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Post-prandial effects of high-polyphenolic extra virgin olive oil on endothelial function in adults at risk for type 2 diabetes: A randomized controlled crossover trial☆

Valentine Y. Njike [a,b,*], Rockiy Ayettey [a,b], Judith A. Treu [a,b], Kimberly N. Doughty [a,b], David L. Katz [a]

[a] Yale-Griffin Prevention Research Center, United States of America
[b] Griffin Hospital, Derby, CT (VN, RA, JT, KD, DK), United States of America

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ABSTRACT

Background: Effects of olive oil on cardiovascular risk have been controversial. We compared the effects of high-polyphenolic extra virgin olive oil (EVOO) and refined olive oil without polyphenols on endothelial function (EF) in adults at risk for Type 2 diabetes mellitus (T2DM).
Methods: Randomized, controlled, double-blind, crossover trial of 20 adults (mean age 56.1 years; 10 women, 10 men) at risk for T2DM (i.e., as defined by either prediabetes or metabolic syndrome) assigned to one of two possible sequence permutations of two different single dose treatments (50 mL of high-polyphenolic EVOO or 50 mL of refined olive oil without polyphenols), with 1-week washout. Participants received their olive oils in a smoothie consisting of ½ cup frozen blueberries and 1 cup (8 oz) low-fat vanilla yogurt blended together. Primary outcome measure was EF measured as flow-mediated dilatation. Participants were evaluated before and 2 h after ingestion of their assigned olive oil treatment.
Results: EVOO acutely improved EF as compared to refined olive oil ($1.2 \pm 6.5\%$ versus $-3.6 \pm 3.8\%$; $p = 0.0086$). No significant effects on systolic or diastolic blood pressure were observed.
Conclusions: High-polyphenolic EVOO acutely enhanced EF in the study cohort, whereas refined olive oil did not. Blood pressure effects were not observed. Reports on the vascular effects of olive oil ingestion should specify the characteristics of the oil.
Clinical trial registration number: NCT04025281

1. Background

Diabetes is a public health problem of epidemic proportions. Type 2 diabetes mellitus (T2DM) accounts for about 90% to 95% of all diagnosed cases of diabetes [1]. Diabetes complications include cardiovascular disease (CVD), stroke, hypertension, blindness, kidney disease, nervous system damage, limb amputations, and biochemical imbalances that can cause acute life-threatening events [1]. Diabetes is the seventh leading cause of death in the U.S. Cardiovascular mortality rates are 2 to 4 times higher among adults with diabetes than among those without diabetes [1].

In a review by Reusch and Wang [2], epidemiologic and cohort studies show a clear and consistent correlation of hyperglycemia with CVD. Elevated blood glucose has been linked to activation of a pro-atherogenic phenotype in the vessel wall of endothelial cells, vascular smooth muscle cells, inflammatory cells, fibroblasts, and platelets, leading to a feed-forward atherogenic response [2]. Low glycemic index diets have been reported to improve serum lipid profiles, reduce C-reactive protein (CRP) levels, and help in weight control [3]. While glycemic control remains a cornerstone of diabetes care, the co-management of hypertension, atherosclerosis, cardiovascular risk reduction, and prevention of long-term consequences are critical to improve long-term survival [4].

Lifestyle change is the foundation of management and prevention of T2DM [3]. Low glycemic index foods are often recommended for individuals at risk for or with T2DM [5]. Extra virgin olive oil (EVOO) is a low glycemic, nutrient-dense food [6]. It is rich in monounsaturated fatty acids (MUFAs) and anti-oxidants that are vital to improve cardiometabolic risk factors in those at risk for T2DM [6]. Among all the oils, olive oils have a relatively high content of MUFAs [6]. Diets rich in MUFAs and polyunsaturated fatty acids have shown favorable effects on cardiovascular health [7–9], glycemic control, insulin sensitivity, and lipid profile [9,10]. All olive oils, inclusive of EVOO, are high in

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* Corresponding author at: Yale-Griffin Prevention Research Center, Griffin Hospital, 130 Division Street, Derby, CT 06418, United States of America.
E-mail addresses: valentine.njike@yalegriffinprc.org (V.Y. Njike), rockiy.ayettey@yalegriffinprc.org (R. Ayettey).

