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Tree Nut consumption is associated with better adiposity measures and cardiovascular and metabolic syndrome health risk factors in U.S. Adults: NHANES 2005–2010

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Abstract

Introduction: Previous research has shown inconsistencies in the association of tree nut consumption with risk factors for cardiovascular disease (CVD) and metabolic syndrome (MetS).

Objective: To determine the association of tree nut consumption with risk factors for CVD and for MetS in adults.

Methods: NHANES 2005–2010 data were used to examine the associations of tree nut consumption with health risks in adults 19+ years (n = 14,386; 51 % males). Tree nuts were: almonds, Brazil nuts, cashews, filberts [hazelnuts], macadamias, pecans, pine nuts, pistachios, and walnuts. Group definitions were non-consumers < $\frac{1}{4}$ ounce/day and consumers of $\geq \frac{1}{4}$ ounce/day tree nuts using data from 24-h dietary recalls. Means and ANOVA (covariate adjusted) were determined using appropriate sample weights. Using logistic regression, odds ratios of being overweight (OW)/ obese (OB) (body mass index [BMI] >25/<30 and ≥ 30 , respectively) and having CVRF or MetS, were determined.

Results: Tree nut consumption was associated with lower BMI (p = 0.004), waist circumference (WC) (p = 0.008), systolic blood pressure (BP) (p = 0.001), Homeostatic Model Assessment—Insulin Resistance (p = 0.043), and higher high density lipoprotein-cholesterol (p = 0.022), compared with no consumption, and a lower likelihood of OB (-25 %), OW/OB (-23 %), and elevated WC (-21 %).

Conclusions: Tree nut consumption was associated with better weight status and some CVRF and MetS components.

Keywords: Tree Nuts, NHANES, Adults, Metabolic Syndrome, Cardiovascular Risk Factors

Introduction

Tree nuts have been part of the diet of humans since paleolithic times [1]. The nutrients found in tree nuts, including almonds, Brazil nuts, cashews, filberts [hazelnuts], macadamias, pecans, pine nuts, pistachios, and walnuts vary by species, but in general, they provide energy, vegetable protein, heart-healthy oils, including monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), dietary fiber, calcium, potassium, folate, magnesium, selenium, and vitamin E. Tree nuts are also low in sodium and have no cholesterol [2]. Coupled with this positive nutrient profile, tree nuts also

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provide phenols, phytosterols, flavonoids, resveratrol, and other bioactive compounds [2, 3], which when coupled with vitamin E and selenium, serve as antioxidants, which may reduce the risk of cardiovascular risk factors (CVRF) [4–7] and cardiovascular disease [8, 9].

Recently, tree nut consumption was shown to have a significant inverse association with all-cause mortality and with death due to heart disease [8, 10]. The study by Bao et al., [8] showed that the frequency of tree nut consumption was associated with decreased risk for heart and cardiovascular disease, with the number of deaths lowest in those consuming tree nuts five or more times a week. That study failed to show a significant association with frequency of tree nut consumption and death due to stroke or type 2 diabetes.



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Most previous feeding studies have shown that consumption of tree nuts has been associated with healthier levels of CVRF, including total cholesterol [7, 11–13], low-density lipoprotein cholesterol (LDL-C) [7, 11–15], high-density lipoprotein cholesterol (HDL-C) [7, 14], triglycerides [7], apolipoprotein A [13, 16], and apolipoprotein B [11]; markers of oxidative stress [17, 18] or inflammatory markers [19]; endothelial dysfunction [7, 20, 21]; insulin resistance [22, 23]; hyperglycemia [15]; and hemoglobin A1c [15]. However, other studies have shown no significant effects on total cholesterol [24, 25], LDL-C [24, 25], HDL-C [24], triglycerides [24, 25], Creactive protein (CRP) [16, 24], fasting blood sugar [25], insulin resistance [21], hemoglobinA1c [21], and serum fructosamine [24].

