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Increasing fruit and vegetable intake has no effect on retinal vessel caliber in adults at high risk of developing cardiovascular disease

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Abstract *Background and aim:* Retinal vessel abnormalities are associated with cardiovascular disease (CVD) risk. To date, there are no trials investigating the effect of dietary factors on the retinal microvasculature. This study examined the dose response effect of fruit and vegetable (FV) intake on retinal vessel caliber in overweight adults at high CVD risk.
Methods and results: Following a 4 week washout period, participants were randomized to consume either 2 or 4 or 7 portions of FV daily for 12 weeks. Retinal vessel caliber was measured at baseline and post-intervention. A total of 62 participants completed the study. Self-reported FV intake indicated good compliance with the intervention, with serum concentrations of zeaxanthin and lutein increasing significantly across the groups in a dose-dependent manner (P for trend < 0.05). There were no significant changes in body composition, 24-h ambulatory blood pressure or fasting blood lipid profiles in response to the FV intervention. Increasing age was a significant determinant of wider retinal venules ($P = 0.004$) whereas baseline systolic blood pressure was a significant determinant of narrower retinal arterioles ($P = 0.03$). Overall, there was no evidence of any short-term dose-response effect of FV intake on retinal vessel caliber (CRAE ($P = 0.92$) or CRVE ($P = 0.42$)).
Conclusions: This study demonstrated no effect of increasing FV intake on retinal vessel caliber in overweight adults at high risk of developing primary CVD.
Clinical trial registration: NCT00874341.
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Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide [1] and diet is known to play a pathogenic role in CVD development [2]. Fruit and vegetables (FV) represent the most botanically diverse food group with potential bioactive properties believed to offer vascular protection [3]. A minimum intake of 400 g FV per day (equivalent to 5 portions excluding potatoes) is recommended as an effective strategy for primary CVD prevention [4], which is supported by observational data [5] and confirmed by meta-analyses [6,7]. While an effect of increased FV intake on CVD events has yet to be demonstrated, there is some evidence of a beneficial effect of increased FV intake on known markers of CVD risk [2] including vascular function [8].

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Microvascular disease has been implicated in the early development of CVD and may be reversible [9]. Therefore, strategies to improve microvascular health are of major public interest for CVD prevention. A recent clinical intervention study in hypertensive adults demonstrated a significant improvement in microvascular function with increasing FV intake in a dose–response manner [8]. In this study, microvascular function was determined by a trained clinician using a high risk, invasive procedure involving brachial artery cannulation and assessment of forearm blood flow response to administration of acetylcholine.

The retinal microvasculature is the only vascular bed that can be directly visualized *in vivo* and may provide a non-invasive, surrogate method of assessing microvascular health. It is possible that changes in the retinal microvasculature reflect structural or functional changes in the overall systemic microcirculation. Observational studies have linked structural changes in the retinal vessels with established CVD risk factors, including hypertension, obesity and diabetes [10–12]. Furthermore, meta-analyses of prospective studies have reported significant associations between narrower retinal arterioles and wider retinal venules and increased coronary heart disease risk [13] and stroke risk [14]. Intriguingly, small clinical trials have demonstrated retinal vessel abnormality regression with low density lipoprotein apheresis in hypercholesterolaemic patients [15,16] and differential effects of antihypertensive medications on the retinal vessels [17]. These data suggest that the retinal microvasculature is dynamic and can respond favourably to drug treatments.

Diet may also influence the retinal vessel caliber but study data are limited to cross-sectional observations. These studies have reported inverse relationships between retinal vessel abnormalities and some dietary constituents such as dietary fibre [18], fish [19] and dairy intake [20]. However, adherence to a healthy or unhealthy derived dietary pattern was not found to be related to retinal vessel caliber measurement in European older adults [21]. To our knowledge, there are no published intervention studies examining the effect of dietary constituents on retinal vessel diameter.

In this study, we examined the dose–response effect of a randomized short-term FV intake intervention on retinal vessel caliber in healthy, overweight individuals at high CVD risk.

Methods

Study population

Details of this dietary intervention trial have been reported elsewhere [22]. Adults without existing diabetes mellitus and CVD but ≥20% CVD risk over the next 10 years as defined by Joint British Societies' Guidelines on prevention of CVD in clinical practice [23] and with a Body Mass Index (BMI) ≥27 and ≤35 kg/m^2 and low habitual FV consumption (≤2 portions per day) were recruited from medical outpatient clinics and from the general public.

Study design

This was a parallel group randomized controlled trial. The Office for Research Ethics Committees Northern Ireland provided ethical approval for this trial according to the principles of the Helsinki Declaration. The study protocol was registered on ClinicalTrials.gov (NCT00874341) and outcomes were stipulated *a priori*. All participants provided written informed consent for the study.

Following a 4 week washout period, where FV intake was limited to no more than 2 portions per day (1 portion = 80 g), participants were block randomized using a computer generated number sequence to one of the three intervention groups: 2 or 4 or 7 portions of FV per day for the next 12 consecutive weeks. Participants were instructed to keep body weight, physical activity and other lifestyle behaviours unchanged during the intervention period.

Several strategies were used to maximize compliance with the FV intervention. Written dietary advice corresponding to the allocated FV group was provided to all participants at baseline. Personalized dietetic advice to increase FV intake and encourage FV variety was also given to participants allocated to the 4- and 7-portion groups. All participants received a weekly FV delivery for the duration of the intervention and were telephoned on a weekly basis to monitor compliance and body weight and discuss any individual difficulties with adherence to the study protocol.

Study assessments

Participants attended a dedicated research facility in the morning following an overnight fast for study measurements performed at baseline (week 4) and repeated at the end of the intervention (week 16). Demographic and lifestyle data were collected via self-administered questionnaires. Anthropometrics including height, weight, waist and hip circumference were measured and body mass index (BMI) (kg/m^2) was calculated as weight (kg)/height (m)^2. Total body fat (%) was assessed using whole body Dual Energy X-ray Absorptiometry (DXA) (GE Medical Systems, Madison, WI). Blood pressure was measured using 24 h ambulatory blood pressure (Meditech ABPM-04 P.M.S. (Instruments) Ltd, Bershire, UK). A fasting venous blood sample was collected, processed and stored within 2 h of collection at −80 °C for analysis.

Retinal photography was performed by a trained researcher using a Topcon TRC *50* EX (Topcon, Tokyo, Japan) digital camera according to a standardized protocol [21]. A colour stereoscopic fundus image of visual field one (centered on the optic disc); using a field of 35° at a flash setting of 50 mW was captured for each eye after dilation. One trained researcher measured the internal retinal vessel diameters from the obtained right eye fundus image using a validated semi-automated software program and measurement protocol (IVAN, Wisconsin, USA) as used in previous studies [10–14,21]. In brief, all retinal vessels >25 μm coursing through a specified area (0.5–1.0 disc diameter

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