



Hypoglycemic effects of antioxidant-rich plant extracts on postprandial glycemic responses in participants with prediabetes (GLARE study)

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ABSTRACT

Background: Plant extracts may help to improve glycemic control in individuals with poor glycemic control. However, few studies have been investigated in the prediabetes cohort, which is a high-risk condition for T2DM. Thus, this study aimed to investigate the acute effect of grape seed, rooibos tea, and olive leaf extracts on postprandial blood glucose and insulin in participants with prediabetes.

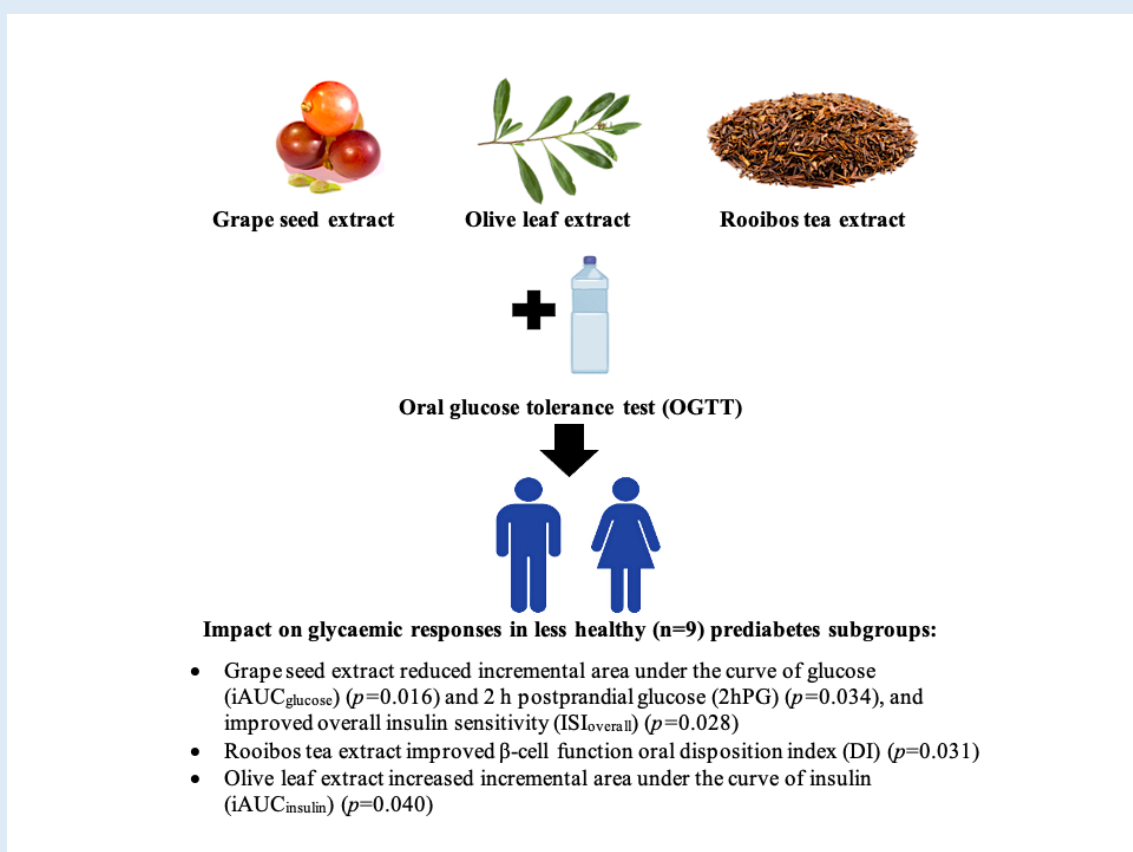
Methods: An acute, single-blind, placebo-controlled, non-randomized, crossover study (ACTRN12617000837325) where placebo and extracts of grape seed, rooibos tea and olive leaf standardized for total antioxidant capacity were given separately during an oral glucose tolerance test to participants (n=19, five men and fourteen women, aged 65.0 ± 1.6 years, Body Mass Index (BMI) 27.3 ± 1.1 kg/m²) with prediabetes (Glycated hemoglobin A1c (HbA1c) 42 ± 1 mmol/mol). The primary outcome incremental area under the curve of glucose (iAUC_{glucose}) was examined with other glycemic measures. Data was analyzed using linear mixed model for repeated measures. Secondary analysis was conducted by stratifying participants into either a healthier or less healthy subgroup based on the postprandial time to glucose and insulin peaks, with the less healthy subgroup experiencing delayed glucose and/or insulin peaks.

Results: There were no overall significant changes to glucose and insulin measures between all plant extracts and placebo ($p>0.05$). Upon secondary analysis, all extracts affected glycemic responses in the less healthy subgroup. Compared to placebo, grape seed reduced plasma $iAUC_{\text{glucose}}$ ($p=0.016$, 21.9% reduction), 2 h postprandial glucose (2hPG) ($p=0.034$, 14.7% reduction) and metabolic clearance rate of glucose (MCR_{glucose}) ($p=0.016$, 16.7% increase). It also improved insulin indices such as 2 h postprandial insulin (2hPI) ($p=0.029$, 22.4% reduction) and Stumvoll overall insulin sensitivity index (ISI_{overall}) ($p=0.028$, 15.0% increase). Rooibos tea extract significantly improved β -cell function as demonstrated by the increased oral disposition index (DI) ($p=0.031$, 32.4% increase) compared to placebo. Olive leaf extract significantly increased incremental area under the curve of insulin ($iAUC_{\text{insulin}}$) ($p=0.040$, 16.7% increase).

Conclusion: Grape seed, rooibos tea and olive leaf extracts demonstrated acute hypoglycemic benefits in adults with prediabetes and having less healthy metabolic profiles. A chronic study on the plant extracts is warranted to determine their longer-term impact on prediabetes.

Trial Registration ID: ACTRN12617000837325

Keywords: grape seed extract; rooibos tea extract; olive leaf extract; impaired glycemic control; hyperglycemia; hypoglycemic effects; insulin sensitivity; beta-cell function



INTRODUCTION

Prediabetes is characterized by isolated impaired fasting glucose (IFG) or isolated impaired glucose tolerance (IGT), or a combination of both (IFG/IGT) [1]. It is the intermediate state of hyperglycemia with blood glucose levels above normal but below the threshold to be classified as type 2 diabetes mellitus (T2DM) [2]. It is estimated that globally, 374 million (ages 20-79 years) (7.5%) people have prediabetes and the global prevalence is projected to be 548 million (8.6%) by 2045 [3]. Prediabetes has an annual conversion rate of 5-10% to T2DM [2, 4]. According to American Diabetes Association expert panel, up to 70% of individuals with prediabetes will eventually develop T2DM [1].

