Chapter 2: Markers of Cardiovascular Disease

Vascular Function

Contents

Background

Vascular function is a general term that has been applied to describe the regulation of blood flow be it arterial or venous, and includes the arterial pressure, capillary recruitment and filtration and the central venous pressure.

Aspects of vascular function, such as arterial stiffness and endothelium-dependent vasodilation, are associated with cardiovascular mortality (Yeboah *et al.*, 2007;Mattace-Raso *et al.*, 2006). Vascular function is, therefore, potentially an important target for dietary modification.

Because of this association, vascular function is often used as a surrogate marker for cardiovascular disease. It is also used as a marker of cardiovascular disease because clinically apparent endpoints such as death, heart attack and stroke have a lag time and any definitive prospective investigation of the link between diet and cardiovascular outcomes would be vast. However, surrogate markers of cardiovascular disease such as vascular / endothelial function may have inherent limitations.

A number of studies have suggested an association between surrogate markers of cardiovascular disease and diet. In particular, studies have indicated that the Mediterranean diet, which is high in fruit and vegetables, whole grains, fish and unsaturated fatty acids may be beneficial with regard to vascular function (Esposito *et al.*, 2006). However, much less evidence is available that has explored the impact of total or type of dietary carbohydrate on vascular function.

There are many markers of vascular function but few are used in routine clinical practice. The following list describes some of the markers of vascular function that have been included in this review. For a more complete review please see (McCall *et al.*, 2011).

Flow mediated dilatation

This is the most commonly used non-invasive technique for assessing endothelial dysfunction (Kelm, 2002). Endothelial dysfunction is the inability of arteries to fully dilate and is suggested to be a crucial early step in the development of atherosclerosis. Endothelial dysfunction predicts both cerebrovascular and cardiovascular events.

Although flow mediated dilatation (FMD) predicts longer-term cardiovascular outcomes (Witte *et al.*, 2005), its additive value to established cardiovascular risk scores/factors is limited (Yeboah *et al.*, 2007). Suggestions have been made that it may be a better predictor of cardiovascular risk in women than men. One cohort study of postmenopausal women indicated that women in the lowest tertile of FMD (<4.5%) had a four-fold elevation in risk of cardiovascular disease compared with women in the highest FMD tertile (>8.1%) after adjustment for other traditional CVD risk factors (Rossi *et al.*, 2008).

Presently, tests to quantify endothelial dysfunction are only used for the purposes of research. Nitric oxide is the key mediator of arterial dilatation, and endothelial dysfunction is predominantly associated with the decreased availability of nitric oxide either because of increased degradation or reduced production. Nitric oxide is responsible for flow-dependent dilatation (Joannides *et al.*, 1995).

Flow mediated dilatation measures endothelial dysfunction by inducing reactive hyperaemia - an artery, often the forearm artery, is occluded temporarily and the resulting increase in artery diameter is measured by ultrasound. The forearm circulation is occluded for 5 minutes. On release of the occlusion inflow through the brachial (forearm) artery is temporarily increased (reactive hyperaemia). The peak flow mediated dilatation is expressed as a percentage change (%FMD), with increases in percentage reflecting improved FMD. In addition, the response of the brachial artery to sublingual glycerol trinitrate is recorded and is often used as a control. As the number of cardiovascular risk factors increases, smooth muscle dysfunction becomes more apparent and the glycerol trinitrate response is progressively impaired independently from endothelial dysfunction (Kelm, 2002). Total, not peak, reactive hyperemia is felt to be more representative of the magnitude of flow mediated dilation (Pyke and Tschakovsky, 2007).

FMD is dependent upon a number of factors including the time of the day, food, smoking, exercise and temperature. There is evidence that flow mediated dilatation measurements are able to detect endothelial dysfunction in hyperlipidaemia, systemic arterial hypertension, diabetes and in the relatives of patients with coronary artery disease (Kelm, 2002). Impaired brachial artery flow mediated dilatation is associated with a greater intima-media thickness of the common carotid artery (Kelm, 2002).

As with intima-media thickness measurement, as yet there is no consensus for normal / pathological reference values in flow mediated dilatation. Nor is there complete consensus concerning the best approach for measurement. For reviews of methodologies associated with assessment see (Deanfield *et al.*, 2007) and (Al-Qaisi *et al.*, 2008).

Pulse wave velocity

Pulse wave velocity is a measure of arterial stiffness and may be measured invasively and non-invasively, with a faster pulse wave velocity indicating a stiffer aorta. Pulse wave velocity (arterial stiffness) is associated with future cardiovascular events and all-cause mortality, and is recognized by the European Society of Hypertension as one of the tools that may be used for the diagnosis and treatment of hypertension (Mancia *et al.*, 2007). However, it is generally only used for research purposes.