Cross-sectional studies of adults that have examined the association between tree nut consumption and CVRF have also shown conflicting results. Tree nut consumers have been shown to have lower values for or decreased risk of higher body mass index (BMI) [5], obesity [4], elevated waist circumference (WC) [5], low HDL-C [5, 6], CRP [6], lower systolic (SBP) or diastolic blood pressure (DBP) [5, 6, 26], elevated fasting glucose [5], hemoglobin A1c [6], insulin [6], and a lower prevalence of metabolic syndrome (MetS) [5]. These studies all looked at multiple CVRF and findings were inconsistent since they also showed that tree nut consumption was not associated with decreased values or decreased risk of higher weight [6], elevated WC [6], components of dyslipidemia [4], hypertension [4], elevated fasting glucose [4, 6], or MetS [4, 6].

Reasons for these conflicting results in feeding and epidemiologic studies are not clear, but may include the populations studied, the type and amount of tree nut consumed, the length of the feeding trial, and, in the epidemiologic studies the method of classifying consumers into groups. Disparities among these studies indicate the need for further studies. There have been no recent studies using nationally representative data that has looked specifically at tree nut consumption and CVRF. The purpose of this study was to examine this association, using current data from participants of the National Health and Nutrition Examination Survey (NHANES) 2005–2010.

Subjects and methods

Study population and analytic sample

Data from the NHANES 2005–2006, 2007–2008, and 2009–2010 datasets were used to evaluate tree nut or tree nut butter (these components were considered together and are referred to as tree nuts below) consumption in the US population. Data from adults 19+ years of age (y) (N = 14,386) participating in the NHANES were combined to increase sample size [27, 28]. Analyses

included only individuals with complete and reliable dietary recalls as determined using the National Center for Health Statistics staff. Females who were pregnant or lactating were excluded from the study. In compliance with federal law, the NHANES use defined strict protocols to ensure confidentiality and protect participants' identity. As this study used secondary data, stripped of individual identifiers, it did not require institutional review [29].

Demographic information, including age, gender, raceethnicity, poverty index ratio (PIR), physical activity levels, and smoking status, used for covariates in the statistical analyses outlined below, was determined via interview [30]. Alcohol was also used as a covariate and was determined using the 24-h dietary recalls described below.

Dietary analyses

Dietary intake was determined using two multiple pass 24-h dietary recalls [31, 32]. The first recall was inperson in the Mobile Examination Center [33] and the second was conducted 3–10 days later via telephone [34]. The US Environmental Protection Agency Food Commodity Intake Database (FCID) commodity codes [35] were used to identify ingredients of survey foods that included tree nuts: almonds, Brazil nuts, cashews, filberts [hazelnuts], macadamias, pecans, pine nuts, pis-tachios, and walnuts.

The gram amount of tree nuts consumed by NHANES 2005–2010 respondents was determined by applying the FDIC tree nut composition data to the respondent's 24h recall dietary interview data. Tree nut intakes were aggregated over the entire day. Usual intake (UI) was determined using the National Cancer Institute method with survey day (one or two) and a weekend day flag (Friday/Saturday/Sunday versus others) as covariates [36]. Tree nut or all nut consumers were defined by a UI of at least ¼ ounce (7.0875 grams) per day [5].

Anthropometric and physiologic measures

Height, weight, and WC were obtained according to NHANES protocols [37]. Body Mass Index (BMI) was calculated as body weight (kilogram) divided by height (meters) squared [38]. Systolic blood pressure and DBP were determined using the standard NHANES protocol [39]. High density lipoprotein-cholesterol were determined on non-fasted individuals [40] while LDL-C [41], triglycerides [41], blood glucose [42], and insulin [42] were determined on only fasted subjects; thus, not all individuals may have values for all tests.

Overweight/obesity and high WC were determined using the National Heart Lung and Blood Institute Clinical Guidelines [38]. Overweight was defined as a BMI >25 and \leq 29.9; obesity was defined as a BMI \geq 30 kg/m². High

WC was defined as >102 cm for males and >88 cm in females. The Homeostatic Model of Assessment-Insulin Resistance (HOMA-IR), used to evaluate insulin resistance, was calculated as: fasting serum insulin/fasting plasma glucose [43]. Metabolic syndrome (MetS) was defined using the National Heart Lung and Blood Institute Adult Treatment Panel III criteria [44]: having 3 or more of the following risk factors: abdominal obesity, WC > 102 cm (males), >88 cm (females); hypertension, SBP ≥130 mmHg or DBP ≥85 mmHg or taking antihypertensive medications; HDL-cholesterol, <40 mg/dL (males), <50 mg/dL (females); high triglycerides, $\geq 150 \text{ mg/}$ dL or taking anti-hyperlipidemic medications; high fasting glucose, $\geq 110 \text{ mg/dL}$ or taking insulin or other hypoglycemic agents. An elevated LDL-C was defined as $\geq 100 \text{ mg/dL}$.

