Impact of provision of abdominal aortic calcification results on fruit and vegetable intake: 12-week randomized phase 2 controlled trial

SUPPLEMENTARY MATERIAL

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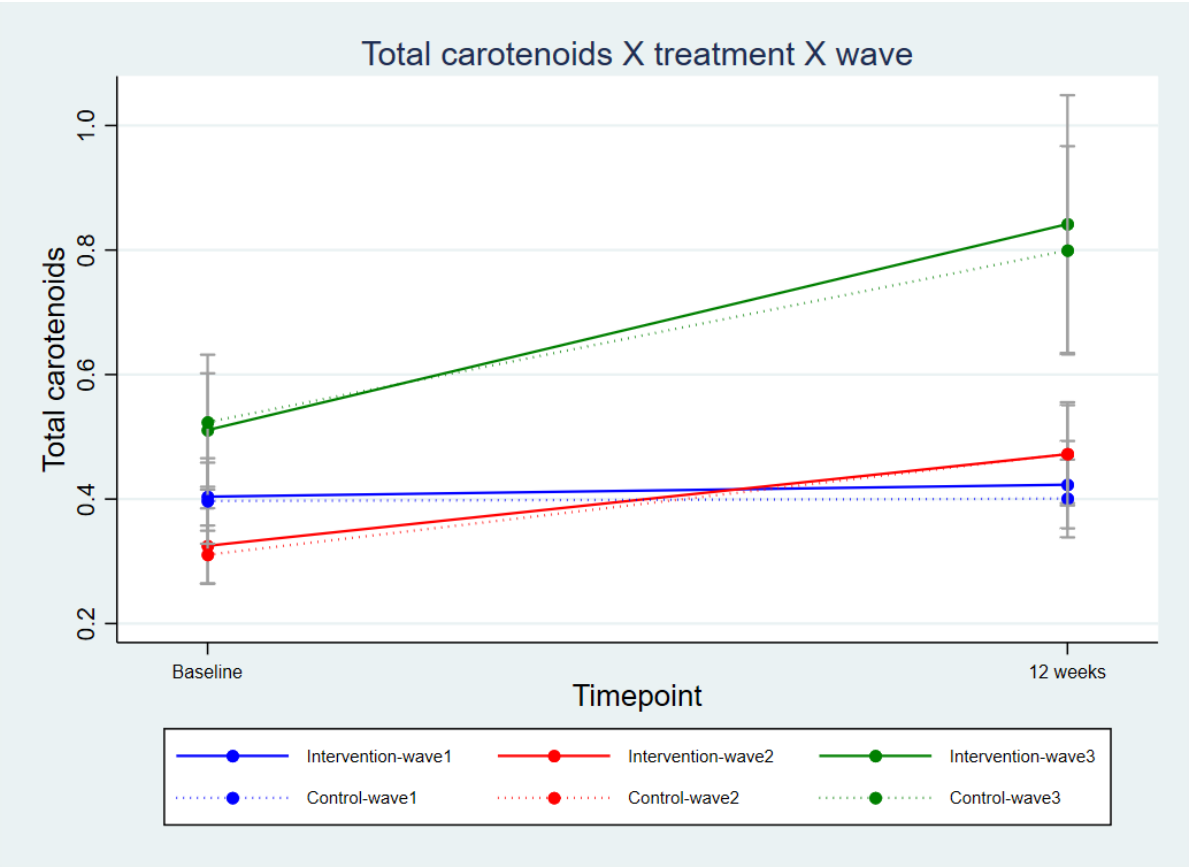

Supplementary Fig. 1 Change in plasma carotenoid concentrations, biomarkers of fruit and vegetables, over 12 weeks by treatment group (AAC+Ed and Control+Ed) and waves (study intakes - wave 1, September 2020 to Apr 2021 [early]; wave 2, May 2021 to September 2021 [mid]; wave 3, October 2021 to May 2022 [late], n=240). Estimated using liner mixed models. Plasma total carotenoid concentration is given in µg/mL. The interaction of total carotenoid concentrations by time and study wave was statistically significant ($p<0.001$).

Supplementary Tables

Supplementary Table 1. Within and between-group dietary components changes in the AAC+Ed (abdominal aortic calcification imaging results plus education) and Control+Ed (education but no AAC results) groups.