The augmentation index is a measure of systemic arterial stiffness. The lack of elasticity of the aortic wall is thought to be a key factor contributing to hypertension in the elderly. The aortic augmentation index is a measure of the stiffness of the aortic wall. Aortic wave reflections are the backwards (reflected) pressure wave. Reflected pressure waves arrive earlier in thoracic aortic and therefore augment the systolic aortic pressure – so accentuating hypertension.

An association exists between the augmentation index and traditional cardiovascular risk factors although the association is reported to attenuate in older patients (Janner *et al.*, 2011).

Aortic calcification

Atheromatous deposits in the aorta are not uncommon. As atherosclerosis progresses, calcium deposits are formed and aortic calcification is a marker of subsequent vascular mortality and morbidity (Wilson *et al.*, 2001). It is believed that atheroma is the result of an inflammatory process, which is accelerated in certain conditions including patients with diabetes mellitus, hypertension, smokers, obesity and hyperlipidaemia.

Coronary artery calcification

Analogous to aortic calcification, the coronary arteries (which supply heart muscle) may become calcified in conjunction with an atherosclerotic process. Coronary calcium may be quantified by cardiac computerised tomography (CT), an investigation supported by the National Institute of Clinical Excellence (NICE) guidance, which assists in the diagnosis of coronary artery disease.

Carotid intima-media thickness

Sometimes the thicknesses of parts of the wall of the carotid artery, or the size of plaque on the wall of the carotid artery, are used as surrogate markers for coronary artery disease and cerebrovascular disease. Just as atheroma develop on coronary arteries and the aorta, it may also be found in the carotid artery, a blood vessel supplying some of the circulation to the brain.

Early atheroma may be evident as a thickening of the intima – the internal layer of the blood vessel wall. The internal carotid artery arises from the common carotid artery, is found in the neck and supplies much of the circulation of the brain.

Carotid intima-media thickness measurements may be acquired by ultrasound of the common carotid artery coupled with sophisticated digital edge detection software. More advanced atherosclerotic disease (evident by plaque formation, with a necrotic core) occurs predominantly in the internal carotid artery. Even so, carotid intima-media thickness and its progression (rate of thickening) is a diffuse process which involves all carotid arteries (Espeland *et al.*, 2003).

Some researchers suggest that the carotid intima-media thickness is a limited marker of cardiovascular disease because thickening of the intima-media occurs in patients with hypertension and with increasing age, which is not always associated with atherosclerosis (Finn *et al.*, 2010). Furthermore, it is suggested that the carotid intima-media thickness measurement does not differentiate lesions with a necrotic core (Finn *et al.*, 2010). Measures of carotid plaque area or volume are considered better predictors of atherosclerosis than the intima-medial thickness (Simon *et al.*, 2010).

Testing for the carotid intima media thickness has been approved by the Food and Drug Administration (FDA) in the USA and has been used in large epidemiologic trials as well as large outcome studies as a surrogate end point for clinical events. Since 2002 it has also been applied in clinical settings in the USA.

Previous studies in COMA reports

Pre-1990 studies included in earlier reports of the Committee on Medical Aspects of Food Policy Policy (Committee on Medical Aspects of Food Policy, 1989;Committee on Medical Aspects of Food Policy, 1994;Committee on Medical Aspects of Food Policy, 1991) were extracted and reviewed in relation to relevant outcomes. Studies were initially scanned by title and abstract for relevance. Those deemed non-relevant were omitted and those of relevance were passed through the inclusion/ exclusion criteria. None of the RCTs and cohort studies that were reviewed reported markers of vascular function as outcomes.

Summary of the evidence base

Cohort Studies

Two cohort studies provided evidence on the relationship between dietary carbohydrates and aspects of vascular function. Their characteristics are included in Table 2.30.

Both were prospective studies conducted in the USA, with modest numbers of middle-aged participants. The Insulin Resistance Atherosclerosis Study (IRAS) (Mellen *et al.*, 2007) included men and women, but the Healthy Women Study included pre-menopausal women only (Park *et al.*, 2006). In these studies the relationship between baseline consumption of total carbohydrate, dietary fibre, whole, and refined grains in relation to subsequent measures of carotid and aortic calcification score and carotid artery dimensions were reported.

With observational studies, especially in the field of diet and nutrition, there is substantial potential for biases caused by incomplete adjustment for confounding, measurement error in the exposure estimate, and other biases in participant selection or data collection. Please interpret observational data with caution: the bias could be large in size, and act in either direction, either towards or away from the null.

Trial Design

Five publications from five randomised controlled trials provided information on the relationship between aspects of vascular function and dietary carbohydrate interventions.

Details concerning the design, participants, duration and nature of the interventions are included in Table 2.31. One study employed a cross-over design (Black *et al.*, 2006), but the remainder used a parallel group design.