Whilst there is unequivocal evidence that many individuals with prediabetes are able to prevent the conversion to T2DM through lifestyle and diet modification [5-8], research is scarce regarding the use of complementary medicines and natural plant extracts to treat prediabetes. Studies of dietary polyphenols from natural plant sources have shown that some could have hypoglycaemic potential [9-13]. Proposed mechanisms by which they can improve glucose metabolism include the inhibition of digestive enzymes (α -amylase and α -glucosidase) and glucose transporters, activation of glucokinase, increase in adiponectin levels, and elevation of incretin levels to stimulate glucose-dependent insulin secretion [9-10,14-15]. Hence, polyphenol-rich plant extracts might be a useful adjunct in the preventing progression of prediabetes to T2DM.

Clinical trials conducted on healthy individuals and those with metabolic syndrome and T2DM have shown equivocal results regarding the consumption of grape

seed [16-25], rooibos tea [25-26], and olive leaf extracts [27-34] likely due to concomitant medications, small sample sizes, as well as the plant extract sources, degree of purification and varying concentrations of extract administered in the trials. More importantly the inherent differences in metabolic profiles of participants might have likely contributed to the differences observed in the studies.

Indeed it is increasingly recognized that individuals with prediabetes are not a homogenous group and that differences in metabolic profiles identified as having different postprandial glucose shapes [35-37], postprandial glucose indices [36,38-41], and patterns of insulin concentrations [42-44] have been associated with varying degrees of risk towards T2DM. Therefore, stratification based on the different metabolic profiles is important in order to elucidate the impact of intervention on both responders and non-responders [45-47]. This would allow the opportunity for optimization of nutritional interventions for each specific metabolic profile.

More recently, Takahashi and colleagues (2018) examined the stratification based on the time to peak in glucose and insulin responses to differentiate between heterogeneous metabolic profiles of participants [48]. They classified participants into four groups: (1) normal type, (2) insulin-late type, (3) insulin- and glucose-late type, and (4) insulin-very late type [48]. They observed that whilst the first two groups (1) and (2) were more metabolically similar and had no significant differences in homeostatic model assessment-insulin resistance (HOMA-IR), homeostatic model assessment-beta-cell function (HOMA- β), area under the curve of glucose

(AUC_{glucose}), and area under the curve of insulin (AUC_{insulin}). Groups (3) and (4) showed distinctly delayed insulin secretory responses and elevated AUC_{glucose} compared to groups (1) and (2). Other studies have corroborated that a delay in glucose peak time has been used as a reproducible index for T2DM risk and a value of above 30 min indicates presence of insulin resistance and poorer glycemic control [39-40,49]. Insulin patterns such as delayed insulin response have been linked to defects in β -cell function and diminished insulin sensitivity in individuals and have been associated with an increased risk of developing T2DM [42,44,50-52].

In our previous work we have shown that antioxidant-rich plant extracts such as the grape seed and rooibos tea extracts had helped to improve acute postprandial blood glucose response by 25-40% in healthy participants [25]. A chronic study conducted by de Bock and colleagues (2013) also demonstrated that with olive leaf insulin sensitivity and pancreatic β -cell responsiveness were significantly improved in normoglycemic, overweight men [29]. To date no study has investigated the hypoglycemic potential of these plant extracts in a group with prediabetes.

The present GLARE (Glucose Lowering Antioxidant-Rich plant Extracts) study aimed to examine the acute impact of grape seed, rooibos tea and olive leaf extracts on postprandial blood glucose response in individuals with prediabetes living in New Zealand. In addition, it will also explore if participants may be stratified into healthier subgroup similar to groups (1) and (2) and a less healthy subgroup similar to groups (3) and (4) after the Takahashi and colleagues (2018) classification, in

order to see if responses to the intervention differ based on participants' prediabetic metabolic profile.

METHODS

Study participants: Participants were recruited from Auckland, New Zealand using poster advertisements within the local university and community (August 2017-May 2019). They were selected if they met all inclusion criteria at screening: (1) having prediabetes with a HbA1c between 41-49 mmol/mol (5.9-6.6%) or a HbA1c of 38-40 mmol/mol (5.6-5.8%) and having at least one of the T2DM risk factors (overweight (BMI \geq 25 kg/m²) or obese (BMI \geq 30 kg/m²), high blood pressure (>120/80 mmHg), had prediabetes before, and family history of T2DM or cardiovascular disease (CVD)), (2) age between 40-80 years, (3) BMI between 18.5-40 kg/m², (4) not taking any form of glucose-lowering medications or medications having an iatrogenic nature that may influence glucose metabolism, particularly β -blockers for cardiovascular diseases and thiazide diuretics for hypertension, (5) no known pancreatic, hepatic, renal or digestive impairments that may alter glucose metabolism or metabolism of plant extracts, (6) no known allergies to the plant extracts under study, and (7) not smoking.

Interested individuals initially completed an online screening questionnaire on T2DM risk [53] before potential participants were invited into Massey University Human Nutrition Research Centre (MUHNRC) in Auckland for more in-depth screening and measurement of HbA1c (Cobas b 101 HbA1c test, CV 0.8-1.7%, Roche Diagnostics).

All participants gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Massey University Human Ethics Committee (MUHEC) (ref: 17/STH/82). The clinical trial was registered prospectively at the Australian New Zealand Clinical Trials Registry and accessible at [http://www.anzctr.org.au/\(ACTRN12617000837325\)](http://www.anzctr.org.au/(ACTRN12617000837325)).

Study design: The study was an acute, single-blind, placebo-controlled, non-randomized, crossover study involving 19 participants. The study involved four visits where participants were required to fast for at least ten hours except water before each visit.

At each trial visit, all participants were checked for dietary and lifestyle compliance, such as having fasted at least 10 hours prior to the visit, keeping their diet constant, no strenuous physical activity, no alcohol, no caffeinated tea or coffee formulations, no health supplements 24 hours to visit, and no consumption of active plant extracts (grape seed, rooibos tea, olive leaf) throughout the duration of the study. No participant had notable changes to their diet or lifestyle during the course of the study.

During the control (placebo) visit, participants were given two placebo capsules to swallow with a few sips of water. This was followed by a 2 h oral glucose tolerance test (OGTT) involving consuming a 300 mL glucose drink containing 75 g of glucose (Carbotest, Fronine, Thermo Fisher Scientific, Australia) within five minutes, and repeated measures of blood glucose during the following two hours. The subsequent three visits involved

consuming one of the three plant extracts in the following order: grape seed, rooibos tea, and olive leaf on separate visits with at least a washout period of one week between visits. The participants did not know what treatment, or in which order the treatment was given. The OGTT procedure was repeated for each visit. All three plant extracts were matched for their total antioxidant capacity and administered in standardised capsule form. Antioxidant capacity has been shown in previous research to correlate with total phenolics [54, 55], and hence a good indicator of overall phenolic composition within each plant extract.

At each visit baseline venous blood samples were taken at 10 min prior to the consumption of the capsules, followed by another baseline sampling at t=0 min before the commencement of the OGTT. Further blood samples were obtained at t=15, 30, 45, 60, 90, and 120 min. Blood draws were done by having the participant lie in a supine position and cannulating the antecubital fossa region of the arm. Blood collected in heparin tubes were immediately centrifuged after collection, whilst blood collected in serum tubes were left to coagulate for at least half an hour before centrifuging. They were then aliquot into Eppendorf tubes and stored at -80°C until analysis.

