









ORIGINAL RESEARCH

Effect of high polyphenol extra virgin olive oil on markers of cardiovascular disease risk in healthy Australian adults (OLIVAUS): A protocol for a double-blind randomised, controlled, cross-over study

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Abstract

Background: Previous clinical studies have suggested that high polyphenol extra virgin olive oil (EVOO) provides a superior cardioprotective effect compared to low polyphenol olive oil. However, further studies are required to replicate these results in non-Mediterranean populations.

Aim: To investigate the effect of high polyphenol EVOO versus low polyphenol olive oil with known polyphenol composition on markers of cardiovascular disease risk in a healthy non-Mediterranean cohort.

Methods: In a double-blind randomised cross-over trial, the present study will examine the effect of high polyphenol EVOO versus low polyphenol olive oil in 50 healthy participants. Each intervention phase will be 3 weeks long with a 2-week washout period between each phase. Outcomes to be assessed include HDL cholesterol efflux, oxidised LDL, blood lipids, C-reactive protein, arterial stiffness, blood pressure and cognitive function. Dietary intake, physical activity levels and anthropometry will also be collected.

Discussion: Because of the rigorous trial design, novel and clinically relevant outcomes, the use of a well-characterised EVOO, and, in contrast to the current literature, the non-Mediterranean study population, the present study will provide a significant contribution to the understanding of the clinical importance of polyphenol intake in the Australian sociocultural context.

Key words: biophenol, cognition, Mediterranean diet, olive oil, oxidative stress, polyphenol.

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Introduction

The traditional Mediterranean diet, known for its cardioprotective effect, has been shown to improve cardiovascular disease (CVD) risk factors including specific measures of blood lipids (HDL cholesterol (HDL-C), triglycerides), markers of inflammation, blood pressure, fasting blood glucose and risk of diabetes.^{1,2} The traditional Mediterranean diet is characterised by an abundance of plant foods (e.g. leafy greens, tomatoes, onions, herbs, wholegrain cereals, legumes and nuts), moderate amounts of fermented dairy foods, seafood, red wine and small quantities of red meat and homemade sweets.^{3–5} Of particular relevance to the proposed study, is the large servings of extra virgin olive oil (EVOO; 60–80 mL daily) as the primary source of culinary fat and a unique culinary component of the

Mediterranean dietary pattern. Olive oil contains highly variable concentrations of polyphenols which can be affected by season, olive variety, region and soil, ripeness of the fruit and processing.⁶ EVOO is characterised by a low-temperature, mechanical processing technique which preserves the higher polyphenol content in comparison to the refining methods such as deodorisation and chemical processing techniques used to produce refined olive oils, which subsequently have significantly lower polyphenol content.^{7,8}

In healthy adults, EVOO has been shown to improve CVD risk factors including blood pressure, low grade inflammation and lipid profile.⁹ The cardioprotective properties of EVOO have been primarily attributed to the high monounsaturated fat content; however, EVOO contains an array of unique polyphenols, also referred to as 'biophenols'.¹⁰ These polyphenols have shown improvements in measures of glucose metabolism, lipid peroxidation and cholesterol markers in clinical trials.^{11–14} Despite this evidence, the unique, cardioprotective polyphenols in EVOO are not currently recognised by CVD guidelines, possibly because of the need for additional high-level evidence.

To further understand the mechanisms involved in the cardioprotective effect of EVOO-derived polyphenols, further clinical research is needed to: (i) replicate previously reported improvements in routinely measured cardiovascular markers (e.g. HDL/LDL cholesterol, blood pressure) in the Australian population; (ii) determine the feasibility of a provision of 60 mL of EVOO per day in a non-Mediterranean population and (iii) investigate the effect of high polyphenol EVOO on novel CVD risk markers. Increased CVD risk has, in part, been attributed to low plasma levels of (HDL-C).¹⁵ However, emerging evidence suggests that impaired HDL function, rather than low HDL-C, may explain HDL-associated CVD risk.¹⁶ HDL-C efflux, as measure of HDL function, has been identified as a marker that may independently predict risk of CVD.¹⁷

To improve the existing evidence base in this area, the proposed trial aims to investigate the effect of a high polyphenol EVOO compared to a low polyphenol olive oil on both routinely measured (e.g. blood pressure and cholesterol) and novel markers (e.g. HDL-C efflux) on CVD risk in a healthy Australian cohort.