None of the trials used children or adolescents as subjects, all were studies of adults. Three studies included both male and female participants. Two trials studied male participants only (Philippou *et al.*, 2009;Black *et al.*, 2006). All trials recruited subjects that had average BMI levels indicative of overweight or obesity. Two trials specifically recruited individuals that had one or more risk factors for the metabolic syndrome or cardiac risk factors (elevated blood pressure, high BMI, or hyperlipidaemias) (Keogh *et al.*, 2008;Philippou *et al.*, 2009).

Trials were conducted in the USA (Phillips *et al.*, 2008), Australia (Keogh *et al.*, 2008;Keogh *et al.*, 2007), and the UK (Philippou *et al.*, 2009;Black *et al.*, 2006). This small evidence base is therefore composed of studies that are fairly well spread across Europe, the Antipodes and North America.

The duration of interventions are detailed in the Trials Characteristics Table, and ranged from 6 weeks to 6 months, with median study duration of 8 weeks.

The number of participants in each trial ranged from 13 to 171, with a mean of 52 and median of 44. The evidence base is therefore composed of a small number of trials with relatively short duration and modest numbers of mainly overweight or obese participants.

Risk of bias

A summary of the risk of bias assessment is provided in Table 2.32. Criteria for judging whether a risk of bias was evident were based on the Cochrane Handbook. A judgement of 'unclear' was provided if there was insufficient evidence within the paper to make a clear judgement. Judgements concerning whether there was evidence of a risk of bias in terms of outcome assessment (the experimenters involved in assessing the outcome were aware which intervention had been followed by each participant) are reported as the final column in each of the specific results tables.

All trials included were randomised controlled trials. All were judged to be either 'unbiased' or 'unclear' (method of random allocation to groups not reported in paper) in terms of allocation sequence generation or allocation concealment. None were judged to be 'biased' in these aspects of trial design. Blinding of participants and researchers to the various dietary approaches was more difficult to achieve, as might be anticipated with dietary intervention trials. None were judged to be 'unbiased' in this respect, for most papers it was unclear whether the researchers were blind to the interventions prescribed to participants. There was some evidence of incomplete outcome reporting in three trials, but no evidence of selective outcome reporting in any of the trials.

Table 2.30 Cohort Study Characteristics

Reference	Study name	Country	Population	Length of follow-up (years)	Dietary assessment method	Total number of cohort participants at baseline	Loss of cohort members to follow-up %
(Mellen *et al.*, 2007)	The Insulin Resistance Atherosclerosis Study (IRAS)	USA	Multicentre observational study Mean age: 55 (40-69) %Male: 46 Ethnicity: Multi-ethnic	5	Diet was assessed using a 114-item FFQ administered twice and it was reported to be validated. The FFQ referred to diet over the previous year.	1625	19
(Park *et al.*, 2006)	The Healthy Women Study	USA	Healthy premenopausal women Mean age: 48 (42-50) Ethnicity: Primarily White	11	Diet was assessed using 24-hour dietary recall administered once and it was reported to be validated.	541	26