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MUFAs, relative to other vegetable oils [6]. However, refined olive oils lack important anti-oxidants and anti-inflammatory properties attributed to naturally occurring components such as polyphenols (i.e., biophenols) [6]. EVOOs specifically contain relatively higher levels of some phenolic compounds, along with more naturally-occurring minerals and vitamins found in olives that have favorable effects on cardio-metabolic health.

Some oils improve, and some impair, endothelial function (EF). Published studies [11–15] have reported both outcomes from olive oils, probably because the grade and quality of the olive oils was not always specified. This study sought to differentiate 'good' from 'bad' olive oils, and to show that high-polyphenolic EVOOs (i.e. EVOOs that naturally contain high levels of polyphenols) have decisively beneficial effects, which are attenuated or lost as the oil is degraded. Specifically, this was randomized crossover controlled trial assessed the impact of high-polyphenolic EVOO, as compared with refined olive oil, on EF in adults at risk for T2DM. Our hypothesis was that ingestion of high-polyphenolic EVOO, compared with refined olive oil, would confer better cardiovascular health benefits in adults at risk for T2DM.

2. Methods

2.1. Study design

This randomized, double-blind crossover trial had 2 treatment assignments to compare post-prandial effects of high-polyphenolic EVOO and a refined olive oil on EF in 20 adults at risk for T2DM. The nutritional information of the tested oils and the bio-phenolic compounds of the extra virgin olive oil are presented in Tables 1 and 2. Participants received their olive oils in a smoothie consisting of ½ cup frozen blueberries, 1 cup (8 oz) low fat yogurt (Crowley low-fat vanilla), and 50 mL olive oil blended together and served in a 20 oz. plastic cup. With the exception of the type of olive oil used, the smoothies were comparable in both intervention phases. The smoothie was provided to participants in the cafeteria of the community hospital where our research laboratory is housed, and consumed either in the cafeteria or the research laboratory waiting area. EF was measured as flow-mediated vasodilation (FMD) of the brachial artery. Participants underwent repeat EF measures following 50 mL single dose administration of each of 2 treatments (high-polyphenolic EVOO or refined olive oil without polyphenols) in random sequence, with a 1-week washout between treatment assignments.

High-Polyphenol Extra Virgin Olive Oil Phase: Participants consumed a smoothie prepared with 50 mL of high-polyphenolic EVOO.

Refined Olive Oil Phase: Participants consumed a smoothie prepared with 50 mL of refined olive oil. The refined olive oil was somewhat comparable to the high-polyphenolic EVOO in terms of calories and nutrient profile, with the exception of the absence of anti-oxidants.

2.2. Participants

We enrolled 20 participants at risk for T2DM who met eligibility criteria. Inclusion criteria included: male or female age 25–75 years; non-smokers; post-menopausal females not currently on hormone replacement therapy; at risk for T2DM as defined by either prediabetes (i.e., fasting blood glucose >100 mg/dL and <126 mg/dL or hemoglobin A1C 5.7%–6.4%) or metabolic syndrome, i.e. meeting 3 out of 5 of the following criteria: (a) blood pressure ≥ 130/85 mmHg or currently taking antihypertensive medication; (b) fasting plasma glucose (FPG) >100 mg/dL (6.1 mmol/L); (c) serum triglycerides level (TG) > 150 mg/dL (1.69 mmol/L); (d) high-density lipoprotein (HDL) cholesterol <40 mg/dL (1.04 mmol/L) in men, and < 50 mg/dL (1.29 mmol/L) in women; (e) overweight (body mass index, or BMI ≥25 kg/m^2) with waist circumference > 40 in. (102 cm) for men and > 35 in. (88 cm) for women.

Table 1
Nutritional profiles of the tested oils.