Furthermore, recently published clinical and animal studies have provided preliminary evidence to suggest that EVOO, as well as other polyphenol-rich interventions, may

improve cognitive performance and prevent age- or experimentally induced cognitive impairment.^{18,19} Hence, as a secondary outcome, the present study will also investigate the effect of high polyphenol EVOO and low polyphenol olive oil on measures of cognitive performance in this healthy cohort.

Methods

The OLIVAUS study is a double-blind, randomised, controlled cross-over trial that aims to investigate the effect of a 3-week intervention of high polyphenol EVOO compared to a retail-purchased low polyphenol olive oil on CVD risk factors in 50 healthy participants (Figure 1). Compared with a low polyphenol olive oil, we hypothesise that a high polyphenol EVOO intervention will result in improved measures of HDL-C efflux, oxidised LDL and low-grade inflammation in a healthy adult population. The trial protocol (registered 30/04/2018, updated 13/02/2019) has been prospectively registered with the Australia New Zealand Clinical Trials Registry ACTRN12618000706279 and was created in accordance with the SPIRIT statement.²⁰

This trial will be conducted in accordance with the Guidelines for Good Clinical Practice and the Declaration of Helsinki and CONSORT reporting guidelines. The trial team has obtained written approval for the protocol and Patient Information and Consent Form from the La Trobe University Human Research Ethics Committee (HEC17-067).

Participants will be recruited in Melbourne, Australia using social media advertisements, and through La Trobe University using email advertisements, mailing lists, word of mouth, and posters on campus and at local medical clinics. Table S1 (Supporting Information) provides the inclusion and exclusion criteria for the present study.

Figure 1 provides a visual representation of the study flow. The participant schedule throughout the trial is shown in Table S2, including data collection time-points. Once enrolled, participants will be asked to undergo an initial washout period where they will be instructed to abstain from consuming all olive oil, olive products, and antioxidant supplements for 2 weeks prior to the scheduled baseline meeting (T1). Participants will be requested to complete a 3-day diet diary including 2 week days and 1 weekend day where they are asked to include details on the foods and beverages consumed including type, brand,

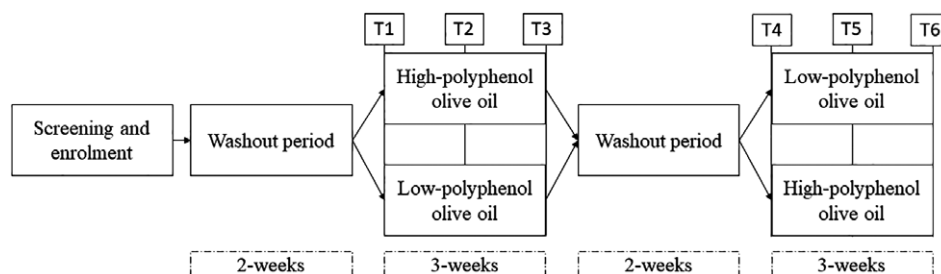


Figure 1 Study flow.

quantity in household measures and cooking methods. Participants will be asked to complete this diet diary in the days preceding the initial appointment and at the conclusion of the intervention phases. Participants will be asked to come to the baseline meeting in a fasted state. At the end of each intervention phase, participants will receive a \$25 AUD gift voucher (\$50 AUD in total).

The research staff will screen against the eligibility criteria during a face-to-face meeting. Following informed consent, participant numbers will be assigned sequentially and will be block randomised to receive either high polyphenol EVOO or low polyphenol olive oil. The block randomisation sequence will be developed using blocks of 6, by a senior researcher (GM), who will not have any direct involvement in the participant recruitment or data collection phase. After baseline measures are taken, a researcher who is not involved in any participant contact (JCW) will email the allocation for each participant to the team. De-identified bottles of high and low polyphenol olive oil will be randomised and coded prior to the recruitment phase and all staff will be blinded to this randomisation.