Table 2.31 Trial characteristics

Authors, Study Name	Subject inclusion criteria	Characteristics of participants	Trial Design (washout duration)	Length of Intervention	Intervention Style	Total n	Intervention group names	Intervention Description	Diet/Supplement nutritional characteristics	Actual diet consumed reported?	Funding source
(Black *et al.*, 2006)	BMI <35 No CHD, T2DM or HTN Not hyperlipidaemic/ hypercholesterolaemic	UK 100% Male Age: (33) BMI: (27)	Crossover (washout 4 weeks)	6 weeks	All food provided	13	1. High sucrose diet 2. Low sucrose diet	1. 25% energy provided as sucrose (solid food & beverages). 55% CHO, 10-15% PRO, 30-35% FAT, 18g/d fibre 2. 10% energy provided as sucrose (solid food & beverages). 55% CHO, 10-15% PRO, 30-35% FAT, 18g/d fibre Diets isocaloric	1. %E: C 55 P 11 F 33 Energy 2484 kcal/d Fibre g/d:17 2. %E: C 55 P 12 F 33 Energy 3176 kcal/d Fibre g/d:18	Yes	The Sugar Bureau and Suikerstichting, the Netherlands
(Keogh *et al.*, 2007)	Age 20-65y BMI 27-40 Moderate alcohol intake No HTN or T2DM No medications which influence outcomes	Australia 32% Male Age: (49) BMI: (33)	Parallel Group	12 weeks Active weight loss phase 1-12 wk, monthly dietician meeting until wk 52	Free living diet plan	44	1. Low carbohydrate diet 2. High carbohydrate diet	1. Energy restricted, low CHO diet, low in saturated fat. 2. Energy restricted, high CHO diet, low in saturated fat.	1. %E: C 33 P 40 F 27 Fibre g/d:26 2. %E: C 60 P 20 F 20 Fibre g/d:40	No, intended diet only	Diabetes Australia Research Trust
(Keogh *et al.*, 2008)	≥ 1 metabolic syndrome risk factor Abdominal obesity No CHD or T2DM	Australia % Male: not reported Age: 24 - 64(50) BMI:27 - 44(34)	Parallel Group	8 weeks	Free living diet plan	117	1. Low carbohydrate, high SFA 2. High carbohydrate, low SFA	1. 30% energy restriction. Some key foods were provided top aid compliance. Intended diet: 4%CHO, 35%PRO, 61%FAT 2. 30% energy restriction. Some key foods were provided top aid compliance. Intended diet: 46%CHO, 24%PRO, 30%FAT	1. %E: C 5 P 35 F 59 g/d: C 20 P 133 F 103 Energy: 6608kJ/d Fibre g/d:13 2. %E: C 47 P 24 F 28 g/d: C 172 P 87 F 47 Energy: 6590kJ/d Fibre g/d:32	Yes	National Heart Foundation of Australia and Medical Research Council of Australia
(Philippou *et al.*, 2009)	≥1 cardiac risk factor (BMI 27-35 kg/m2, waist ≥94 cm, total cholesterol to high-density lipoprotein ratio ≥5.0, raised BP up to a maximum of 140/90 mm Hg) No medication	UK 100% Male Age: 35 - 65 BMI: mean not reported	Parallel Group	6 months	Substitution	56	 1. High GI 2. Low GI	Those with BMI>25 also received weight management advice 1. High GI, carbohydrate foods (e.g. white/wholemeal bread, cornflakes, weetabix, potatoes, couscous, risotto rice, melon, pineapple, rice cakes) 2. Low GI, carbohydrate foods	Both groups decreased EI (greater in low GI group), but no macronutrient differences between arms	Yes	British Heart Foundation

Authors, Study Name	Subject inclusion criteria	Characteristics of participants	Trial Design (washout duration)	Length of Intervention	Intervention Style	Total n	Intervention group names	Intervention Description	Diet/Supplement nutritional characteristics	Actual diet consumed reported?	Funding source
								(e.g. seeded bread, wholemeal pita, muesli, porridge, sweet potatoes, pasta, noodles, basmati slow-cook rice, beans, lentils, apples, dried fruit, nuts)			
(Phillips *et al.*, 2008)	Age 18-50y BMI 29-39 Generally healthy No CHD, T2DM or HTN Non smokers Not hyperlipidaemic/ hypercholesterolaemic	USA 25% Male Age: mean not reported BMI: mean not reported	Parallel Group	6 weeks	All food provided	28	1. Low carbohydrate diet 2. Low fat diet	1. Isocaloric arms. Low carbohydrate Atkins-style diet (20g/d CHO). 750kcal/d energy deficit weeks 1-4 weeks. 2. American Heart Association low fat diet (30% total energy from fat). 750kcal/d energy deficit weeks 1-4.	1. g/d: C 20 2.%E: F 30	No, intended diet only	NIH & the Medical College of Wisconsin Cardiovascular Centre

Table 2.32 Sources of bias in trials

Authors	Allocation sequence generation	Allocation concealment	Participant blinding	Researcher Blinding	Incomplete outcome reporting	Selective outcome reporting	Any other bias
(Black *et al.*, 2006)	No Bias	Unclear	Bias	Unclear	Unclear	No Bias	No Bias
(Keogh *et al.*, 2007)	Unclear	Unclear	Unclear	Unclear	Bias	No Bias	No Bias
(Keogh *et al.*, 2008)	No Bias	Unclear	Bias	Unclear	Bias	No Bias	No Bias
(Philippou *et al.*, 2009)	Unclear	Unclear	Bias	Unclear	Bias	No Bias	No Bias
(Phillips *et al.*, 2008)	No Bias	No Bias	Bias	Bias	No Bias	No Bias	No Bias

Markers of vascular function, total carbohydrate and high carbohydrate diets

Summary of cohort results

The Healthy Women Study was the only cohort study to investigate total carbohydrate intake and markers of vascular function (Park *et al.*, 2006). Coronary and aortic calcification scores (measured by electron beam CT scans) and carotid plaque index (measured by a carotid ultrasound scan) were used to assess vascular function, and one 24-hour dietary recall was employed to assess diet. Adjusted relative risk estimates for aortic calcification and carotid plaque index were close to 1, suggesting no evidence of an association with per cent energy derived from carbohydrate. However, when examining coronary calcification, there was evidence of a reduction in risk associated with increased percentage of energy derived from carbohydrate. The adjusted relative risk comparing the highest quartile of intake against the lowest was 0.37 (95% CI: 0.18, 0.74).