Treatments: All the plant extract samples were obtained commercially. Grape seed extract (*Vitis vinifera*) (Nutra-Life, Vitaco Health (NZ) Ltd.) was produced with 40 g of dry grape seed and 10 g of fresh grape seed per capsule and contained 640 mg of procyanidins in the concentration given to participants. Rooibos tea extract (*Aspalathus linearis*, E2CCJ) (Rooibos Limited, South

Africa) contained at least 485 mg of total polyphenols in the concentration given to participants. Olive leaf extract (*Olea europaea*) (Comvita NZ Ltd) was made from 3.5 g of fresh leaf suspended in olive oil per capsule and contained 264 mg of oleuropeins in the concentration given to participants. The plant extracts were commercialised products and had no known adverse toxic effects associated with them or similar products at the levels consumed [56-58]. All extracts were prepared according to good manufacturing practice (GMP). The placebo capsule was made of 500 mg microcrystalline cellulose.

Sample extracts of grape seed, rooibos tea, and olive leaf were sent to Callaghan Innovation (Wellington, New Zealand) to determine their total antioxidant capacity (TAC) using the Oxygen Radical Absorbance Capacity (ORAC) assay. Following a previous study, but with some modifications [59], the ORAC assay was carried out with each sample of plant extract dissolved in a ratio of ethanol: water mixture (70:30, v/v) and their TAC determined.

Extracts of grape seed, rooibos tea, and olive leaf were matched at similar TAC content at 8,499 trolox equivalent (TE) μmol , 8,496 TE μmol , and 9,152 TE μmol , respectively, with concentrations based on a previous study [25]. Due to the nature of the olive leaf extract that was suspended in oil in a soft-gel and packed in a hard casing, contents could not be modified to obtain closer TAC values with grape seed and rooibos tea extracts.

Measures of glucose and insulin responses during the

OGTT: The primary outcome of this study was incremental area under the curve of glucose (iAUC_{glucose}).

The mean iAUC of the blood glucose (iAUC_{glucose}) and insulin (iAUC_{insulin}) were analyzed using the trapezoidal rule [60, 61]. Other indices of glucose such as 2 h postprandial glucose value (2hPG), and glucose peak time were measured. Time to glucose peak was defined as the time point on the OGTT when the glucose level was highest. If two equal peaks occurred during the OGTT, the earlier peak was considered as the peak. Similarly, 2 h postprandial insulin (2hPI), and insulin peak time were calculated. Insulin sensitivity indices such as the Matsuda Insulin Sensitivity Index (ISI/M) [62], Stumvoll overall insulin sensitivity (ISI_{overall}) [63], and Oral Glucose Insulin Sensitivity (OGIS) [64] were calculated. The insulin early phase responses were captured by insulinogenic index (IGI₃₀) [65] and Stumvoll first (ISI_{first}), whilst the late phase by Stumvoll second phase (ISI_{second}) insulin sensitivity indices [63]. The pancreatic β -cell function was calculated using the Insulin Secretion-Sensitivity Index-2 (ISSI-2) [66] and oral disposition index (DI), which was calculated as ratio of IGI₃₀ to fasting insulin [67]. The metabolic clearance rate of glucose (MCR_{glucose}) was also calculated [63].

Analysis of blood samples: Plasma glucose and insulin samples were measured in a commercial laboratory (Waitemata District Health Board North Shore Hospital, Auckland, New Zealand). Plasma glucose concentrations were measured by the hexokinase method [68] (Vista GLU Flex[®] reagent cartridge, total CV 2-3%, Dimension Vista[®] 1500 System, Siemens Healthcare Limited). Plasma insulin was measured by a two-site sandwich immunoassay using direct chemiluminescent technology [69] (ADVIA Centaur Insulin assay, total CV 6.3-7.5%,

Siemens ADVIA Centaur XP, Siemens Healthcare Limited).

Sample size calculation and statistical analysis: A sample size calculation was performed based on the study done by Moore and colleagues (2000 and 2001) with an effect reduction of 100 mmol.min/L targeted and hence at least 16 participants were required [70, 71]. Statistical analyses were performed using IBM SPSS statistics version 27 (IBM corporation, New York, US). A linear mixed model for repeated measures using the repeated covariance compound symmetry with estimation employing restricted maximum likelihood (REML). A p value of ≤ 0.05 was considered to be significant (95% confidence level), based on 80% power with $\alpha=0.05$ and $\beta=0.10$. The data are expressed as model-adjusted mean \pm standard error of the mean (SEM).

Secondary analysis of participants stratified into two prediabetes subgroups: As impaired glycemic control involves a set of heterogeneous disorders [47], exploratory, secondary analysis was performed where participants were stratified into two prediabetes subgroups following the Takahashi and colleagues (2018) stratification method based on time to glucose and insulin peaks [48]. Participants in each subgroup would have more similar metabolic profiles. The healthy subgroup would have metabolic profiles that were distinctly different from the less healthy subgroup. This would help to elucidate the impact of plant extracts on glycemic responses in each prediabetes subgroup.

The healthier subgroup ($n=10$) consisted of (1) normal type: participants with both normal glucose and insulin peaks (30 min) and (2) insulin-late type: participants with normal glucose peak (30 min) but a delayed insulin peak (60 min).

The less healthy subgroup ($n=9$) consisted of (3) insulin- and glucose-late type: participants with both delayed glucose (60 min) and delayed insulin peaks (≥ 60 min), and (4) insulin-very late type: participants with normal glucose peak (30 min) but very delayed insulin peaks (120 min).

Statistical calculation was conducted similarly to the primary analysis. Differences between the two subgroups were analyzed using unpaired independent Student t-test assuming equal variances.

RESULTS

Nineteen participants with prediabetes (five men and fourteen women) completed the study (**Figure 1**). The overall mean age of the participants was 65.0 ± 1.6 years of age, with a mean BMI of 27.3 ± 1.1 kg/m², and mean HbA1c value of 42 ± 1 mmol/mol. Participant demographics for the complete group and after stratification are reported in **Table 1**. There were no adverse events that occurred during the study trial. The plant extracts at their given doses were observed to be well-tolerated by all the participants.

At the control (placebo) visit, participants in the healthier subgroup had significantly lower 1 h and 2 h postprandial glucose ($p < 0.05$) and shorter glucose peak time ($p = 0.017$), lower 2hPI ($p = 0.022$) and shorter insulin peak time ($p = 0.002$), higher insulin sensitivity ($p < 0.05$) and higher early-phase insulin sensitivity ($p < 0.05$),

higher β -cell function ($p < 0.05$), as well as higher MCR_{glucose} ($p = 0.004$) compared to the less healthy subgroup. The significant differences in glucose and insulin levels, as well as insulin sensitivity justify the existence of two distinct metabolic groups in the participants.

Seven out of 19 participants had other accompanying comorbidities such as hypertension, high cholesterol and heart disease and were taking medications on a consistent basis. These medications were assessed to have no to minimal influence on glucose metabolism.

All nineteen participants completed both the control (placebo) and grape seed trials, whilst only 17 of them completed the subsequent rooibos tea and olive leaf trials, as two participants were withdrawn from the study at study visit 3 due to problems with obtaining enough blood via cannulation (**Figure 1**).