	AAC+Ed group		Control+Ed group			
	n	**Mean ± SD or (95% CI)**	**n**	**Mean ± SD or (95% CI)**	**Mean net difference in change (95%CI) [a]**	**p-value Interaction [b]**
Energy intake (kJ/day)						
Baseline	120	8,724 ± 2,308	117	8,644 ± 2,127		
12 weeks	115	8,456 ± 2,587	112	8,392 ± 2,161		
Within-group change [c]	115	-191 (-516, 135)	112	-278 (-608, 52)	87 (-376, 551)	0.711
Protein (g/d)						
Baseline	120	92.26 ± 26.68	117	87.63 ± 24.64		
12 weeks	115	90.50 ± 27.31	112	85.85± 21.99		
Within-group change [c]	115	-1.18 (-4.94, 2.59)	112	-1.89 (-5.71, 1.92)	0.71 (-4.65, 6.08)	0.794
Fibre (g/d)						
Baseline	120	26.40 ± 9.45	117	25.30 ± 8.21		
12 weeks	115	28.10 ± 10.44	112	26.54 ± 9.23		
Within-group change [c]	115	1.86 (0.59, 3.12)**	112	1.13 (-0.15, 2.41)	0.72 (-1.08, 2.52)	0.431
Fat (g/d)						
Baseline	120	93.37 ± 29.64	117	95.68 ± 30.30		
12 weeks	115	89.96 ± 33.84	112	92.72 ± 31.58		
Within-group change [c]	115	-2.11 (-7.33, 3.12)	112	-3.24 (-8.54, 2.05)	1.13 (-6.31, 8.57)	0.765
Saturated fats (g/d)						
Baseline	120	29.19 ± 9.70	117	29.19 ± 9.61		
12 weeks	115	26.87 ± 9.33	112	28.02 ± 9.67		

Within-group change [c]	115	-2.02 (-3.43, -0.62)[*]	112	-1.19 (-2.62, 0.23)	-0.83 (-2.84, 1.17)	0.416
Carbohydrates (g/d)						
Baseline	120	178.83 ± 64.92	117	173.63 ± 54.15		
12 weeks	115	173.84 ± 63.70	112	168.00 ± 51.90		
Within-group change [c]	115	-3.97 (-12.01, 4.06)	112	-5.82 (-13.96, 2.32)	1.85 (-9.59, 13.28)	0.752
Sugar (g/d)						
Baseline	120	94.24 ± 38.71	117	92.77 ± 36.27		
12 weeks	115	90.91 ± 33.63	112	92.66 ± 31.33		
Within-group change [c]	115	-2.67 (-8.24, 2.90)	112	-0.22 (-5.87, 5.42)	-2.45 (-10.38, 5.49)	0.545
Total carotenoids (µg/d)						
Baseline	120	6,939 ± 3,990	117	6,592 ± 3,723		
12 weeks	115	8,196 ± 4,954	112	7,730 ± 4,225		
Within-group change [c]	115	1,279 (590, 1,968)[***]	112	1,156 (457, 1,854)[***]	12 (-858, 1,104)	0.806
β-carotene (µg/d)						
Baseline	120	4,153 ± 2,395	117	3,831 ± 2,136		
12 weeks	115	4,838 ± 2,743	112	4,489 ± 2,342		
Within-group change [c]	115	692 (318, 1,066)[***]	112	668 (290, 1,047)[***]	23 (-509, 556)	0.931
Total folates (µg/d)						
Baseline	120	450 ± 196	117	481 ± 198		
12 weeks	115	474 ± 212	112	465 ± 178		
Within-group change [c]	115	24 (1, 49)[*]	112	-16 (-40, 8)	41 (6, 75)	**0.020**
Vitamin B1 (mg/d)						
Baseline	120	1.67 ± 0.80	117	1.73 ± 0.86		
12 weeks	115	1.69 ± 0.89	112	1.62 ± 0.70		
Within-group change [c]	115	0.02 (-0.08, 0.12)	112	-0.12 (-0.22, -0.02)[*]	0.14 (0.00, 0.29)	0.056
Vitamin B2 (mg/d)						