The Healthy Women Study (Park *et al.*, 2006) appeared to adjust appropriately for confounders.

Please interpret observational data with caution: With observational studies, especially in the field of diet and nutrition, there is substantial potential for biases.

Summary of RCT data

Three trials provided data on high carbohydrate diets and aspects of vascular function (Phillips *et al.*, 2008;Keogh *et al.*, 2007;Keogh *et al.*, 2008).

Keogh *et al.* (Keogh *et al.*, 2007) assessed the effect of a conventional high carbohydrate (60% energy), low fat diet compared to a low carbohydrate (33% energy), low saturated fat (7% energy) weight loss diet in 33 obese adults. Flow mediated dilation (ultrasound measurements before and after forearm ischaemia induced by blood pressure cuff), pulse wave velocity (Doppler recording at carotid and femoral artery) and the augmentation index (using the SphygmoCor™ blood pressure analysis system) were assessed at baseline, and at 6, 12 and 52 weeks on the diets. The participants lost weight on both diets (5.6% of initial body weight at 1 year, with no difference between diets), but there was no differential effect on flow mediated dilation, pulse wave velocity or the augmentation index. The authors estimated that they had sufficient power to detect a 12% difference in FMD with 80% probability despite the relatively small number of participants and high dropout rate.

Keogh *et al.* (Keogh *et al.*, 2008) conducted a further 8-week study with more extreme reductions in carbohydrate intake. The effect of a high carbohydrate (47% energy), low fat diet was compared with a low carbohydrate (5% energy), high saturated fat (21% energy) weight loss diet in 99 obese adults. As earlier, FMD, pulse wave velocity and augmentation index were assessed at baseline and after 8 weeks on the diets. Energy intakes were similar and both groups lost weight, but losses were higher on the low carbohydrate diet than the higher carbohydrate diet (-7.5 vs. -6.2 kg). Pulse wave velocity improved in both diets (in association with weight loss) but was not statistically different between diets. FMD, flow independent dilation and the augmentation index did not differ by diet group.

Both of the studies by Keogh *et al.* indicated that low carbohydrate weight loss diets did not have an adverse effect on FMD or other measures of vascular function when compared with conventional high carbohydrate, low fat weight loss diets. Conversely, data from a different research group suggests that low carbohydrate diets are associated with a reduction in endothelial function compared with isoenergetic high carbohydrate diets. Phillips *et al.* (Phillips *et al.*, 2008) conducted a 6-week study of a range of measures of endothelial health as listed in table 2.34. The objective of this study was to compare the effects of isoenergetic, energy restricted low carbohydrate (<20g/day) and high carbohydrate, low fat (30% energy) diets provided by research staff for 4 weeks, followed by a further 2 weeks where energy intake increased but the macronutrient manipulation continued. Weight losses were similar between the two diets, and most markers of endothelial health also did not differ between diets. However, brachial FMD deteriorated (a 14% reduction) after 6 weeks on the low carbohydrate diet, whereas an improvement was recorded for the low fat, higher carbohydrate diet (34% improvement) (difference between diets p=0.003).

Flow mediated dilation and high carbohydrate diets – meta-analysis

Three studies were included in the meta-analyses comparing different carbohydrate intakes and changes in FMD reported as percentage change. There were insufficient studies to stratify by fat and protein difference. All studies included adults as participants. The first follow up reported at the end of the intervention was used. This varied from 6 to 8 weeks.
The pooled estimate indicated that FMD was 0.68% (95% CI -0.47 to 1.83%) higher with consumption of higher carbohydrate diets compared with lower carbohydrate diets. This was not significantly different from zero (p=0.25). Overall heterogeneity denoted by I^2 was 15% (95% CI 0 to 91%). Statistically, there was no evidence that diets higher in carbohydrate are associated with differences in FMD. A funnel plot was not prepared as there were too few studies.

Figure 2.20 Forest plot for high carbohydrate diets and FMD (% change brachial artery diameter)