Participants will receive a 3-week supply of either the low polyphenol olive oil or high polyphenol EVOO (1.26 L) at the commencement of the first intervention (T1) and the commencement of the second intervention (T4). Participants will be required to consume 60 mL per day for each of the 3-week intervention phases. Measuring cups will be provided for participant use, where appropriate, to demonstrate the required volume. Emphasis on strategies that incorporate olive oil into their habitual diet in a raw, uncooked form will be provided by researchers. This will include dressing salads or vegetables, drizzling the oil on prepared meals such as soups or casseroles, and ensuring leftover amounts are also consumed. Participants will be supplied with the full amount of EVOO and olive oil required per 3-week intervention period.

Total polyphenol and polyphenol subclasses for each olive oil intervention were analysed by Modern Olives Laboratory Services (Lara, Australia), a Commonwealth Government accredited testing agency, using high-performance liquid chromatography. Samples were prepared and blinded for the researcher. Table S3 provides a comparison of the total polyphenol and polyphenol subclasses of each olive oil intervention. All high polyphenol EVOO was sourced from Cobram Estate Pty. Ltd. from the same harvest and lot and stored under the same conditions. An EVOO with a confirmed polyphenol count of approximately 320 ppm will be provided to participants as the high polyphenol EVOO intervention. A low polyphenol olive oil was sourced from a local supermarket where a bulk purchase of the same brand from the same lot number was made. This oil was confirmed to have a polyphenol count of approximately 86 ppm.

At the commencement of the first and second intervention phase meeting (T1 and T4), participants will attend a 1-hour appointment in the morning with research staff at

the nutrition clinical rooms, Bundoora campus, La Trobe University. Data collection including 3-day diet diaries, medical history and lifestyle (e.g. physical activity) questionnaires, anthropometry, fasting blood collection, blood pressure, arterial stiffness measures and cognitive performance will take place at each face to face appointment. Basic demographic data will also be collected at baseline including age, gender and ethnicity. These are described in detail below.

The research staff will contact participants by phone or email approximately 1.5 weeks into each intervention phase to discuss progress, adherence to the intervention and to ask participants if they have experienced any adverse events during the study period.

At the end of each intervention phase (T3 and T6) participants will attend a face to face appointment where they will complete all the data collection indicated at the T1 and T3 appointment. In addition, participants will be required to return their olive oil bottles so that research staff can record the weight of any remaining oil as an additional marker of adherence.

For T3 only: Research staff will instruct the participants to undergo a 2-week washout period whereby they cease consumption of all olive oil and olive products during this period, until their next meeting (T4, start of second olive oil phase).

For T6 only: Research staff will assess blinding by asking the participant about the order they think they received the two olive oil interventions and whether there were any differences in taste.

All outcomes described below will be measured pre and post the olive oil intervention phases (T1, T3, T4, T6) as per Table S2. Blood collection will also take place at each pre- and post-time-point. Research staff will confirm that participants have fasted for 8–12 hours. If so, fasting venous blood samples will be obtained, by a researcher trained in venepuncture, from the antecubital vein using standard venous puncture techniques. If blood collection is unsuccessful research staff will arrange for blood collection at a local pathology centre within 48 hours of the scheduled appointment.

HDL-C efflux, the primary outcome, will be analysed using a Cholesterol Efflux Fluorometric Assay Kit (Biovision, Milpitas, California). Participants will be invited to participate in an optional cognitive performance assessment. If they have consented to this aspect of the trial, the participant will conduct the full cognitive assessment at each face to face appointment. The Swinburne University Computerised Cognitive Assessment Battery (SUCCAB) is a validated, computer-based cognitive battery, administered using a 5-button control box.²¹ Eight tests of cognitive function will be assessed by both accuracy and response time. These tests include Simple and Choice Reaction Times, Immediate and Delayed Recognition, Congruent and Incongruent Stroop colour-words, Spatial Working Memory and Contextual Memory. This battery has been used in numerous studies to assess the cognitive effects of dietary supplementation and other interventions.^{22–24}

Total, HDL and LDL cholesterol, high sensitivity C-reactive protein and triglyceride levels will be measured using standard enzyme assays. Oxidised LDL will also be analysed using a solid phase two-site enzyme immunoassay (ELISA; Mercodia, Uppsala, Sweden).