Baseline	120	2.34 ± 0.85	117	2.40 ± 0.94		
12 weeks	115	2.34 ± 0.94	112	2.32 ± 0.83		
Within-group change [c]	115	0.01 (-0.11, 0.12)	112	-0.08 (-0.20, 0.04)	0.09 (-0.08, 0.25)	0.294
Vitamin B3 (mg/d)						
Baseline	120	24.19 ± 7.64	117	23.28 ± 7.23		
12 weeks	115	24.26 ± 8.07	112	22.69 ± 6.41		
Within-group change [c]	115	0.21 (-0.84, 1.27)	112	-0.64 (-1.70, 0.43)	0.85 (-0.65, 2.35)	0.267
Vitamin B5 (mg/d)						
Baseline	120	3.79 ± 1.32	117	3.69 ± 1.12		
12 weeks	115	3.83 ± 1.42	112	3.72 ± 1.31		
Within-group change [c]	115	0.07 (-0.12, 0.26)	112	0.04 (-0.15, 0.23)	0.03 (-0.24, 0.30)	0.845
Vitamin B6 (mg/d)						
Baseline	120	1.12 ± 0.37	117	1.13 ± 0.45		
12 weeks	115	1.17 ± 0.42	112	1.18 ± 0.39		
Within-group change [c]	115	0.06 (-0.01, 0.13)	112	0.04 (-0.02, 0.11)	0.01 (-0.08, 0.11)	0.778
Vitamin B12 (mg/d)						
Baseline	120	3.48 ± 1.48	117	3.38 ± 1.32		
12 weeks	115	3.47 ± 1.48	112	3.38 ± 1.61		
Within-group change [c]	115	0 (-0.21, 0.22)	112	0.02 (-0.20, 0.23)	-0.01 (-0.32, 0.29)	0.938
Vitamin C (mg/d)						
Baseline	120	115.75 ± 59.64	117	120.46 ± 65.26		
12 weeks	115	136.17 ± 64.07	112	136.60 ± 78.89		
Within-group change [c]	115	21.56 (11.15, 31.98)***	112	16.31 (5.76, 26.86)**	5.26 (-9.57, 20.08)	0.487
Vitamin E (mg/d)						
Baseline	120	12.75 ± 5.21	117	13.37 ± 5.02		
12 weeks	115	12.93 ± 5.60	112	13.46 ± 5.18		

Within-group change [c]	115	0.42 (-0.47, 1.32)	112	0.03 (-0.87, 0.94)	0.39 (-0.88, 1.66)	0.548

Baseline and 12-week data were estimated using descriptive statistics in Stata, with margins ± SD reported. Within-group changes and mean between-group differences for the change over time were estimated using linear mixed models with values representing means and 95%CI. [a] Difference in change between AAC+Ed vs. Control+Ed groups. [b] p-values for the interaction between treatment group and timepoint from linear mixed models. [c] Within-group change from post-estimation margins. * Indicate statistical significance within-group changes from baseline to 12 weeks (***p<0.001, **p<0.01 and *p<0.05). Bold p-values indicate statistical significance between-group differences.

Supplementary Table 2. Correlation between AAC scores and 12-week change in outcomes according to evidence *vs.* no evidence of abdominal aortic calcification (AAC) in the AAC plus education (AAC+Ed) group.

	AAC score	
Change variables	**ρ**	**p-value**
Total plasma carotenoid (μg/mL) no lycopene	-0.1848	0.053
Total fruit and vegetable intake (g/d)	0.0851	0.377
Body weight (kg)	**-0.2515**	**0.008**
Physical activity (total h/week)	0.0610	0.526
Physical activity – moderate to vigorous (total h/week)	0.1109	0.249
Systolic blood pressure (mmHg)	-0.1332	0.165
Diastolic blood pressure (mmHg)	-0.1156	0.229
Heart Rate (beats/min)	0.0151	0.876
Triglyceride (mmol/L)	-0.0445	0.644
Total cholesterol (mmol/L)	-0.0616	0.523
HDLc (mmol/L)	-0.0113	0.907
LDL-c (mmol/L)	-0.0617	0.522
Non-HDL-c (mmol/L)	-0.0747	0.438
Glucose (mmol/L)	0.1015	0.291
Australian CVD-risk (%)	-0.1314	0.171

Rho (ρ) is from Spearman's correlation. Bold indicates a significant correlation between higher AAC score and greater change over 12 weeks.
Abbreviations: AAC, Abdominal Aortic Calcification; CVD, Cardiovascular disease; HDL-c, high density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

Supplementary Table 3. Changes in outcomes according to evidence *vs.* no evidence of abdominal aortic calcification (AAC) in the AAC plus education (AAC+Ed) group.