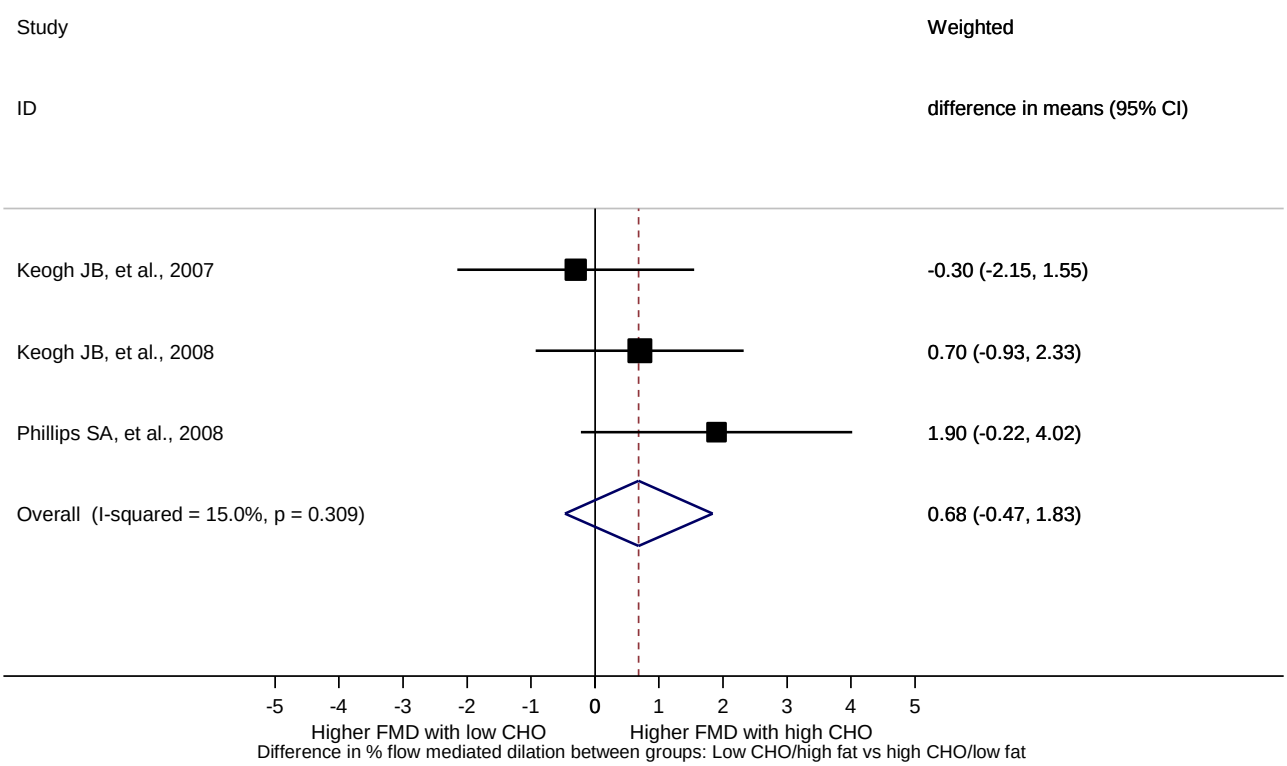

Table 2.33 Markers of vascular function and total carbohydrate: cohort study in adults

Result ID/ Reference/ Cohort Name	Country, Ethnicity, Inclusion criteria	Age range (mean) %Male	(Cases)/ Total	Follow Up (% loss)	Diet method	Exposure	Outcome/ Assessment Details	Contrast (mean)	Exposure Units	RR (CI)	Adjustments
(Park *et al.*, 2006) 14517 Healthy Women Study	USA, Primarily White, Generally healthy, No hypertension, No medications which influence outcomes, Pre-menopausal	42-50 (48) %M 0	(261) /541	11 y (26)	Dietary recall	Carbohydrate, total (% energy)	Aortic calcification Medical testing	Q4 vs Q1	%Energy	1.2 (0.52, 2.79)	Age, Alcohol, Blood glucose, Education, Energy intake, HRT, Parental CHD, Parental Stroke, Physical activity, Smoking, systolic blood pressure
14518 Healthy Women Study			(199) /541				Carotid plaque index Medical testing	Q4 vs Q1	%Energy	1.08 (0.55, 2.15)	As above
14516 Healthy Women Study			(169) /541				Coronary calcification Medical testing	Q4 vs Q1	%Energy	0.37 (0.18, 0.74)	As above