Cardiovascular function will be assessed using the non-invasive SphygomoCor XCEL system (AtCor Medical, Australia) once the participant has rested for 5 minutes in the supine position. Assessments will include standard brachial blood pressures, aortic (central) blood pressures, pulse wave analysis of peripheral arterial stiffness, and carotid-femoral pulse wave velocity analysis of central arterial stiffness.

Three-day diet diaries will be collected at each face to face appointment. Research staff will conduct a baseline interview with all participants and will confirm that they underwent the required 2-week washout period. The research staff will also review the 3-day dietary intake data to ensure sufficient detail has been recorded for nutrient analysis and to clarify any missing data on responses that look inaccurate. Participants will self-report details regarding their intake of food and liquids over a 3-day period including the quantity (via household measures), type and timing of items consumed. Furthermore, a specific section to capture timing and amount of olive oil will be incorporated. Participant weight, height and waist circumference will be measured using standard techniques, in duplicate by the research staff. If there is >10% variation between the two measures, a third measure will be obtained. The mean of the closest two measures will be used. Self-reported physical activity will be completed prior to the commencement of the trial (T1) and at the end of the trial (T6) via the Active Australia Survey,²⁵ a validated tool within the Australian population and consists of eight questions to assess the previous 7 days. The questionnaire captures a range of activity types including walking, work in the yard, vigorous physical activity and moderate physical activity. Adverse events will be monitored at all time-points. If a participant experiences significant adverse events, they will be withdrawn from the study. All adverse events will be reported to the trial steering committee, comprised of the Principal Investigator and trial staff. The Human Research Ethics Committee will also be notified, as appropriate. Emergency unblinding will occur for serious adverse events deemed related to the study product. All participant data will be securely stored either in onsite locked cabinets or password protected documents on secured university servers with restricted access to the study team only.

All outcomes will be analysed by using linear mixed-effects (LMEs) models with random intercepts and slopes to account for within-participant correlation over time and varying treatment effect among participants. The effect of intervention order, because of potential carry-over effect, on all outcomes will be tested and adjusted for in the LME model if necessary by including an interaction term between the treatment and period effects. A senior statistician (LAP) will oversee the fitting of

the LME models and be responsible for assessing model validity.

Participant 3-day dietary records will be analysed and dietary changes will be used as a covariate. Adjusted results will be calculated using a multiple linear regression model including the stratification factors (e.g. gender, physical activity levels). A sensitivity analysis comparing the LME analyses and pooled estimates from the multiple imputation procedures will be conducted to prevent against bias. All reported *P*-values will be 2-tailed. The levels of statistical significance will be set at $P < 0.05$ and estimates will be accompanied with 95% confidence intervals. All statistical analyses will be conducted using the SPSS statistical software for Windows (version 25); IBM, Armonk, New York. Based on the results of previous research, a sample size of 40 was considered adequate to provide sufficient statistical power to detect a statistically significant 5% difference in HDL-C efflux between the two intervention phases with 80% power and 5% level of significance.²⁶ To account for a 20% level of potential attrition, this sample size was expanded to 50 participants.

Results

Recruitment commenced in July 2018 and is expected to be completed by late-2019. Currently, a total of $n = 21$ participants have been enrolled in this trial, leading to an average recruitment rate of 7 per month. Sixty-five per cent of participants are female with a mean age of 37 years. Five of the currently recruited cohorts have completed the intervention with 100% of outcome data collected. Incomplete data have been collected on one participant due to withdrawal from the study because of inability to consume the required amount of olive oil. Ten per cent of participants that have completed the intervention consumed at least 80% of the provided oils. There have been no reported serious adverse events related to the study intervention. Reported adverse events include diarrhoea, bloating, reflux and heartburn.