Plant extracts and their impact on overall glucose and insulin indices in participants prior to stratification into healthier and less healthy prediabetes subgroups: The impact of each plant extract (grape seed, rooibos tea and olive leaf) on glucose and insulin responses in comparison to placebo are shown in **Table 2**. Overall, there were no significant differences in glucose and insulin indices between plant extracts and placebo, nor amongst the plant extracts prior to stratifying into the prediabetes subgroups ($p > 0.05$).

Plant extracts and their impact on glucose and insulin indices after stratification of participants into healthier and less healthy prediabetes subgroups **Figure 2 and Table 3:** show the postprandial glucose and insulin responses of the participants in the healthier and less healthy subgroups after stratification comparing each treatment with plant extract to the control (placebo).

In the less healthy subgroup, grape seed was shown to significantly reduce $iAUC_{\text{glucose}}$ ($p = 0.016$, 21.9% reduction), 2hPG ($p = 0.034$, 14.7% reduction), 2hPI ($p = 0.029$, 22.4% reduction). It also improved ISI_{overall} ($p = 0.028$, 15.0% increase) and MCR_{glucose} ($p = 0.016$, 16.7% increase) compared to placebo. Rooibos tea extract significantly improved DI ($p = 0.031$, 32.4% increase). Olive leaf extract was observed to significantly increase $iAUC_{\text{insulin}}$ ($p = 0.040$, 16.7% increase).

In the healthier subgroup, a significantly higher 2hPI in the olive leaf extract treatment compared to placebo ($p = 0.030$, 49.5% increase) was observed. The higher insulin concentration inevitably led to a reduced ISI_{overall} ($p = 0.032$, 12.5% reduction) and MCR_{glucose} ($p = 0.040$, 10.2% reduction).

Comparing between the healthier and less healthy subgroups, there were no significant differences in both glucose and insulin indices amongst the treatments ($p > 0.05$), except in the treatment with olive leaf extract, where IGI_{30} was significantly higher in the healthier subgroup than in the less healthy subgroup (1.6 ± 0.4 vs 0.6 ± 0.1 , $p = 0.042$).

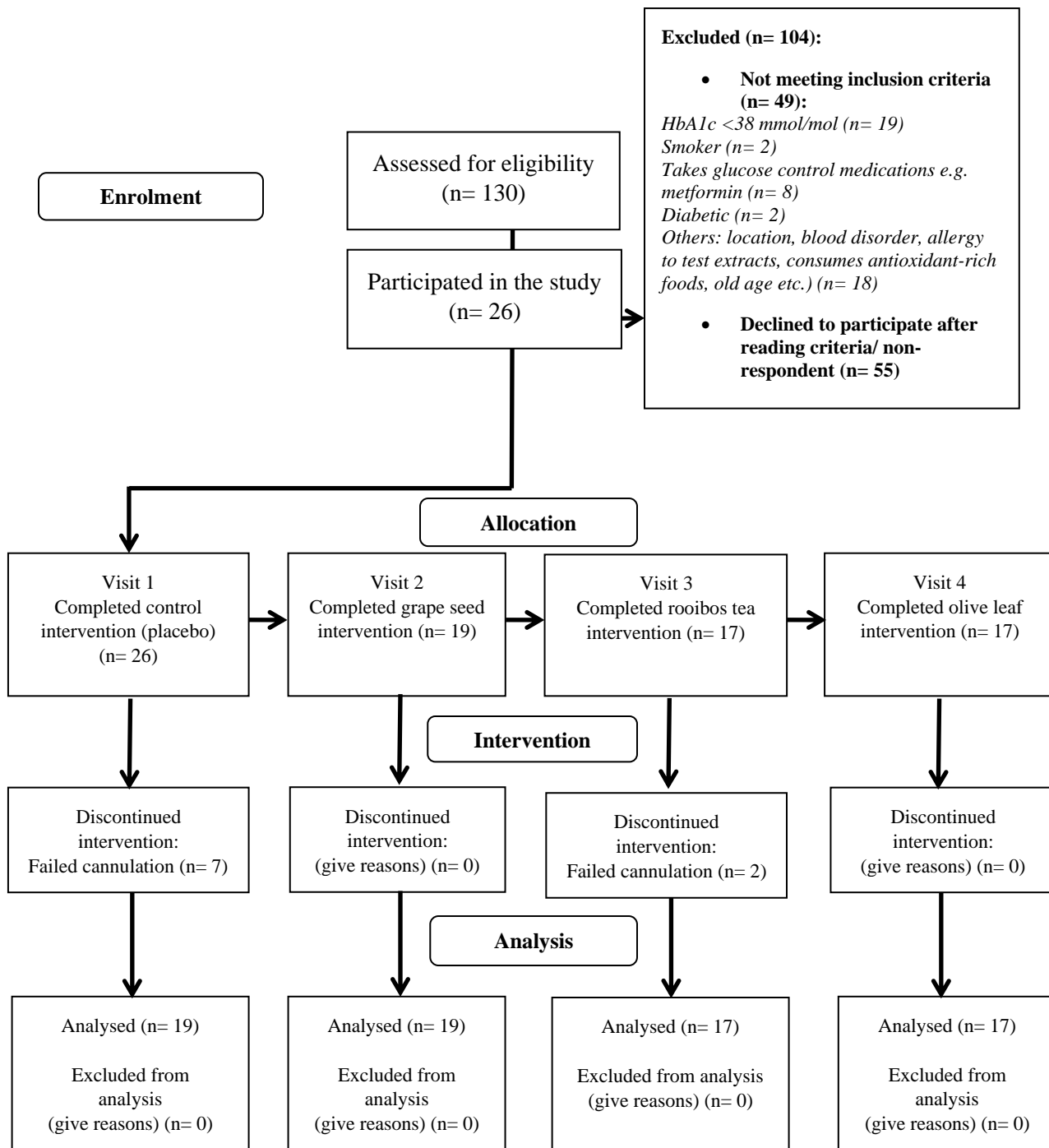


Figure 1. CONSORT flow diagram for the GLARE study.

Table 1. Overall and stratified participant demographics at the control (placebo) visit.