Variable	High-polyphenolic extra virgin olive oil	Refined olive oil
Free Fatty Acids (%)	0.14	0.04
Peroxides Value (meq/kg)	4.70	0.70
K232	1.273	1.754
K270	0.120	0.391
Saturated Fatty Acids (%)	15.6	15.1
Monounsaturated Fatty Acids (%)	80.2	73.7
Polyunsaturated Fatty Acids (%)	4.1	10.0
Trans Fats (%)	0.06	0.20
Phytosterols (ppm)	1346.0	1485.0
Squalene (ppm)	13,587.4	2317.5
Alpha Tocopherols (ppm)	152.2	102.9
Hydroxytyrosol (ppm)	4.1	–
Tyrosol (ppm)	2.7	–
Vanillic Acid + Caffeic Acid (ppm)	3.9	–
Vanillin (ppm)	2.1	–
P-Coumaric Acid (ppm)	13.5	–
Hydroxytyrosol Acetate (ppm)	–	–
Ferulic Acid (ppm)	10.6	–
O-Coumaric Acid (ppm)	–	–
Decarb. Oleuro Aglycone, Ox Al (ppm)	2.6	–
Oleacein (ppm)	30.9	–
Oleuropein (ppm)	7.2	–
Oleuro Aglycone, Al (ppm)	3.6	–
Tyrosol Acetate (ppm)	2.3	–
Decarb. Ligstr Aglycone, Ox Al (ppm)	14.0	1.4
Oleocanthal (ppm)	17.3	1.7
Pinoresinol +1 Acetoxy Pinoresinol (ppm)	5.0	–
Cinnamic Acid (ppm)	4.8	2.5
Ligstroside Aglycone, Al (ppm)	1.5	0.3
Oleuro Aglycone, Ox Al Hy (ppm)	5.9	3.7
Luteolin (ppm)	16.1	0.8
Oleuro Aglycone, Al Hy (ppm)	20.2	2.2
Ligstro Aglycone, Ox Al Hy (ppm)	4.6	2.8
Apigenin (ppm)	8.4	0.5
Methyl-Luteolin (ppm)	–	–
Ligstroside Aglycone, Al Hy (ppm)	7.8	1.6
Total Biophenols- HPLC (ppm)	189.1	17.5

Table 2
Bio-phenolic Compounds of the Extra Virgin Olive Oil.

Flavones	Apigenin
	Luteolin
Phenolic acids	4-hydroxybenzoic
	Syringic
	Vanillic
	Caffeic
	Cinnamic
	Ferulic
	Sinapic
	Sinapine
	Hydroxycaffeic
	m-Coumaric
	p-Coumaric
	4-hydroxyphenylacetic
	Homovanillic
	Tyrosol
	Hydroxytyrosol
Lignans	1-Acetoxypinoresinol
	Pinoresinol
	Lariciresinol
	Matairesinol
	Secoisolariciresinol
Secoiridoids	Oleuropein-aglycone
	Oleuropein
	Oleocanthal
	Ligstroside-aglycone
	Ligstroside

Exclusion criteria included: failure to meet inclusion criteria; anticipated inability to complete the study protocol for any reason; current eating disorder; use of lipid-lowering or antihypertensive medications unless stable on medication for at least 3 months and willing to refrain from taking medication for 12 h prior to EF scanning; regular use of high doses of vitamin C or E; use of insulin, glucose-sensitizing medication, vasoactive medication (including glucocorticoids, antineoplastic agents, psychoactive agents, or bronchodilators) or nutraceuticals; regular use of fiber supplements; diabetes; sleep apnea; restricted diet by choice (e.g., vegetarian, vegan); coagulopathy, known bleeding diathesis, or history of clinically significant hemorrhage, or current use of warfarin; regular exercise as defined by participating in moderate-intensity ≥150 min/week.

2.3. Recruitment and screening

This study was approved by the Griffin Hospital Institutional Review Board (IRB) and registered at the clinicaltrials.gov website (NCT04025281) prior to participant recruitment and enrollment. Participants were recruited from the Lower Naugatuck Valley in Connecticut through flyers and newspaper advertisements. Potential participants were pre-screened for eligibility via a structured telephone interview using established inclusion criteria. Those who met preliminary eligibility criteria and agreed to participate were invited to undergo clinical eligibility screening, and were asked to sign a consent form approved by the IRB. They were informed of the option of discontinuing participation at any time during the study. All potential participants underwent clinical screening to assess whether they met additional eligibility criteria based on the clinical screening results. The clinical screening physical examination included weight, height, and blood pressure measures obtained by experienced study personnel using calibrated equipment. Participants also underwent a fasting blood profile for lipids, fasting plasma glucose, and hemoglobin A1C. All screening laboratory assays were performed at the Griffin Hospital laboratory. Participants' flow through the study is presented in Fig. 1.

2.4. Randomization

Eligible participants who were enrolled were randomized to 1 of 2 sequence permutations of high-polyphenolic EVOO enriched smoothie and refined olive oil enriched smoothie. Each permutation included a first treatment assignment, followed by a 1-week washout phase, followed by a second treatment assignment.