Statistical analyses

Sample-weighted data were used in all statistical analyses; and, all analyses were performed using SUDAAN Release11.0 (Research Triangle Institute, Research Triangle Park, NC) to adjust the variance for the complex sample design. For the 6-years 2005–2010, a 6-year weight variable was created by assigning $\frac{1}{3}$ of the 2 year weight for 2005–2006, 2007–2008, and 2009–2010 [27, 28]. A 6-year MEC-examined sample weight was used in analyses of intake, body measurements, blood pressure, and laboratory data, except a 6-year fasted sample weight was used in analyses of LDL-*C*, triglycerides, plasma glucose, insulin, and MetS.

The sample-weighted percentages (and standard error of the percentages) of the adults in tree nut consumers were calculated using PROC DESCRIPT of SUDAAN. Least-square means (and the standard errors of the least-square means) were calculated using PROC RE-GRESS of SUDAAN. The adjusted prevalence of a risk factor was determined by calculating the least-square mean of a dichotomous variable using PROC REGRESS, and odds ratios were calculated using PROC LOGISTIC of SUDAAN.

Covariates for least-square mean values and odds ratios of weight/adiposity related variables were, gender, age (years), race-ethnicity, poverty index ratio, physical activity level, smoking status and alcohol intake. The least-square mean values and odds ratios of BP, blood lipids, fasting glucose, and insulin were adjusted for BMI (kg/m²) as well. A p value of <0.05 was considered significant.

Results

Nut consumption

Tree nut consumers (n = 755; 50.2 % female; mean age 51.18 years ± 0.42 SE) constituted approximately 6.8 % of

the population. Details of the demographics of this population have been published previously [45]. Mean UI of tree nut consumers was 44.3 ± 1.6 g/d; whereas, *per captia* UI was 3.3 ± 0.1 g/d.

Weight/adiposity measures/blood pressure

Table 1 shows tree nut consumers had better weight/ adiposity parameters than non- consumers. BMI $(27.9 \pm 0.3 \text{ v} 28.7 \pm 0.1 \text{ kg/m}^2; p = 0.004)$, and WC $(95.8 \pm 0.7 \text{ v} 98.1 \pm 0.3 \text{ cm}; p = 0.008)$ were all significantly lower in tree nut consumers. Systolic blood pressure was lower in tree nut consumers (119.5 ± $0.8 \text{ v} 122.1 \pm 0.2 \text{ mm}$ Hg; p = 0.001).

Physiologic measures

Table 2 shows that tree nut consumers had higher HDL-C levels ($54.4 \pm 0.6 \text{ v} 52.9 \pm 0.3 \text{ mg/dL}$; p = 0.022) and lower HOMA-IR values ($3.0 \pm 0.1 \text{ v} 3.3 \pm 0.1$; p = 0.043) than non-consumers. Odds ratio analyses (Table 3) showed that tree nut consumers had a 25 % lower likelihood of obesity (OR = 0.75; 95 % confidence interval [CI] 0.60-0.95), a 23 % lower likelihood of overweight or obesity (0.77; 0.62-0.95), and a 21 % lower likelihood of an elevated WC (0.79; 0.64-0.99) than non-consumers.

Discussion

This study showed that those consuming tree nuts had better weight/adiposity measures and a lower risk of obesity, overweight/obesity, and elevated WC than nonconsumers. Tree nut consumers also had lower SBP and higher levels of HDL-C. The association of tree nut consumption and weight and cardiovascular risk factors using NHANES data has not been examined since the 1999–2004 data sets were published. An advantage to using data sets published after 2001–2002 cycle is that two 24-h dietary recalls are available from participants. Thus, since UI can be calculated [36], concerns about using a single dietary recall in data analysis should be assuaged.