	AAC=0 (n=50)	**AAC≥1 (n=65)**	**p-value**
Total plasma carotenoid (μg/mL) no lycopene	0.24 (0.08, 0.40)	0.11 (0.05, 0.17)	0.103
Total fruit and vegetable intake (g/d)	65.7 (13.0, 118.3)	116.0 (68.1, 163.8)	0.161
Total fruit intake (g/d)	43.2 (9.9, 75.6)	56.7 (28.3, 85.0)	0.538
Total vegetable intake (g/d)	22.1 (-10.4, 54.6)	60.8 (28.7, 92.2)	0.098
Body weight (kg)	-0.07 (-0.53, 0.39)	-0.98 (-1.43, -0.53)	0.006
Physical activity (h/week)	-0.07 (-4.11, 3.97)	-0.73 (-3.30, 1.83)	0.774
Physical activity – moderate/vigorous (h/week)	0.06 (-2.25, 2.38)	-0.03 (-1.80, 1.73)	0.947
Systolic blood pressure (mmHg)	2.82 (-0.53, 6.16)	-2.56 (-6.31, 1.18)	0.040
Diastolic blood pressure (mmHg)	1.33 (-0.54, 3.19)	-1.45 (-3.07, 0.16)	0.026
Heart Rate (beats/min)	-3.06 (-5.13, -0.99)	-4.12 (-6.37, -1.88)	0.499
Triglyceride (mmol/L)	-0.06 (-0.25, 0.12)	-0.21 (-0.37, -0.06)	0.212
Total cholesterol (mmol/L)	-0.12 (-0.33, 0.09)	-0.29 (-0.51, -0.08)	0.260
HDLc (mmol/L)	-0.02 (-0.07, 0.04)	-0.04 (-0.10, 0.01)	0.496
LDLc (mmol/L)	-0.08 (-0.26, 0.10)	-0.17 (-0.35, -0.01)	0.475
Non-HDLc (mmol/L)	-0.10 (-0.30, 0.09)	-0.25 (-0.43, -0.06)	0.290
Glucose (mmol/L)	-0.16 (-0.44, 0.12)	0.05 (-0.16, 0.26)	0.213
Australian CVD-risk (%)	0.17 (0.02, 0.32)	-0.19 (-0.48, 0.10)	0.050

Independent t-test was used for the estimated difference in the change. Abbreviations: AAC, Abdominal Aortic Calcification; CVD, Cardiovascular disease; HDL-c, high density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

Supplementary Table 4. Changes to the MODEL randomized controlled trial in response to the COVID-19 pandemic and institutional, State and national restrictions.

	Pre-COVID-19 [a]		**Post-COVID-19** [b]	
Participants	32 participants attended a baseline visit, but the trial was halted due to COVID-19 and all participants received their results and were not included in any analyses		240 participants from waves 1-3 were randomized to either the AAC+Ed or Control+Ed group	
Primary outcome	Fruit and vegetable intake assessed by a FFQ		Fruit and vegetable intake assessed by plasma carotenoids (biomarkers) and FFQ	
Sample size	n=200		n=300	
Inclusion Criteria	Community-dwelling males and females aged 60–80 years		Community-dwelling males and females aged 60–80 years	
Exclusion criteria	BMI<18 or >37 kg/m2 (or body weight >125 kg); Type 1 diabetes; Type 2 diabetes if greater than 10 years since diagnosis; History of CVD events; Prior CAC or coronary angiograms (within the last 2 years); Use of anticoagulant therapy (warfarin); Chronic kidney disease (eGFR <30 mL/min/1.73 m2), cancer (current) or heart failure (class 3 and 4); SBP >160 or DBP >100 mmHg; Inability to assess AAC due to poor-image quality of the DXA scans		Inability to assess AAC due to poor-image quality of the DXA scans; Those without an email address and phone number; Those who were not able to complete online questionnaires.	
	Baseline	***12 weeks***	***Baseline***	***12 weeks***
Enrolment				
Pre-screening	Via phone		Via phone	
Randomization/allocation	Sex and age		Enrolment order	
Intervention				
AAC+Ed group: Information on AAC	Face-to-face		Via email	
Control+Ed group: Information on AAC		Face-to-face		Via email