2.5. Blinding

The olive oils were provided to the investigators in dark bottles color-coded with red or blue stickers to differentiate between the two oils. They were mixed by into the smoothies with a blender and served to the participants. The appearance and consistency of the smoothies were comparable. The types of olive oils corresponding to the two different color codes were revealed to the investigators at the conclusion of the data analysis by unsealing an envelope with the color-coded assignments provided to the investigators at the initiation of the trial.

2.6. Compliance

During each visit, after participants consumed the smoothie, the study coordinator met with them to answer questions, document any study-related health or other issues, and assess, via observation, compliance to the treatment assignment. Good compliance was defined as consumption of >80% of the smoothie provided.

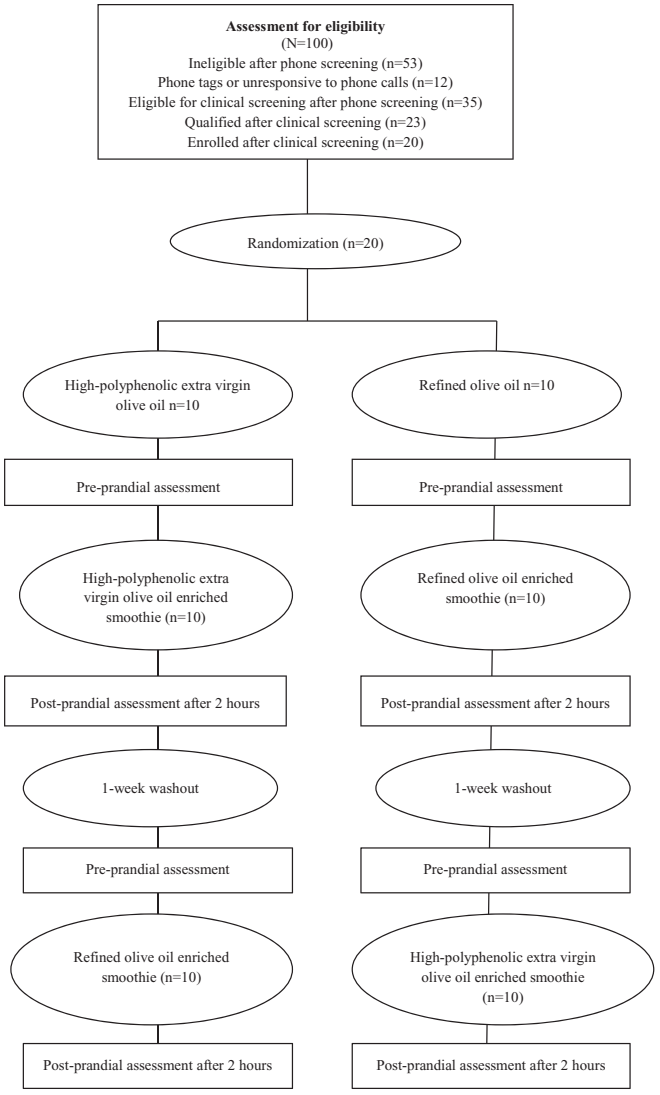

Fig. 1. Study flow diagram.

2.7. Primary outcome measure

Endothelial Function (EF) Assessment: EF was measured noninvasively in the right brachial artery with a high-frequency, 10-15 MHz, vascular ultrasound transducer (Philips iU22, Philips Medical Systems, Bothell, WA) in accordance with published guidelines [16]. The timing of each image frame with respect to the cardiac cycle was determined with simultaneous electrocardiogram (ECG) gating during image acquisition via the high-quality mainframe ultrasound system. All images were coordinated with a continuous ECG monitor and obtained at end-diastole. Measurements were taken from the anterior to posterior "m" line in diastole over a consistent segment of vessel at least 10-15 mm in length. The transmit (focus) zone was set to the depth of the near wall due to difficulty in differentiating the near from far wall "m" line (the interface between media and adventitia). Images were saved on the ultrasound machine and transferred to a research DICOM on a computer to read on the brachial analyzer for research version 6 Medical Imaging Application, Iowa City, IA. Participants were required to rest in a quiet, temperature-controlled, softly lit room for 15 min before scanning was initiated. The right brachial artery was imaged longitudinally, 2–5 cm above the antecubital fossa, by an experienced registered vascular technologist blinded to the treatment assignments. A resting scan was performed, and arterial flow velocity was measured.