Always of interest is to compare secular trends in consumption of healthful foods, like tree nuts. However, it is difficult to compare the percentage of individuals consuming tree nuts and the amount consumed by individuals in three previous NHANES studies [5, 6, 46], since the earlier studies used a single 24 h dietary recall and this study used UI. Further, this study used the FCID commodity codes [35] to determine intake, as opposed to the food codes found in Food and Nutrient Database for Dietary Studies [47] which are often used [5, 6]. The advantage is that the FDIC database provides estimates of food consumption, in terms of ingredients or as the food "as eaten." In this study, approximately 6.8 % of the study population consumed tree nuts; although this seems low, the weighted number actually represented

±SE p							
0.102							
0.004							
0.008							
0.001							
0.221							

Table 1 The association of consuming tree nuts with weight/adiposity and blood pressure measurements in adults participating in the 2005–2010 National Health and Nutrition Examination Survey

Abbreviations: BMI = Body mass index, WC = Waist circumference, BP = Blood pressure

^aCovariates: Gender, Ethnicity, Age, Poverty Index Ratio, Physical Activity Level (sedentary, moderate, active), Current Smoker Status, and Alcohol

^bCovariates: Gender, Ethnicity, Age, Poverty Index Ratio, Physical Activity Level (sedentary, moderate, active), Current Smoker Status, Alcohol, and BMI *Significantly Different

over 12,440,000 million individuals—a significant number. Those consuming tree nuts consumed an average of 44.3 g which is higher than the $\frac{1}{2}$ ounce (~14 g) that is considered an ounce equivalent of a protein food by MyPlate and higher than the US Food and Drug Administration's reference amount customarily consumed of 30 g for all types of nuts and mixtures except butters [48]. It is similar to the $1\frac{1}{2}$ ounces (42.5 g) recommended in the qualified health claim for tree nuts and heart disease [49].

In this study tree nut consumers had lower mean weight, BMI, and WC than non-consumers. There was also a lower risk of obesity/overweight, obesity, and elevated WC. Although tree nuts are an energy dense food, an inverse association between tree nut consumption and weight parameters or weight gain has been shown previously in cross-sectional studies [4, 5], prospective long-term cohort studies [50, 51], and feeding studies [52, 53]. A recent meta-analysis of controlled clinical trials looking at nut consumption and weight has also

shown that diets "enriched with nuts" did not increase weight or measures of adiposity [54].

The biological plausibility for these findings has been offered previously [54, 55]. Due to their high vegetable protein, dietary fiber, MUFA, and PUFA content, tree nuts are a satiating food and following consumption, appetite and consequently intake may be suppressed at subsequent eating occasions. Nuts must be chewed so that the particles are small enough to be swallowed; mastication may modify appetite. Further, the energy in nuts may be inefficiently absorbed. Finally, Atwater factors, when applied to almonds [56] and pistachios [57] resulted in a 32 % and 5 % overestimation, respectively, of their measured energy content. Obesity also contributes to the major causes of morbidity and mortality in the US; thus, any dietary changes that can lower the risk of obesity should be encouraged.

This study and both earlier studies [5, 6] of NHANES participants have shown lower SBP in tree nut consumers than in non-consumers. With relatively high levels of

Table 2 The association of	consuming tree nut	ts with physiolog	ic measures in	adults par	ticipating in t	the 2005–2010	National Health
and Nutrition Examination S	Survey						

Variable	Number	Tree Nut Consumers LS Mean \pm SE	Non-Tree Nut Consumers LS Mean ± SE	
LDL-C (mg/dL) ^a	6480	115.5 ± 2.4	115.8 ± 0.6	0.902
HDL-C (mg/dL) ^a	13,666	54.4 ± 0.6*	52.9 ± 0.3*	0.022
Triglycerides (mg/dL) ^b	6621	127.4 ± 6.5	134.1 ± 1.9	0.344
Glucose (mg/dL) ^c	6662	102.1 ± 1.2	104.3 ± 0.4	0.061
Insulin (uU/mL)	6581	11.3 ± 0.5	12.1 ± 0.2	0.125
HOMA-IR	6568	$3.0 \pm 0.1^{*}$	$3.3 \pm 0.1^{*}$	0.043
CRP (mg/dL) ^d	13,709	0.4 ± 0.04	0.4 ± 0.01	0.276

Abbreviations: BMI = body mass index, WC = waist circumference, BP = blood pressure, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = Homeostatic Model of Assessment - Insulin Resistance, CRP = C-reactive protein