Table 2.34 Markers of vascular function and high carbohydrate diets: RCT data

Author / Result ID	Intervention groups	Completers/ Allocated	Baseline	Follow-up	Within group Δ from baseline	p-value within group Δ from baseline	p-value difference between groups	Outcome/Assessment method	Result/ Outcome details	Result-specific follow-up	Weight change	Outcome Assessment Bias
(Keogh *et al.*, 2007) *15599	High carbohydrate diet	12/12	5.9 (SE 0.5)	5 (SE 0.8)				Flow mediated dilation (% change artery diameter - ultrasound measurements before and after forearm ischaemia induced by blood pressure cuff)	Clinical assessment (%)	6 weeks	Decrease	No bias
	Low carbohydrate diet	13/13	5.3 (SE 0.6)	5.3 (SE 0.5)			NS				Decrease	
15604	High carbohydrate diet	12/12	10.1 (SE 0.7)	8.6 (SE 2)				Pulse wave velocity (Doppler recording at carotid and femoral artery)	Clinical assessment (m/s)	6 weeks	Decrease	unclear
	Low carbohydrate diet	13/13	9.6 (SE 1.1)	9.2 (SE 0.5)			NS				Decrease	
15605	High carbohydrate diet	12/12	10.1 (SE 0.7)	4.9 (SE 2.8)				Pulse wave velocity (Doppler recording at carotid and femoral artery)	Clinical assessment (m/s)	12 weeks	Decrease	unclear
	Low carbohydrate diet	13/13	9.6 (SE 1.1)	10 (SE 0.8)			NS				Decrease	
15606	High carbohydrate diet	completers not reported/12	7 (SE 1.3)	4.8 (SE 0.6)				Flow mediated dilation (% change artery diameter - ultrasound measurements before and after forearm ischaemia induced by blood pressure cuff)	Clinical assessment (%)	1 year	Decrease	No bias
	Low carbohydrate diet	completers not reported/13	5.3 (SE 0.8)	4.4 (SE 0.7)			NS				Decrease	
15609	High carbohydrate	completers	11.2 (SE 0.7)	8.8 (SE 0.6)				Pulse wave velocity	Clinical	1 year	Decrease	unclear

Author / Result ID	Intervention groups	Completers/ Allocated	Baseline	Follow-up	Within group Δ from baseline	p-value within group Δ from baseline	p-value difference between groups	Outcome/Assessment method	Result/ Outcome details	Result-specific follow-up	Weight change	Outcome Assessment Bias
	diet	not reported/12						(Doppler recording at carotid and femoral artery)	assessment (m/s)			
	Low carbohydrate diet	completers not reported/13	8.3 (SE 0.7)	10.3 (SE 0.6)			NS				Decrease	
15628	High carbohydrate diet	12/12	20 (SE 4)	24 (SE 3)		NS		Augmentation Index (using the SphygmoCor™ blood pressure analysis system)	Clinical assessment (%)	6 weeks	Decrease	unclear
	Low carbohydrate diet	13/13	27 (SE 2)	27 (SE 2)		NS	NS				Decrease	
15630	High carbohydrate diet	12/12	20 (SE 4)	22 (SE 3)		NS		Augmentation Index (using the SphygmoCor™ blood pressure analysis system)	Clinical assessment (%)	12 weeks	Decrease	unclear
	Low carbohydrate diet	13/13	27 (SE 2)	27 (SE 2)		NS	NS				Decrease	
15631	High carbohydrate diet	completers not reported/12	21 (SE 5.2)	22.3 (SE 4.7)		NS		Augmentation Index (using the SphygmoCor™ blood pressure analysis system)	Clinical assessment (%)	1 year	Decrease	unclear
	Low carbohydrate diet	completers not reported/13	26.5 (SE 3.7)	24.4 (SE 2.2)		NS	NS				Decrease	
(Keogh *et al.*, 2008) *16725	High carbohydrate, low SFA	47/50	6.0 (SD 3.8)	6.3 (SD 4.4)		NS		Flow mediated dilation (% change brachial artery diameter - ultrasound measurements before and after forearm ischaemia induced by blood pressure cuff)	Clinical assessment (%)	8 weeks	Decrease	unclear
	Low carbohydrate, high SFA	52/57	5.4 (SD 3.4)	5.6 (SD 3.6)		NS	NS				Decrease	
16726	High carbohydrate, low SFA	30/50	18.9 (SD 7.2)	21.0 (SD 6.2)		0.05		Flow-independent dilation (% change brachial artery diameter - ultrasound measurements before after 300µg sublingual glyceryl-trinitrate)	Clinical assessment (%)	8 weeks	Decrease	unclear
	Low carbohydrate, high SFA	36/57	20.1 (SD 5.1)	20.7 (SD 6.4)		0.05	NS				Decrease	
16728	High carbohydrate, low SFA	30/50	11.1 (SD 2.9)	9.5 (SD 1.5)		<0.05		Pulse wave velocity (Doppler recording at carotid and femoral artery)	(m/s)	8 weeks	Decrease	unclear
	Low carbohydrate, high SFA	36/57	10.7 (SD 3.0)	9.9 (SD 2.4)		<0.05	NS				Decrease	
16729	High carbohydrate, low SFA	45/50	28.1 (SD 12.8)	27.6 (SD 9.4)		NS		Augmentation Index (using the SphygmoCor™ blood pressure analysis system)	(%)	8 weeks	Decrease	unclear
	Low carbohydrate, high SFA	51/57	29.4 (SD 9.6)	28.9 (SD 10.2)		NS	NS				Decrease	
(Phillips *et al.*, 2008) 174341 7436	Low carbohydrate diet	10/~14	3.42 (SE 0.21)	3.58 (SE 0.23)		NS	0.317	Baseline brachial artery diameter Assessed using ultrasound probe (11 mHz) positioned at 90° to the vessel to visualize anterior and posterior lumen-intimal interfaces before cuff	Clinical assessment Fasting (mm)	6 weeks	Decrease	No bias
	Low fat diet	10/~14	3.94 (SE 0.25)	3.85 (SE		NS					Decrease	