Characteristics	Overall mean (\pm SEM)	Healthier subgroup mean (\pm SEM)	Less healthy subgroup mean (\pm SEM)	<i>p</i> value between healthier and less healthy subgroups
N	19	10	9	NA
Gender (M/F)	5/14	2/8	3/6	NA
Age (years)	65.0 \pm 1.6	63.0 \pm 2.5	67.2 \pm 1.9	0.209
Body Mass Index, BMI (kg/m²)	27.3 \pm 1.1	26.0 \pm 1.7	28.6 \pm 1.3	0.249
Fasting blood glucose, FBG (mmol/L)	5.7 \pm 0.1	5.6 \pm 0.2	5.9 \pm 0.2	0.367
1 h postprandial glucose, 1hPG (mmol/L)	8.5 \pm 0.7	6.6 \pm 0.5	10.6 \pm 0.9	0.001*
2 h postprandial glucose, 2hPG (mmol/L)	6.9 \pm 0.7	5.0 \pm 0.3	8.9 \pm 1.2	0.003*
Fasting insulin, FI (mU/L)	8.0 \pm 1.1	7.2 \pm 1.2	9.0 \pm 2.0	0.418
1 h postprandial insulin, 1hPI (mU/L)	70.5 \pm 9.2	78.4 \pm 12.1	61.8 \pm 14.2	0.385
2 h postprandial insulin, 2hPI (mU/L)	50.5 \pm 5.8	38.6 \pm 6.5	70.1 \pm 11.0	0.022*
Glycated hemoglobin A1c, HbA1c (mmol/mol)	42 \pm 1	41 \pm 1	43 \pm 1	0.252
Systolic blood pressure (mm Hg)	135 \pm 5	130 \pm 5	141 \pm 8	0.237
Diastolic blood pressure (mm Hg)	79 \pm 3	79 \pm 5	80 \pm 4	0.919

Values were presented as means \pm SEM. The healthier subgroup consisted of participants with both normal glucose and insulin peaks (30 min) (normal type) and participants with normal glucose peak (30 min) but a delayed insulin peak (60 min) (insulin-late type). The less healthy subgroup consisted of participants with both delayed glucose (60 min) and insulin peaks (\geq 60 min) (insulin- and glucose-late type), and participants with normal glucose peak (30 min) but very delayed insulin peaks (120 min) (insulin-very late type). *Signifies significant difference between healthier and less healthy subgroups, $p < 0.05$ using the student t-test assuming equal variance.

Table 2. Overall glycaemic outcomes of participants before stratification into two prediabetes subgroups.

Analysis	Control (placebo)	Grape seed	Rooibos tea	Olive leaf	Overall <i>p</i> value
Glucose indices					
Mean incremental area under the curve of glucose, $iAUC_{\text{glucose}}$ (mmol/L.min)	266.4 ± 46.3	256.5 ± 46.3	286.2 ± 46.8	284.2 ± 46.8	0.605
2 h postprandial glucose, 2hPG (mmol/L)	6.9 ± 0.7	6.6 ± 0.7	6.9 ± 0.7	7.0 ± 0.7	0.819
Glucose peak time (min)	39.5 ± 4.1	43.4 ± 4.1	43.5 ± 4.2	36.8 ± 4.2	0.097
Metabolic clearance rate of glucose, MCR_{glucose} (mL/kg.min)	7.5 ± 0.6	7.6 ± 0.6	7.5 ± 0.6	7.1 ± 0.6	0.307
Insulin sensitivity indices					
Matsuda index	5.3 ± 0.7	5.3 ± 0.7	5.4 ± 0.7	5.1 ± 0.7	0.870
Oral glucose insulin sensitivity, OGIS (mL/min.m ²)	380.9 ± 13.2	379.3 ± 13.2	385.9 ± 13.4	377.4 ± 13.4	0.799
Stumvoll overall insulin sensitivity index, ISI_{overall} (pmol/L)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.153
Early and late phase insulin response and sensitivity indices					
Insulinogenic index, IGI_{30}	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	0.631
Stumvoll first phase insulin sensitivity index, ISI_{first} (pmol/L)	1138.1 ± 144.8	1180.0 ± 144.8	1113.9 ± 146.7	1165.4 ± 146.7	0.865
Stumvoll second phase insulin sensitivity index, ISI_{second} (pmol/L)	304.7 ± 34.7	314.6 ± 34.7	299.4 ± 35.1	311.8 ± 35.1	0.880
β-cell function indices					
Insulin-secretion-sensitivity-index, ISSI-2	34.0 ± 3.5	33.7 ± 3.5	33.7 ± 3.6	34.5 ± 3.6	0.995
Oral disposition index, DI (by insulin)	0.12 ± 0.03	0.14 ± 0.03	0.13 ± 0.03	0.15 ± 0.03	0.684
Insulin secretion and response indices					
Mean incremental area under the curve of insulin, $iAUC_{\text{insulin}}$ (mU/L.min)	5663.3 ± 696.7	5794.7 ± 696.7	5714.6 ± 705.3	6300.4 ± 705.3	0.354
2 h postprandial insulin, 2hPI (mU/L)	53.5 ± 7.1	50.9 ± 7.1	51.4 ± 7.3	62.4 ± 7.3	0.153
Insulin peak time (min)	75.8 ± 6.4	74.2 ± 6.4	82.9 ± 6.7	67.1 ± 6.7	0.154

Values were adjusted means ± SEM based on linear mixed model using compound symmetry as repeated covariance with restricted maximum likelihood (REML) estimation.

Table 3. Glycemic outcomes of participants in each prediabetes subgroup comparing plant extract treatment with control (placebo).

Glycemic measurements	Healthier subgroup				Less healthy subgroup			
	Control (placebo)	Grape seed	Rooibos tea	Olive leaf	Control (placebo)	Grape seed	Rooibos tea	Olive leaf
Glucose indices								
Mean incremental area under the curve of glucose, $iAUC_{\text{glucose}}$ (mmol/L.min)	126.3 ± 38.5	190.8 ± 38.5*	203.1 ± 40.1*	171.0 ± 40.1	422.0 ± 71.4	329.5 ± 71.4*	381.0 ± 71.4	405.7 ± 71.4
2 h postprandial glucose, 2hPG (mmol/L)	5.0 ± 0.5	5.8 ± 0.5	5.4 ± 0.5	5.7 ± 0.5	8.9 ± 1.2	7.6 ± 1.2*	8.4 ± 1.2	8.5 ± 1.2
Glucose peak time (min)	31.5 ± 3.2	39.0 ± 3.2	34.2 ± 3.5	31.2 ± 3.5	48.3 ± 7.3	48.3 ± 7.3	53.3 ± 7.3	43.3 ± 7.3
Metabolic clearance rate of glucose, MCR_{glucose} (mL/kg.min)	8.9 ± 0.7	8.4 ± 0.7	8.6 ± 0.7	8.0 ± 0.7*	5.8 ± 0.8	6.8 ± 0.8*	6.3 ± 0.8	6.0 ± 0.8
Insulin sensitivity indices								
Matsuda index	5.9 ± 1.0	5.7 ± 1.0	5.9 ± 1.0	5.9 ± 1.0	4.6 ± 0.8	4.7 ± 0.8	4.8 ± 0.8	4.2 ± 0.8
Oral glucose insulin sensitivity, OGIS (mL/min.m ²)	405.4 ± 16.8	392.2 ± 16.8	410.9 ± 17.7	402.3 ± 17.7	353.7 ± 18.3	365.0 ± 18.3	360.0 ± 18.3	351.6 ± 18.3
Stumvoll overall insulin sensitivity index, ISI_{overall} (pmol/L)	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01*	0.06 ± 0.01	0.07 ± 0.01*	0.07 ± 0.01	0.06 ± 0.01
Early and late phase insulin response and sensitivity indices								
Insulinogenic index, IGI_{30}	1.3 ± 0.4	1.4 ± 0.4	1.2 ± 0.4	1.6 ± 0.4	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Stumvoll first phase insulin sensitivity index, ISI_{first} (pmol/L)	1389.8 ± 209.9	1335.3 ± 209.9	1321.5 ± 218.0	1295.2 ± 218.0	858.4 ± 185.4	1007.5 ± 185.4	890.3 ± 185.4	1011.1 ± 185.4
Stumvoll second phase insulin sensitivity index, ISI_{second} (pmol/L)	362.2 ± 50.3	351.2 ± 50.3	348.6 ± 52.4	339.5 ± 52.4	240.8 ± 44.9	273.9 ± 44.9	246.9 ± 44.9	278.4 ± 44.9
β-cell function indices								
Insulin-secretion-sensitivity-index, ISSI-2	43.7 ± 4.4	41.5 ± 4.4	38.9 ± 4.6	42.6 ± 4.6	23.2 ± 4.2	25.0 ± 4.2	27.4 ± 4.2	25.6 ± 4.2
Oral disposition index, DI (by insulin)	0.18 ± 0.04	0.20 ± 0.04	0.16 ± 0.05	0.22 ± 0.05	0.07 ± 0.02	0.08 ± 0.02	0.09 ± 0.02*	0.08 ± 0.02
Insulin secretion and response indices								
Mean incremental area under the curve of insulin, $iAUC_{\text{insulin}}$ (mU/L.min)	5857.1 ± 991.3	6352.2 ± 991.3	5935.7 ± 1024.6	6158.6 ± 1024.6	5448.0 ± 1031.8	5175.2 ± 1031.8	5451.0 ± 1031.8	6359.3 ± 1031.8*
2 h postprandial insulin, 2hPI (mU/L)	38.6 ± 8.4	47.8 ± 8.4	42.8 ± 9.0	57.8 ± 9.0*	70.1 ± 11.3	54.4 ± 11.3*	60.8 ± 11.3	68.4 ± 11.3
Insulin peak time (min)	57.0 ± 7.5	69.0 ± 7.5	64.7 ± 8.3	49.7 ± 8.3	96.7 ± 7.7	80.0 ± 7.7	100.0 ± 7.7	83.3 ± 7.7