Counselling: Dietary and lifestyle advice	Face-to-face	Face-to-face	Follow-up phone call/email	Follow-up phone call/email
Assessments				
Anthropometry	Body weight, height, waist and hip circumference	Body weight, height, waist and hip circumference	Body weight and height	Body weight and height
DXA scans (hip, spine, whole-body and AAC)	4 scans	4 scans	4 scans	4 scans
Blood collection	Fasting	Fasting	Non-fasting	Non-fasting
24h urine collection	Home/Clinic	Home/Clinic	Removed	Removed
Saliva samples	Clinic	Clinic	Removed	Removed
Blood pressure	Clinic	Clinic	Clinic	Clinic
Arterial stiffness	Clinic	Clinic	Removed	Removed
Physical activity actigraphy	Actigraph	Actigraph	Removed	Removed
Physical function	Grip strength, timed-up-and-go and gait speed	Grip strength, timed-up-and-go and gait speed	Grip strength	Grip strength
Food frequency questionnaire	Administered by investigators	Administered by investigators	Self-administered / online	Self-administered / online
Lifestyle questionnaires	Online	Online	Online	Online

[a] Recruitment for the MODEL study was initiated in December 2019, with study participants beginning the protocol in February 2020. The trial was terminated soon after in March 2020 due to the COVID-19 pandemic. [b] The protocol was amended to comply with government COVID-19 recommendations and restriction and Ethics approval was granted in August 2020, allowing the study to restart in September 2020.
Abbreviations: AAC, Abdominal Aortic Calcification; BMI, Body Mass Index; CAC, Coronary Artery Calcification; CVD, Cardiovascular Disease; DXA, Dual X-ray Absorptiometry; DBP, Diastolic Blood Pressure; eGFR, estimated Glomerular Filtration Rate; FFQ, Food Frequency Questionnaire; SBP, Systolic Blood Pressure.

Supplementary Table 5. Complementary intervention methods.

Trial intervention
The intervention consisted of a pre-scheduled 1) results communication email; and 2) follow-up phone call. The emails were sent approximately 2 weeks after the baseline clinic visit, early in the morning, and phone calls received on the same day in the afternoon.
1. Pre-scheduled communication email content
Group allocation information Participants were informed whether they were in the AAC+Ed or Control+Ed group. *Video link* A link to a ~12 minute video was provided and comprised: i) information on the burden of CVD in Australia (from the Heart Foundation, Australia) [1]; ii) education about CVD (plus, for the AAC+Ed group only, an explanation of AAC and its link of CVD risk [AAC video: ~13 minutes; control video ~11 minutes]); and iii) dietary and physical activity recommendations based on the Australian Dietary Guidelines [2] and how to achieve the 3 goals of the study [3]. *Goals of the study* *Goal 1*, increase fresh fruit intake by at least 1 serve/d (150 g/d) and increase vegetable intake by at least 1 serve/d (75 g/d). *Goal 2*, improve other aspects of diet (i.e., reduce intake of salt, sugar, processed foods and increase wholegrains and nuts). *Goal 3*, increase physical activity and reduce sitting time. *E-booklet* Contained a summary of the dietary and physical activity recommendations provided in the video, information on food portion sizes and tips on how to incorporate healthy foods into the diet. *Frequently asked questions (FAQ)* Comprised dietary information for both groups, and AAC information for the AAC+Ed group. *AAC results letter and explanatory letter to general practitioner (GP)* A result letter showing the individual's AAC scan was provided in the email at baseline for the AAC+Ed group. For the Control+Ed group, these letters were delayed to the end of the study. One of three different version of the AAC results letter was provided to participants based on their AAC scores: i) evidence of AAC (scores 1-5); or ii) evidence of extensive AAC (scores ≥6) - for any AAC (i.e., 'i' and 'ii'), the results letter included the actual AAC scan showing the spine and the calcified abdominal aorta indicated by arrows, an illustrative representation of the regions with advanced blood vessel disease marked in red based on AAC-24 scoring, and a written description of the results (**Figure 2**); iii) no evidence of AAC - the letter showed a clear image and diagram plus a green circle indicated absence of calcification. Participants were encouraged to discuss their results with their GP. A copy of the AAC results letter and the GP letter was sent directly to each participant's GP by the study investigators, via email or fax, within 3 days of the follow up phone call. There were no recommendations provided on medications.
2. Follow-up phone call
The scheduled 30-minute follow-up phone call was provided to all participants by an investigator (CPB, JDV, MS). It allowed reinforcement of the messages provided in the videos, including those related to AAC measurement (AAC+Ed group only) and study goals; and provided participants the opportunity to ask any questions about their results.