Discussion

Previous clinical studies have reported that EVOO provides a cardioprotective effect through mediating improvements in cardiovascular risk factors;^{1,9} however, few studies have investigated the contribution of the polyphenol component of olive oil to these improvements. The present study will compare the effect of high polyphenol EVOO to low polyphenol olive oil on markers of CVD risk that are related to cholesterol transport and metabolism, LDL oxidation, blood pressure (peripheral and central), arterial stiffness, and inflammation, as well as measures of cognitive function. By implementing a study design that will be able to differentiate between the effect of polyphenols from the other components of olive oil (e.g. monounsaturated fat will remain consistent between study arms), this trial will provide important information regarding the effect of EVOO polyphenols on a range of cardiovascular risk factors and cognition. In contrast to the current literature which has predominantly been conducted within Mediterranean

populations, this will assess the use of high polyphenol EVOO in the Australian western sociocultural context. In addition, previous research has primarily assessed the effect of a Mediterranean diet and EVOO in populations with existing comorbidities such as coronary heart disease, type 2 diabetes, cancer and cognitive decline while the present study aims to recruit healthy participants.^{2,27} The present study is one of the first trials to comprehensively assess the polyphenol composition within each of the oils provided to participants. Other studies, even those which compare oils with varying polyphenol content, do not report the composition of the polyphenols contained within.⁹ Finally, this study will report HDL efflux, oxidised LDL and other biomarkers of CVD that have not been extensively studied in previous dietary intervention studies. If shown to be beneficial, the present study will provide evidence for a widely accessible, low cost dietary intervention to reduce CVD risk and will significantly contribute to the existing literature on the clinical importance of polyphenol intake.

Funding source

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Conflict of interest

ESG, CI, CJT and HLM have received food donations for previous trials (Cobram Estate Pty Ltd., Jalna Dairy Foods Pty Ltd., Almond Board of Australia, Simplot Australia Pty Ltd., Birds Eye, HJ Heinz Company Australia, Carmen's Kitchen).

Authorship

All authors contributed to the development of the protocol. WM and ESG lead the development of the manuscript. KS provided data on current trial results. LAP provided statistical support. GK and AP provided the information regarding the cognitive function component. CJT, HLM, CI and JCW all contributed to the manuscript. We declare that the content of this manuscript has not been published elsewhere. We would like to acknowledge the work of Mr Siddharth Shivantha (Honours student) in the early data collection phase of this trial.