Values were adjusted means ± SEM based on linear mixed model using compound symmetry as repeated covariance with restricted maximum likelihood (REML) estimation. The healthier subgroup consisted of participants with both normal glucose and insulin peaks (30 min) (normal type) and participants with normal glucose peak (30 min) but a delayed insulin peak (60 min) (insulin-late type). The less healthy subgroup consisted of participants with both delayed glucose (60 min) and insulin peaks (≥60 min) (insulin- and glucose-late type), and participants with normal glucose peak (30 min) but very delayed insulin peaks (120 min) (insulin-very late type). * indicates significant difference ($p < 0.05$) between treatment and placebo.

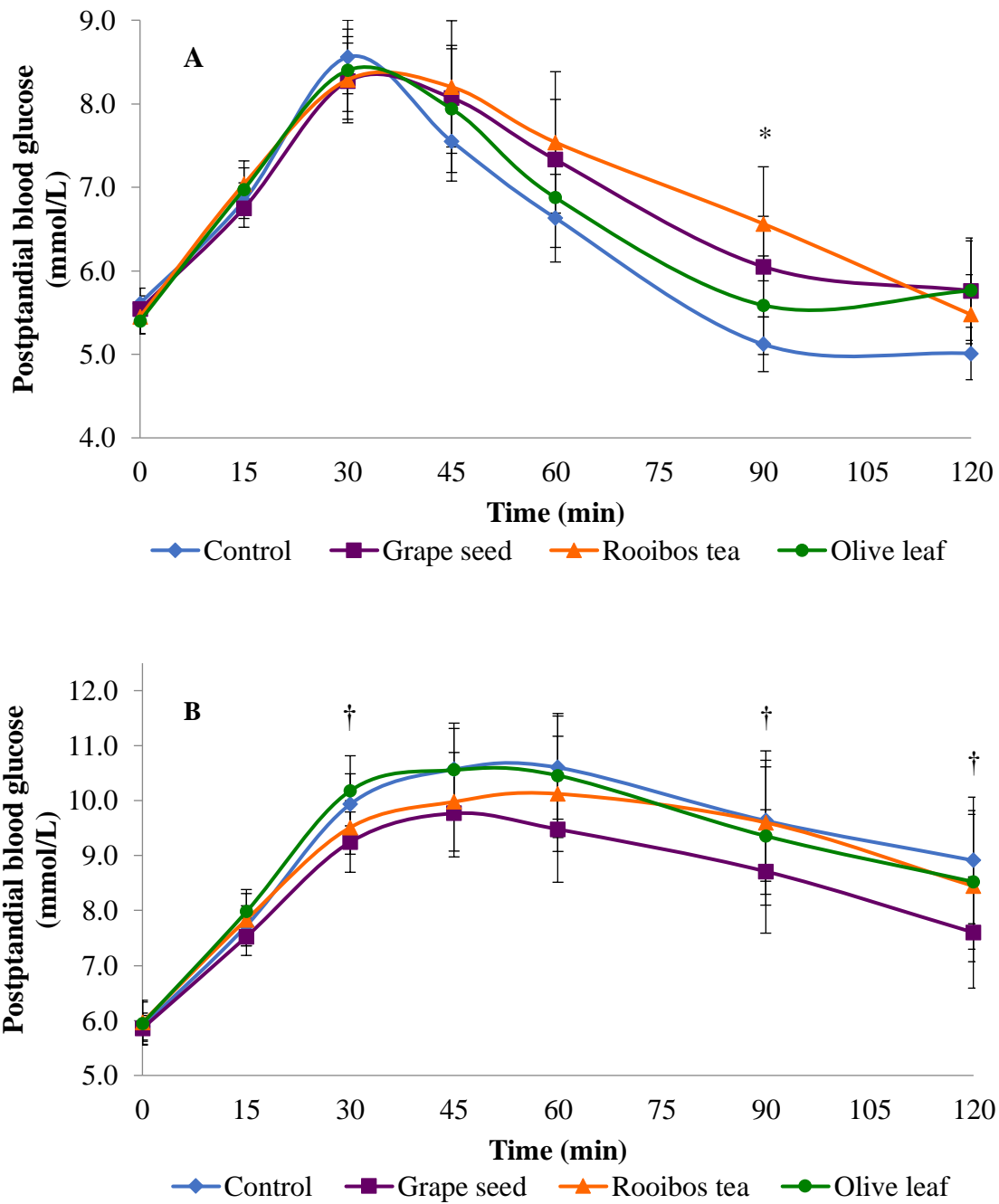


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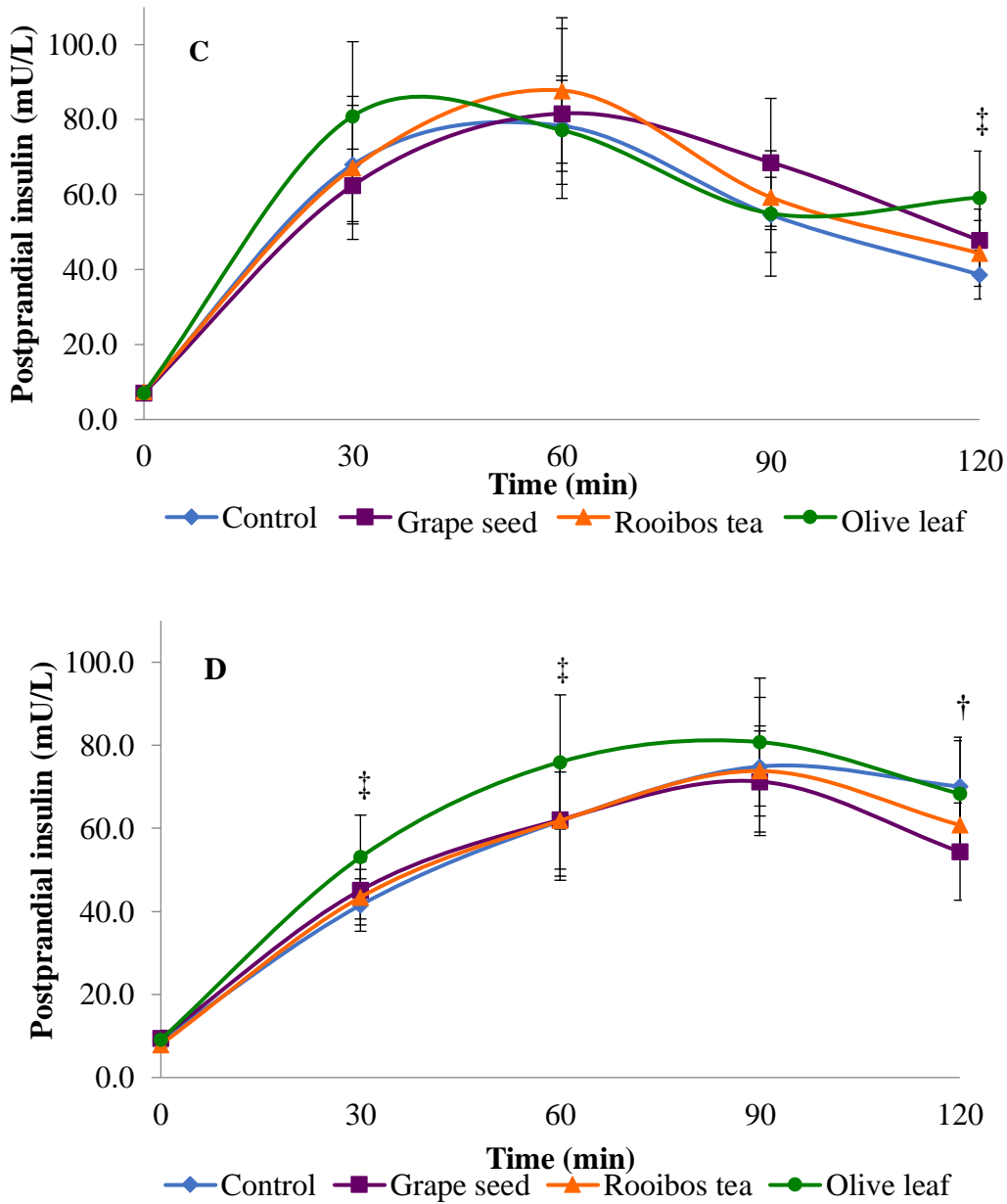


Figure 2. Unadjusted mean postprandial plasma glucose and insulin of participants during an oral glucose tolerance test (OGTT) with control (placebo), grape seed, rooibos tea and olive leaf extracts. (A) Postprandial glucose of participants in the healthier subgroup. (B) Postprandial glucose of participants in the less healthy subgroup. (C) Postprandial insulin of participants in the healthier subgroup. (D) Postprandial insulin of participants in the less healthy subgroup. The healthier subgroup consisted of participants with both normal glucose and insulin peaks (30 min) (normal type) and participants with normal glucose peak (30 min) but a delayed insulin peak (60 min) (insulin-late type). The less healthy subgroup consisted of participants with both delayed glucose (60 min) and insulin peaks (≥ 60 min) (insulin- and glucose-late type), and participants with normal glucose peak (30 min) but very delayed insulin peaks (120 min) (insulin-very late type). Linear mixed model for repeated measures with repeated covariance compound symmetry with restricted maximum likelihood (REML) estimation was used to derive statistical significance. * Indicates treatment with rooibos tea extract was significantly higher than placebo ($p < 0.05$). † indicates treatment with grape seed extract was significantly lower than placebo ($p < 0.05$). ‡ indicates treatment with olive leaf extract was significantly higher than placebo ($p < 0.05$).

DISCUSSION

To the best of our knowledge the present study is the first to investigate the hypoglycemic impact of grape seed, rooibos tea, and olive leaf extracts in a prediabetes cohort. No significant results were seen for the group as a whole. However, when we stratified the GLARE study participants into two distinct metabolic subgroups (healthier versus less healthy subgroup) based on the work by Takahashi and colleagues (2018) [48] we observed differences in responses to the intervention.