Covariates: Gender, Ethnicity, Age, Poverty Index Ratio, Physical Activity Level (sedentary, moderate, active), Current Smoker Status, Alcohol, and BMI

^aTo convert mg/dL to mmol/L divide by 38.67

^bTo convert mg/dL to mmol/L divide by 38.67

^cTo convert mg/dL to mmol/L multiply by 0.055

^dTo convert mg/dL to mmol/L multiply by 9.524

*Significantly Different

Table 3 Risk of overweight and obesity and cardiovascular and metabolic syndrome risk factors in adult consumers and Non-

consumers of tree nuts participating in the 2005–2010 National Health and Nutrition Examination Survey

Variable	OR	LCL	UCL
Overweight ^{a,b}	1.02	0.67	1.24
Obese ^{a,b}	0.75*	0.60	0.95
Overweight or obese ^{a,b}	0.77*	0.62	0.95
WC elevated ^{a,c}	0.79*	0.64	0.99
Elevated systolic BP ^d	0.91	0.71	1.17
Elevated diastolic BP ^d	1.03	0.80	1.33
LDL-C elevated ^{d,e}	0.80	0.57	1.14
HDL-C reduced ^{d,e}	0.85*	0.67	1.07
Triglycerides elevated ^{d,e}	0.81	0.58	1.13
Glucose elevated ^{d,e}	0.81	0.59	1.11
Insulin elevated (>85th Pctl) ^d	0.82	0.54	1.26
Metabolic syndrome ^{a,f}	0.74	0.52	1.05

Abbreviations: WC = Waist circumference, BP = Blood pressure, LDL-C = Lowdensity lipoprotein cholesterol, HDL-C = High-density lipoprotein cholesterol For Tree Nut Consumers, the reference group was no tree nut consumption ^aCovariates: Gender, Ethnicity, Age, Socioeconomic Status (PIR 0–1.25, 1.25-3.4, \geq 3.25), Physical Activity Level (sedentary, moderate, active), Current Smoker Status, and Alcohol

^bOveweight was defined as a BMI 25–29.9; obese was defined as a BMI \geq 30; overweight or obese was defined as a BMI \geq 25

^cElevated WC was defined as >102 cm (males), >88 cm (females)

^dCovariates: Gender, Ethnicity, Age, Socioeconomic Status (PIR 0–1.25, 1.25-3., \geq 3.25), Physical Activity Level (sedentary, moderate, active), Current Smoker Status, Alcohol, and BMI

^eReduced HDL-cholesterol was defined as <40 mg/dL

(males), <50 mg/dL (females); high triglycerides, \geq 150 mg/dL or taking antihyperlipidemic medications; high fasting glucose, \geq 110 mg/dL or taking insulin or other hypoglycemic agents. Elevated LDL-C \geq 100 mg/dL ^fMetabolic syndrome was defined using the National Heart Lung and Blood Institute Adult Treatment Panel III criteria; that is having 3 or more of the following risk factors: abdominal obesity, WC > 102 cm (males), >88 cm (females); hypertension, SBP \geq 130 mmHg or DBP \geq 85 mmHg or taking anti-hypertensive medications; HDL-cholesterol, <40 mg/dL (males), <50 mg/ dL (females); high triglycerides, \geq 150 mg/dL or taking anti-hyperlipidemic medications; high fasting glucose, \geq 110 mg/dL or taking insulin or other hypoglycemic agents

*p < 0.05

unsaturated fatty acids, calcium (almonds), potassium, magnesium, and dietary fiber, coupled with low levels of sodium [2], tree nuts would appear to be a food associated with low blood pressure and they are encouraged in the DASH diet [58]. However, studies have shown that the effect of tree nut consumption on blood pressure is inconsistent. The cross-sectional PREDIMED study did not show an effect of tree nut consumption on hypertension [4]. Data from prospective cohorts are limited. Participants in the Physicians' Health Study I [59] reported a lower incidence of hypertension in lean men only; however, in the SUN study there was not relationship between nut consumption and incident hypertension [60]. A review of 19 clinical trials looking at blood pressure and nut consumption showed inconsistent results, with 13 studies showing no changes in blood pressure, one showing an increase in blood pressure, and the remaining five studies showing a reduction [61].