An occluding cuff placed on the upper arm was inflated to a pressure of 50 mmHg above the systolic blood pressure for 5 min and rapidly deflated to induce reactive hyperemia. Brachial artery scans were acquired on the ultrasound machine continuously between 30 and 180 s after cuff deflation, including a repeated flow-velocity measurement during the first 15 s after cuff release. Brachial artery diameters were analyzed by commercially available software (Brachial Analyzer; Medical Imaging Application, Iowa City, IA). Dilatation from baseline was measured at 30–180 s after cuff deflation to assess endothelium-dependent vasodilatation.

Participants were asked to fast 8 h prior to testing. All brachial artery reactivity studies (BARS) were completed prior to noon.

BARS Quality Control Assurance: To assess the stability of ultrasound readings obtained from the same clinical research specialist at different time points, a random sample of 30 previously read brachial artery reactivity scans were provided to the clinical research specialist for a blinded second reading. The correlation coefficient between the readings obtained at different time points from the same clinical research specialist was 0.9996. This was used as a quantitative measure of intra-observer reliability.

Handling of BARS Images and Data: Measures of vessel diameter and flow velocity were generated after each scanning session. Velocity measures were generated automatically using the pulsed wave Doppler. Diameter measurements were obtained by automatic identification using edge-detection software (Brachial Analysis Tools, Medical Imaging Applications 2001), an automated method for near and far wall detection and vessel diameter measurements in brachial ultrasound image sequences. The method automatically learns properties for the analyzed vessel in one frame of a sequence that is analyzed under training parameters. Vessel properties are reflected in the cost function used in a graph-search-based border detection process. This automated method decreases variability by generating an average diameter measurement derived from multiple measures along a segment of the vessel. Several automated quality control steps are incorporated to improve accuracy and reproducibility. It can then create and output a data file with study information and export it into either Excel or SAS file formats, as stipulated by the data manager. These computerized readings are saved and charted on a standardized scan form developed by the study investigators, then scanned into the database for analysis.

Flow-mediated dilatation (FMD) was measured as the percent change in brachial artery diameter from pre-cuff inflation to 60-s post-cuff release. In addition to brachial diameter at 60 s post-cuff release, flow after cuff deflation within the first 15 s was used as an indicator of stimulus strength, hyperemic flow being the stimulus for endothelial reactivity. To account for potential variability in stimulus strength, a secondary analysis was performed in which FMD is divided by flow at 15 s post-cuff deflation to create a stimulus-adjusted response measure (SARM).

2.8. Secondary outcome measure

Blood Pressure: Systolic and diastolic blood pressures were measured at each visit using an approved automated device (SunTech® 247™ Automated Blood Pressure Device). Blood pressures were measured (average of two measurements with five minutes between measurements) with the participant sitting in a quiet room.

2.9. Statistical analysis

Linear mixed model regressions were used to assess the difference in outcome measures between high-polyphenolic EVOO included meals as compared with a refined olive oil included meal. Paired student *t*-tests were used to assess changes in outcome measures from baseline. In addition, other factors (i.e., age, gender, race, compliance, and treatment sequence) were incorporated into the regression models to adjust for potential confounding factors. A p-value of <0.05 was considered statistically significant. SAS software for Windows version 9.3 (SAS Institute, Cary, NC) was used to conduct all statistical analyses. The sample size was estimated to allow for 20% attrition and noncompliance and to provide 80% power to detect a minimal difference of 3.5% in FMD between high-polyphenolic EVOO and refined olive oil, with alpha set at 5%.

3. Results

3.1. Participants

Twenty participants were enrolled in the study. Fifty percent were females. Almost all the participants (95%) were Caucasian. Participants' average age was about 56 years. On average, participants were obese (BMI = 31.4 kg/m^2) with elevated waist circumference (102.8 cm). Prior to the initiation of study interventions, participants' mean FMD was 14.0%, and their average systolic and diastolic blood pressures were above normal limits. Eight participants were on blood pressure-lowering medications, 7 were on lipid-lowering medications, and 2 were on aspirin. All demographic and baseline characteristics are presented in Table 3. All participants completed the study.