In the less healthy subgroup, grape seed extract was shown to be the most effective in improving glucose and insulin responses compared to rooibos tea or olive leaf extract. Postprandial glucose and insulin concentrations were significantly reduced, whilst there were significant improvements in overall insulin sensitivity and MCR_{glucose} compared to placebo.

Observed differences in the healthier subgroup was an increased $iAUC_{\text{glucose}}$ with grape seed and rooibos tea extracts compared to placebo but nonetheless the $iAUC_{\text{glucose}}$ values were significantly lower ($p < 0.05$) than that of the less healthy subgroup at the control visit. There was also an increase in 2hPI leading to reduced insulin sensitivity in the olive leaf extract compared to placebo due to the increased insulin response in the healthier subgroup.

The study has thus demonstrated that within the less healthy subgroup, participants were significantly more responsive to the intervention with plant extracts resulting in glycemic improvements, in contrast to the healthier subgroup. This observation was in corroboration with other research that

elucidated heterogeneity in metabolic profiles could often lead to very different responses towards interventions and therefore stratification provides a platform for a more precise, targeted treatment [45-47].

Acute, randomised controlled trials investigating grape seed extracts of various concentrations (100-500 mg) have shown reductions in $iAUC_{\text{glucose}}$ or 2hPG in healthy individuals and those with metabolic syndrome, in agreement with our findings [19, 23, 25].

In the present study, rooibos tea extract was shown to significantly improve the DI indicating the possibility of potential improvement to the β -cell function in the less healthy subgroup. Restoring β -cell function is pivotal to restoring glucose homeostasis and delaying T2DM development [72-75]. Rooibos tea also showed improvements in $iAUC_{\text{glucose}}$ (9.7% reduction), 2hPG (5.2% reduction) and MCR_{glucose} (8.4% increase) in the less healthy subgroup compared to placebo, but these did not reach statistical significance ($p > 0.05$). This is in contrast to a previous clinical trial showed significant reduction in $iAUC_{\text{glucose}}$ ($p < 0.05$, 35.5% reduction) in healthy participants after consuming 760 mg of rooibos tea extract [25]. The difference in results might be due to the metabolic differences in the participants in the two studies and the higher dose used in the previous study.