Consistent with other cross-sectional studies [5, 6], this study showed higher HDL-C levels in tree nut consumers than in non-consumers. Cross-sectional studies are hypothesis generating; thus, these findings led, in part, to clinical trials (hypothesis testing) that examined diets containing nuts versus those not containing nuts. These clinical trials have shown inconsistent results with regard to HDL-C levels. For example, Tappsell, et al., [62] showed an increase in HDL-C levels in individuals after 6 months of consuming a diet containing walnuts as compared to those consuming a control diet; whereas, Sabaté, et al., [63] showed that HDL-C levels were lower in those consuming 20 % of energy from walnuts, as compared with those consuming the control diet. Both of these studies were conducted in specific groups, the first in diabetics and the second in men only. To help reconcile these findings, a recent pooled analysis of primary data from 25 tree nut consumption trials with a total of 583 participants failed to show a significant difference in mean HDL-C levels between tree nut consumers and non-consumers [64]. Reasons for the differences between the results of cross-sectional studies, such as NHANES, and clinical trials are not clear but may reflect the population used, the length of the study, the amount of specific tree nuts consumed, and the assignment to consumption groups in cross-sectional studies. Overall, the relationship between lipid levels and tree nut consumption has been inconsistent, but overall, the association is positive [63]. This is likely due to the low saturated fatty acids, high MUFA, PUFA, and phytochemical content of most tree nuts.

Metabolic Syndrome is characterized by dyslipidemia, hypertension, abdominal obesity, insulin resistance, and hyperglycemia; it is a major risk factor for cardiovascular disease and type 2 diabetes [65]. It has previously been shown that tree nut consumers have a lower prevalence of MetS [5], but a previous cross-sectional study that looked only at out-of-hand tree nut and peanut consumption [6] failed to show that nut consumption was associated with a reduced risk of MetS. One reason may be that that study failed to show a difference in several of the risk factors for MetS, including elevated WC, triglycerides, and fasting glucose. Since this study also failed to show a reduced risk of MetS in tree nut consumers, additional studies are warranted.

Differences among results from the cross-sectional, cohort, and feeding studies seen in the CVRF examined may be the result of different tree nuts used in individual feeding studies, which may reflect the different nutrient profile of individual nut species; how consumption was determined, e.g., amount or frequency; the inflammatory markers studied; the characteristics of the population tested, including gender or whether participants were healthy or had been diagnosed with obesity, MetS, hyperlipidemics, or diabetes; or the length of the study.

Strengths and limitations

The strengths of this study included a large, nationally representative population and use of UI in the analyses. The limitations of the study are that results from any cross-sectional epidemiologic study cannot be used to determine cause and effect. Also since these data are based on self-reported intake, it must be considered whether tree nuts are reported differently than other foods. If self-reported intake of nuts is different from other foods it may make it more likely that consumers and non-consumers were misclassified.

Conclusions and implications

The prevalence of tree nut consumers was low; however, consumption was associated with a better weight/adiposity and CVRF profile than seen in non-consumers. Health professionals, especially registered dietitians and other health educators should provide diet counseling and nutrition education programs that increase awareness of the health benefits of tree nut consumption. Tree nuts should be consumed as part of an overall healthful meal pattern. Because of the conflicting results produced when studying the health benefits of tree nuts, future research should include more longitudinal studies and intervention trials examining these potential benefits.

Abbreviations

BMI: Body mass index; BP: Blood pressure; CPR: C-Reactive protein; CVRF: Cardiovascular risk factors; DBP: Diastolic blood pressure; FCID: Food commodity intake database; HDL-C: High density lipoprotein-cholesterol; HOMA-IR: Homeostatic model of assessment—insulin resistance; LDL-C: Low density lipoprotein-cholesterol; MetS: Metabolic syndrome; MUFA: Monounsaturated fatty acids; NHANES: National health and nutrition examination survey; OB: Obesity; OW: Overweight; PUFA: Polyunsaturated fatty acids; SBP: Systolic blood pressure; UI: Usual intake; WC: Waist circumference.

Competing interests

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Authors' contributions

VLF, TAN, and CO'N designed the study; VLF conducted the statistical analyses and had principal responsibility for assessment; TAN and CO'N also assessed the data; CO'N was the principal author of the manuscript; TAN and

VLF revised and edited the manuscript. Thus, all authors participated equally in this study and in the preparation of this manuscript. All authors read and approved the final manuscript.

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