3.2. Efficacy endpoint

A single-dose ingestion of a high-polyphenolic EVOO smoothie, compared with a single-dose ingestion of a refined olive oil smoothie, improved FMD ($1.2 \pm 6.5\%$ versus $-3.6 \pm 3.8\%$; $p = 0.0086$). However, SARM did not change when comparing high-polyphenolic EVOO with refined olive oil (-0.01 ± 0.09 versus -0.01 ± 0.06; $p = 0.7653$). Single-dose ingestion of high-polyphenolic EVOO, compared with a single-dose ingestion of refined olive oil, did not improve systolic or diastolic blood pressure (-0.9 ± 7.1 mmHg versus -0.6 ± 9.8 mmHg; $p = 0.9122$ and -1.6 ± 5.0 mmHg versus -1.1 ± 7.6 mmHg; $p = 0.8061$ respectively). Efficacy endpoint data are presented in Table 4.

3.3. Adverse events

There were 3 reported cases of upset stomach and/or heartburn after smoothie ingestion: two after ingestion of the high-polyphenolic EVOO smoothie, and one after ingestion of the refined olive oil smoothie.

4. Discussions

Our data suggest that ingestion of a single dose of 50 mL of high-polyphenolic EVOO, compared with 50 mL of refined olive oil without

Table 3
Demographic and Baseline Values of Study Participants.

Variable	Values (n = 20)
Gender	
Female, n (%)	10 (50%)
Male, n (%)	10 (50%)
Race	
Caucasian, n (%)	19 (95%)
Asian, n (%)	1 (5%)
Age (years)	56.1 ± 14.1
Weight (kg)	85.6 ± 15.8
Waist (cm)	102.8 ± 9.3
Body Mass Index (kg/m^2)	31.4 ± 5.7
Flow Mediated Dilatation (%)	14.0 ± 6.0
Stimulus Adjusted Response Measure	0.13 ± 0.08
Systolic Blood Pressure (mmHg)	130.2 ± 14.4
Diastolic Blood Pressure (mmHg)	81.2 ± 7.5
Medication Use	
Blood Pressure Lowering Medications, n (%)	8 (40%)
Lipids Lowering Medications, n (%)	7 (35%)
Aspirin, n (%)	2 (10%)

Values are mean ± SD except otherwise stated.

Table 4
Post-prandial changes in outcome measures.

Variable	High-polyphenolic extra virgin olive oil	Refined olive oil	*p*-Value
Flow Mediated Dilatation (%)	1.2 ± 6.5	−3.6 ± 3.8[a]	0.0086
Stimulus Adjusted Response Measure[b]	−0.01 ± 0.09	−0.01 ± 0.06	0.7653
Systolic Blood Pressure (mmHg)	−0.9 ± 7.1	−0.6 ± 9.8	0.9122
Diastolic Blood Pressure (mmHg)	−1.6 ± 5.0	−1.1 ± 7.6	0.8061

Values are mean ± SD.
[a] Indicate significant (p-value < 0.05) compared with pre-prandial value.
[b] Indicates occasional missing.

polyphenols, improved FMD, a marker of cardiovascular risk, by an absolute improvement of 4.8% in adults at risk for T2DM. Based on EF studies guidelines, an absolute improvement of as little as 1.5% in FMD is considered clinically significant [16]. Therefore, the FMD improvement observed was clinically meaningful and statistically significant. No acute effect on blood pressure was observed with either olive oil. The ingestion of high-polyphenolic EVOO reduced cardiovascular risk in those at risk for T2DM.