Olive leaf extract was consistently shown to elevate insulin levels in the present study, with a higher 2hPI in the healthier subgroup and an elevated $iAUC_{\text{insulin}}$ in the less healthy subgroup whilst having no significant reductions in postprandial glucose

compared to placebo. The finding agrees with the acute study conducted by Kerimi and colleagues who showed no significant improvement in postprandial glucose in healthy participants after consuming olive leaf extract (125-1000 mg extract, 50-200 mg oleuropein, respectively). Significant postprandial glucose reduction was only observed with 400 g of olive leaf extract (160 mg oleuropein) with a sucrose load [32]. In contrast, Komaki and colleagues (2003) showed that 1000 mg of olive leaves consumed with 300 g of cooked rice in an acute study significantly reduced postprandial glucose at 30 min and 1 h in a borderline diabetic subgroup [27]. Prior research has demonstrated that olive leaf might be an insulin secretagogue and might be suitable for hyperglycaemic individuals secreting low levels of insulin due to impairment of the pancreatic β -cell function [76-77]. More research is warranted regarding the potential impact on glycaemia with olive leaf consumption.

Although the plant extracts tested were matched for total antioxidant capacity to allow for efficacy comparison as antioxidant capacity has been correlated to phenolic concentrations in several studies [54, 55], it was observed that the outcomes were not similar amongst the extracts. There is a possibility that the phenolic or antioxidant content of plant extracts might not fully correlate with the hypoglycemic actions exhibited [78-81]. Rather, the presence of different phenolic compositions in the extracts work collectively to structurally interact to inhibit digestive enzymes and glucose transporters including utilising several glucose metabolism pathways to impact glucose uptake and absorption [9-10, 14-15].

The strengths of the GLARE study included the use of a crossover design where participants were their own control resulting in a smaller sample size required. The plant extracts examined were also matched for TAC using the ORAC assay that is a well-researched method, and allowed the comparison of efficacy amongst the extracts. Additionally, the present study has included a secondary analysis exploring two distinctly different prediabetes subgroups and using a number of glucose and insulin indices to quantify their responses to the plant extracts.

Nevertheless, the present study is not without limitations. Even though the exploratory, secondary analysis demonstrated observable glycemic improvements with the plant extracts, the sample size in each prediabetes subgroup was small. A larger sample size is thus recommended in future studies to elucidate the importance of considering prediabetes subgroups in nutrition interventions.

The current definition of prediabetes is somewhat arbitrary and is still expanding, and hence it is yet unclear whether it is truly a continuum or spectrum of heterogeneous worsening of glycemic control [82], or whether it is defined by distinct metabolic phenotypes [38]. Studies have elucidated the existence of prediabetes spectrum: IFG, IGT and IFG/IGT, and demonstrated them to be driven by different underlying dysfunctional metabolic profiles of glucose metabolism that included differences in glucose and insulin patterns [83, 84]. The present study had recruited participants with prediabetes based solely on their HbA1c values and not on these distinct metabolic phenotypes as a much larger sample size would be required.

The GLARE study consisted of participants who were only borderline prediabetic (mean HbA1c 42 ± 1 mmol/mol), and study outcomes observed in this

study might not be replicable to those with worsening glycemic control and having a higher HbA1c reading closer to the diabetic range, and who may also be in greater need of intervention. However, a study conducted by Marini and colleagues (2014) on 338 non-diabetic offspring of T2DM parents using American Diabetes Association criteria for prediabetes revealed that those with HbA1c values above 39 mmol/mol (5.7%) already suffered from a significant faltering insulin sensitivity and β -cell function [85]. Edelman and colleagues (2004) in their research also recommended closer scrutiny for individuals having high-normal HbA1c (37.7-42.1 mmol/mol, 5.6-6.0%) to elevated HbA1c (43.2-51.9 mmol/mol, 6.1-6.9%) especially if they were obese because they had a higher diabetes incidence [86]. Therefore, it may be useful to consider intervention even for those with borderline prediabetes.

For future studies measurement of C-peptide would be more advantageous. C-peptide is co-secreted in equimolar amounts with insulin and does not undergo hepatic first pass prior to circulation unlike insulin and is not affected by impaired insulin clearance in insulin resistant individuals. It is therefore a better reflection of true insulin secretion [87-89].

CONCLUSION

Plant extracts have been increasingly gaining attention over the past decade as functional agents to improve glycaemic control. The current study has shown the potential acute hypoglycemic effects of grape seed, rooibos tea and olive leaf extracts and their impact on indices of glucose and insulin responses in individuals with prediabetes and having less healthy metabolic profiles. However, findings have been mixed likely due to differences in the extract types/grade, doses, sample sizes, and

metabolic profile of participants between the present study and previous studies. Future studies should look into standardizing the extraction methods and concentrations of phenolic compounds in the plant extracts, and to investigate the relative impact of plant extracts on different metabolic profiles for a more targeted treatment. Where acute effects are observed, chronic studies are then needed to examine the longer-term impact of plant extracts in a prediabetes cohort.

List of Abbreviations: AUC_{glucose}: area under the curve of glucose, AUC_{insulin}: area under the curve of insulin, BMI: body mass index, CVD: cardiovascular disease, DI: oral disposition index, FBG: fasting blood glucose, HbA1c: glycated hemoglobin A1c, HOMA-IR: homeostatic model of assessment-insulin resistance, HOMA- β : homeostatic model of assessment-beta-cell function, iAUC_{glucose}: incremental area under the curve of glucose, iAUC_{insulin}: incremental area under the curve of insulin, IFG: impaired fasting glucose, IGI₃₀: insulinogenic index, IGT: impaired glucose tolerance, IFG/IGT: combined impaired fasting glucose and impaired glucose tolerance, ISI/M: matsuda insulin sensitivity index, ISI_{first}: Stumvoll first phase insulin sensitivity index, ISI_{overall}: Stumvoll overall insulin sensitivity index, ISI_{second}: Stumvoll second phase insulin sensitivity index, ISSI-2: insulin-secretion-sensitivity-index-2, MCR_{glucose}: metabolic clearance rate of glucose, NZSSD: New Zealand Society for the Study of Diabetes, OGIS: oral glucose insulin sensitivity, OGTT: oral glucose tolerance test, ORAC: oxygen radical absorbance capacity, TAC: total antioxidant capacity, TE: trolox equivalent, T2DM: type 2 diabetes mellitus, 1hPG: 1 h postprandial glucose, 2hPG: 2 h postprandial glucose, 1hPI: 2 h postprandial insulin, 2hPI: 2 h postprandial insulin.

Competing Interests: The authors report no conflicts of interest.

Authors' Contributions: The authors' contributions were as follows – all authors: designed and provided oversight of the GLARE study; Owen Mugridge and Wen Xin Janice Lim: conducted the research; Wen Xin Janice Lim: analyzed the data, performed the statistical analyses, wrote the paper, and had primary

responsibility for the final content; all authors: have revised, read, and approved the final manuscript.

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