References

- 1 Estruch R, Martinez-Gonzalez MA, Corella D *et al.* Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med* 2006; **145**: 11.
- 2 Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *Am J Clin Nutr* 2010; **92**: 1189–96.
- 3 Martinez-Gonzalez MA, Garcia-Arellano A, Toledo E *et al.* A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. *PLoS One* 2012; **7**: e43134.
- 4 Willett WC, Sacks F, Trichopoulos A *et al.* Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr* 1995; **61**: 1402s–6s.
- 5 George ES, Kucianski T, Mayr HL, Moschonis G, Tierney AC, Itsiopoulos C. A Mediterranean diet model in Australia: strategies for translating the traditional Mediterranean diet into a multicultural setting. *Nutrients* 2018; **10**: 465.
- 6 Ghanbari R, Anwar F, Alkharfy KM, Gilani AH, Saari N. Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.)—a review. *Int J Mol Sci* 2012; **13**: 3291–340.
- 7 International Olive Oil Council. Designations and definitions of olive oil, 2018. (Available from: <http://www.internationaloliveoil.org/estaticos/view/83-designations-and-definitions-of-olive-oils>, accessed 15 June 2018).
- 8 Standards Australia Limited. *AS 5264-2011: Olive Oils and Olive-Pomace Oils*. Sydney: Standards Australia Limited, 2011.
- 9 George ES, Marshall S, Mayr HL *et al.* The effect of high-polyphenol extra virgin olive oil on cardiovascular risk factors: a systematic review and meta-analysis. *Crit Rev Food Sci Nutr* 2018; 1–24.
- 10 Obied HK, Prenzler PD, Omar SH *et al.* Pharmacology of olive biophenols. In: Fishbein JC, ed. *Advances in Molecular Toxicology*, Vol. 6. Atlanta, Georgia: Elsevier, 2012; 195–242.
- 11 Santangelo C, Filesi C, Vari R *et al.* Consumption of extra-virgin olive oil rich in phenolic compounds improves metabolic control in patients with type 2 diabetes mellitus: a possible involvement of reduced levels of circulating visfatin. *J Endocrinol Invest* 2016; **39**: 1295–301.
- 12 Ruano J, Lopez-Miranda J, Fuentes F *et al.* Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. *J Am Coll Cardiol* 2005; **46**: 1864–8.
- 13 Covas MI, Nyyssonen K, Poulsen HE *et al.* The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Ann Intern Med* 2006; **145**: 333–41.
- 14 Farras M, Castaner O, Martin-Pelaez S *et al.* Complementary phenol-enriched olive oil improves HDL characteristics in hypercholesterolemic subjects. A randomized, double-blind, crossover, controlled trial. The VOHF study. *Mol Nutr Food Res* 2015; **59**: 1758–70.
- 15 Lewington S, Whitlock G, Clarke R *et al.* Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* 2007; **370**: 1829–39.
- 16 Triolo M, Annema W, Dullaart RP, Tietge UJ. Assessing the functional properties of high-density lipoproteins: an emerging concept in cardiovascular research. *Biomark Med* 2013; **7**: 457–72.
- 17 Khera AV, Cuchel M, de la Llera-Moya M *et al.* Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med* 2011; **364**: 127–35.

- 18 Martinez-Lapiscina EH, Clavero P, Toledo E *et al.* Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. *J Nutr Health Aging* 2013; **17**: 544–52.
- 19 Marx W, Kelly JT, Marshall S *et al.* Effect of resveratrol supplementation on cognitive performance and mood in adults: a systematic literature review and meta-analysis of randomized controlled trials. *Nutr Rev* 2018; **76**: 432–43.
- 20 Chan A-W, Tetzlaff JM, Altman DG *et al.* SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013; **158**: 200–7.
- 21 Pipingas A, Harris E, Tournier E, King R, Kras M, Stough C. Assessing the efficacy of nutraceutical interventions on cognitive functioning in the elderly. *Curr Top Nutraceutical Res* 2010; **8**: 79–87.
- 22 Masee LA, Ried K, Pase M *et al.* The acute and sub-chronic effects of cocoa flavanols on mood, cognitive and cardiovascular health in young healthy adults: a randomized, controlled trial. *Front Pharmacol* 2015; **6**: 93.
- 23 Pase MP, Grima N, Cockerell R *et al.* The effects of long-chain omega-3 fish oils and multivitamins on cognitive and cardiovascular function: a randomized, controlled clinical trial. *J Am Coll Nutr* 2015; **34**: 21–31.
- 24 Kennedy G, Meyer D, Hardman RJ, Macpherson H, Scholey AB, Pipingas A. Physical fitness and aortic stiffness explain the reduced cognitive performance associated with increasing age in older people. *J Alzheimers Dis* 2018; **63**: 1307–16.
- 25 Australian Institute of Health Welfare. *The Active Australia Survey: A Guide and Manual for Implementation, Analysis and Reporting*. Australia: Australian Institute of Health and Welfare, 2003.
- 26 Hernaez A, Fernandez-Castillejo S, Farras M *et al.* Olive oil polyphenols enhance high-density lipoprotein function in humans: a randomized controlled trial. *Arterioscler Thromb Vasc Biol* 2014; **34**: 2115–9.
- 27 Sofi F, Casini A. Mediterranean diet and non-alcoholic fatty liver disease: new therapeutic option around the corner? *World J Gastroenterol* 2014; **20**: 7339–46.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Study eligibility criteria.

Table S2 Study procedure and time points in the OLIVAUS trial.

Table S3 Polyphenol composition of the HPOO and LPOO provided in the OLIVAUS trial.