The consumption of diets containing polyphenol-rich olive oils has been associated with improvement in endothelial function in women with high-normal or stage 1 essential hypertension [17]. In a previous multicenter study [18], each 10 g daily increase in EVOO consumption in the context of Mediterranean diets was associated with reduced risk of cardiovascular disease and mortality by 10% and 7%, respectively, among individuals with increased cardiovascular risk. In a recent meta-analysis [19], high-polyphenolic EVOO consumption in the context of Mediterranean diets was associated with lower cardiovascular risk. In addition, a Mediterranean diet enriched with EVOO without caloric restrictions was associated with reduced risk of T2DM among individuals at risk for cardiovascular disease [20]. Our study also demonstrated that a single dose of 50 mL (i.e., approximately 58.5 g) of high-polyphenolic EVOO, when compared with refined olive oil without polyphenols, was associated with EF improvement among individuals at risk for T2DM. The acute effects of EVOO on EF observed may be explained by differences in the composition of EVOO and refined olive oils. EVOO contains relatively higher levels of some polyphenolic compounds, naturally-occurring minerals, and vitamins found in olives that have favorable effects on cardio-metabolic health, while refined olive oils lack important anti-oxidants and anti-inflammatory properties. The mechanisms through which EVOO improves EF may be through nitric oxide synthase activation to increase nitric oxide production, and also through modulation of gene receptors expression for endothelin-1 [15]. Previous studies [21–23] have demonstrated that EVOO has anti-inflammatory, anti-oxidant, and vasodilatation effects that help contribute to cardiovascular risk reduction. EVOOs when administered with fish oil are shown to have beneficial synergistic effects on oxidative stress and lipid metabolism among individuals at risk for T2DM [24]. A meta-analysis [25] suggests that high-polyphenolic olive oil improves the oxidative state that plays a vital role in cardiovascular prevention. In fact, high-polyphenolic olive oils have been shown to modulate the oxidative/anti-oxidative status among healthy men who consumed diets that were very low in antioxidants [26]. Polyphenols in olive oils have been shown to decrease LDL concentration and LDL atherogenicity [27,28]. Olive oil polyphenols have been shown to increase HDL size, promote greater HDL stability, and enhance HDL oxidative status through an increase in olive oil polyphenol metabolites in lipoproteins [29–34].

In contrast with previous studies [17,35], our study did not show improved systolic and/or diastolic blood pressure among participants following a single dose ingestion of 50 mL of high-polyphenolic EVOO. In one meta-analysis [25], the ingestion of high-polyphenolic EVOO was associated with systolic blood pressure reduction. In women with excess body fat, the ingestion of 25 mL of EVOO daily for 9 weeks has been shown to reduce systolic and diastolic blood pressure while improving body fat [36]. High-polyphenolic EVOO has been found to contain peptides and water-soluble extracts that possess an angiotensin-converting enzyme inhibitory activity which has anti-hypertensive effects [37]. In addition, the polyphenols in EVOO stimulate nitric oxide production and inhibit the expression of endothelin-1 that plays a vital in reducing blood pressure [15]. We did not see blood pressure improvement in our study, perhaps due to small sample size, insufficient dose, and/or inadequate duration of intervention.

4.1. Limitations

This study has several limitations. First, the sample size was small, limiting the ability to detect significant findings in blood pressure. Nonetheless, the crossover study design, intended to reduce variability, helped to improve our statistical power. Second, the study population was predominantly Caucasian; this limits our ability to generalize the study findings to other racial and ethnic groups. Third, we administered just a single dose of each type of olive oil. The effects of olive oils may differ after sustained consumption over a longer period of time. Fourth, since this was a crossover trial, there was a possibility for potential carryover effects from a previous phase of the trial. Our randomization process by sequence permutation, together with our statistical analytical plan that controlled for treatment sequence, minimized or eliminated any potential carryover effects. It is also unlikely that a single dose of either oil would continue to exert any significant effects after the one-week washout period.

5. Conclusions

Our data suggest that ingestion of high-polyphenolic EVOO, relative to refined olive oil without polyphenols, improved EF among individuals at risk for T2DM. No acute effects on blood pressure were observed with either olive oil. Therefore, the inclusion of high-polyphenolic EVOO, rather than refined olive oil without polyphenols, in the diets of those at risk for T2DM may reduce their cardiovascular risk. This study helps to clarify some confusion concerning claims of health benefits or harms (including impaired EF) of olive oil reported in some previous studies that did not specify the type of oil. This study importantly shows that the vascular effects of truly 'good' olive oil are good, while those of 'bad' olive oil are not. A larger study with longer intervention duration is warranted to elucidate these findings. In addition, more mechanistic studies are needed to understand how high-polyphenolic EVOOs exert their cardiovascular risk benefits.

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Statement of authorship

All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Author contributions

Valentine Y. Njike, MD, MPH: Investigation; Conceptualization; Funding acquisition; Methodology; Data curation; Formal data analysis; Supervision; Writing - original draft; Writing - review & editing. Rockiy G. Ayettey MS: Project administration; Data curation; Resources. Judith A. Treu, MS, RD: Investigation; Validation; Visualization; Writing - review & editing. Kimberly N. Doughty, MPH, PhD: Investigation; Funding acquisition; Writing - review & editing. David L. Katz, MD, MPH: Investigation; Conceptualization; Methodology; Funding acquisition; Writing - review & editing.

Declaration of Competing Interest

No competing conflict of interest to disclose.

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