The phone call was rescheduled if participants had not watched the video provided in the email.

Communication of results to study participants

At the end of each wave, participants were provided with a report summarizing their individual results. This included:

a) Changes in participants diet and physical activity, as well as in systolic and diastolic blood pressure.
b) A copy of participants' biochemistry results.
c) A copy of participants' bone density results (and a bone density report for those diagnosed with osteoporosis).
d) A letter explaining the tests performed (e.g., what each one of the blood test measures were used for).
e) A flyer for the Nutrition Clinic at the Joondalup Community Clinical School for dietary support.

Study outcomes

The MODEL study trial is registered in the Australian and New Zealand Clinical Trials Registry (ANZCTR) and includes a randomised 12-week controlled trial phase as well as a long-term non-randomized follow up phase. The ANZCTR requires that all outcomes included in the trial and their assessment timepoints be individually recorded. However, this paper only reports on the primary and major secondary outcomes of the randomized controlled trial phase and does not include data on longer-term changes in lifestyle and mental/physical health-related outcomes, such as body composition, mental health, physical function, and gut health. Longer-term changes for these outcomes will be evaluated by level of AAC (including both intervention and control groups as they all have received their AAC results at either baseline or 12 weeks) with the comparator being those with evidence of AAC vs. those with no evidence of AAC after a 4-year follow up has been completed.

Supplementary Table 6. Fruit and vegetables items from the food frequency questionnaire used to calculate total fruit and vegetable intake.

Fruit items (18 items)	Vegetable items (30 items)
Apples	Asian greens (bok choy)
Pears	Coleslaw
Oranges and citrus	Brussels sprouts, cabbage
Bananas	Cauliflower
Apricot	Broccoli
Berries	Iceberg lettuce
Cherries	Other lettuce and salad leaves
Figs	Other cooked leafy vegetables (spinach)
Plums	Celery
Mango or paw-paw	Fresh tomatoes
Grapes	Canned tomatoes
Peaches	Tomato products
Strawberries	Sweet Potato
Cantaloupe, rockmelon	Capsicum
Watermelon	Pumpkin
Pineapple	Beetroot
Kiwi	Carrots
Tinned fruit	Onion or leeks
	Garlic
	Green beans
	Green peas
	Baked beans
	Dried beans, dried peas, chickpeas
	Asparagus
	Avocado
	Cucumber
	Mushrooms
	Zucchini or squash
	Eggplant
	Sweet corn

Total fruit and vegetable intake was calculated by summing all types of fruit and vegetables listed in the table (in grams per day), based on the frequency of intake. Total fruit comprised 18 fruit items, excluding dried and canned fruit and fruit juice as they have high amounts of sugar [2]. Total vegetable intake included 30 vegetables items, excluding potatoes as they may not be considered as part of a healthy diet, aligning with recommendations provided in the video for both AAC+Ed and Control+Ed groups.

Supplementary References

1 Heart Foundation. *Heart Healthy Eating Principles*, https://www.heartfoundation.org.au/for-professionals/food-and-nutrition/heart-healthy-eating-principles (2017).

2 NHMRC. National Health and Medical Research Council (2013).

3 Radavelli-Bagatini, S. *et al.* Modification of diet, exercise and lifestyle (MODEL) study: a randomised controlled trial protocol. *BMJ Open* **10**, e036366 (2020).

Supplementary Note

Supplementary Note 1 – Link for the original study protocol.

https://pubmed.ncbi.nlm.nih.gov/33177129/