skeletal muscle mass was slightly lowered but likely not clinically relevant.

Funding Sources: Hort Innovation, Sydney, Australia.

Appendix 2.

Authors: Heskey C, Rajaram S, Clarke A, Jones JL, Sirirat R, Sabaté J.

Abstract Title: The Perceived Impact of Macadamia Nut consumption on Feelings of Satisfaction and Bowel Function.

Objectives: To assess subject perception of their level of various components of satisfaction, and changes in bowel habits, while consuming macadamia nuts during a free-living intervention study.

Methods: This was a randomized, crossover study in which free-living subjects (n=35) were allocated to an 8 week macadamia nut (15% kcals) intervention and control (usual diet) phases. Eligibility criteria included a BMI of 25-39 kg/m², abdominal obesity, and presence of at least 1 other cardiometabolic risk factor. Subjects attended a biweekly clinic with the study dietitians to receive a supply of pre-weighed daily portions of nuts (mixture of raw and roasted), instructions and reinforcement on ways to incorporate the nuts into their habitual diet. A 14-item macadamia nut satisfaction questionnaire was administered at the end of the study, to assess various factors including perceptions of taste, ease of use, ease of preparation of the nut, and fullness. A 9-item bowel habits questionnaire was administered at the end of both the treatment and control phases, assessing factors including bowel frequency, perceptions of change in digestion, and stool consistency, using the Bristol Stool chart. Subject responses to the satisfaction questionnaire were calculated as proportions. Bowel movement frequency, and stool type were compared between the macadamia nut and control phases using the Wilcoxon signed rank sum test.

Results: Most subjects liked the taste (89%) of macadamia nuts, found the nuts easy to use (95%), and incorporate (95%) into their daily diet, and did not feel deprived (86%) while eating macadamia nuts. About half of the subjects felt strongly that macadamia nuts added flavor and variety to their diets, and 80% really liked including macadamia nuts into their habitual diet. In terms of bowel function, no significant difference in bowel movement frequency was noted between the macadamia and control phases. Stool consistency (Bristol stool assessment) was significantly different between the macadamia and control phases (p=0.049).

Conclusions: Most subjects indicated favorable perceptions towards, and satisfaction with macadamia nuts. Self-reported stool consistency was different between the macadamia and control phases.

Funding Sources: Hort Innovation, Sydney, Australia

Appendix 3.

Authors: Clarke A, Jones JL, Sirirat R, LeBeau K, Heskey C, Oda K, Rajaram S, Sabaté J.

Abstract Title: Daily Macadamia Nut Intake and Its Effects on Macronutrient Intake and Nutrient Displacement in Overweight and Obese Adults.

Objectives: To determine the effect of daily intake of macadamia nuts on macronutrient intake and nutrient displacement in overweight and obese adults in a randomized crossover study.

Methods: This randomized cross-over study was comprised of two phases, each lasting eight weeks. Participants (n=35) were randomly assigned first to either the intervention (15% of calorie needs from macadamia nuts) or control (habitual diet) phase, with a two-week washout followed by the other phase. A total of six 24-hour recalls, two at baseline and two during each phase were conducted. Period adjustment t-test was used to determine the differences in macronutrient intake between the two phases. The displacement of nutrients was done by subtracting the observed intake of a particular nutrient from its' expected intake.

Results: Compared to the control phase, there were marginal increases in the consumption of total energy (mean diff= 145 kcal), total fiber (mean diff= 2g) and a significant increase in the consumption of total fat (mean diff=35g, P< 0.001). In addition, there was a significant increase in consumption of palmitoleic acid (mean diff= 5g) and oleic acid (mean diff= 11g, P< 0.001). However, the total saturated fat intake between the two phases was non-significant. There was a non-significant decrease in protein and carbohydrate intake. The displacement analysis revealed that participants had lower intakes of energy, protein and carbohydrates than predicted, with carbohydrates having the highest displacement of the nutrients.

Conclusions: The inclusion of macadamia nuts is associated with a marginal reduction of carbohydrate intake and an increase intake of monounsaturated fat which may have favorable health outcomes, but remains to be investigated.

Funding Sources: Hort Innovation, Sydney, Australia