Author / Result ID	Intervention groups	Completers/ Allocated	Baseline	Follow-up	Within group Δ from baseline	p-value within group Δ from baseline	p-value difference between groups	Outcome/Assessment method	Result/ Outcome details	Result-specific follow-up	Weight change	Outcome Assessment Bias
				0.28)				constriction				
17437	Low carbohydrate diet	10/~14	3.77 (SE 0.24)	3.82 (SE 0.24)		NS	0.722	Peak brachial artery diameter Determined during peak hyperemia after release of the cuff from the forearm. To assess vasodilation, 10 seconds of images captured at a rate of 10 images/second (total=100 images) at 30 seconds, one minute and two minutes after cuff release.	Clinical assessment Fasting (mm)	6 weeks	Decrease	No bias
	Low fat diet	10/~14	4.29 (SE 0.3)	4.26 (SE 0.27)		NS					Decrease	
*17438	Low carbohydrate diet	10/~14	8.2 (SE 0.7)	6.8 (SE 0.6)		0.05	0.003	Maximum flow mediated dilation Percent FMD was calculated using the average brachial artery diameter at baseline compared to the largest averaged values of the 3 time points measured after release of forearm occlusion	Clinical assessment Fasting (%)	6 weeks	Decrease	No bias
	Low fat diet	10/~14	6.8 (SE 0.7)	8.7 (SE 0.9)		0.05					Decrease	
17439	Low carbohydrate diet	10/~14	60.8 (SE 5.9)	65.7 (SE 3.2)		NS	0.278	Baseline peak velocity Flow velocity was recorded at baseline before forearm occlusion	Clinical assessment Fasting (cm/s)	6 weeks	Decrease	No bias
	Low fat diet	10/~14	54 (SE 3)	53 (SE 3.7)		NS					Decrease	
17440	Low carbohydrate diet	10/~14	36.2 (SE 3.7)	38.5 (SE 2.5)		NS	0.466	Baseline mean velocity Flow velocity was recorded at baseline before forearm occlusion	Clinical assessment Fasting (cm/s)	6 weeks	Decrease	No bias
	Low fat diet	10/~14	32.3 (SE 1.7)	31.5 (SE 2)		NS					Decrease	
17441	Low carbohydrate diet	10/~14	105.2 (SE 10)	116.9 (SE 6.3)		NS	0.955	Reactive hyperemia peak velocity Peak flow velocity measured after release of forearm occlusion.	Clinical assessment Fasting (cm/s)	6 weeks	Decrease	No bias
	Low fat diet	10/~14	104 (SE 7)	115.1 (SE 7)		NS					Decrease	
17442	Low carbohydrate diet	10/~14	58.6 (SE 6)	67.7 (SE 3.2)		NS	0.391	Reactive hyperemia mean velocity Mean flow velocity measured after release of forearm occlusion.	Clinical assessment Fasting (cm/s)	6 weeks	Decrease	No bias
	Low fat diet	10/~14	59.4 (SE 6.15)	61.4 (SE 3.5)		NS					Decrease	
17443	Low carbohydrate diet	10/~14	76 (SE 12)	80 (SE 8)		NS	0.246	Peak change in flow velocity Flow velocity was recorded at baseline and just after cuff release where maximal velocity is observed	Clinical assessment Fasting (%)	6 weeks	Decrease	No bias
	Low fat diet	10/~14	83.8 (SE 9)	101 (SE 10)		NS					Decrease	
17444	Low carbohydrate diet	10/~14	20.9 (SE 0.02)	20.6 (SE 0.02)		NS	0.305	Maximum nitroglycerin dilation Response to NTG was calculated using the average brachial artery diameter at baseline compared to the largest averaged values of the 3 time points measured after administration of NTG	Clinical assessment Fasting (%)	6 weeks	Decrease	No bias
	Low fat diet	10/~14	21.3 (SE 0.023)	19.3 (SE 0.02)		NS					Decrease	

*This result was used in the meta-analysis for high carbohydrate diets and flow mediated dilation

Markers of vascular function and dietary sugars

No cohort studies reported outcomes concerning sugar intake and markers of vascular function.

Summary of RCT data

One cross-over trial explored the impact of high and low sucrose diets on measures of vascular compliance in 13 healthy males (Black *et al.*, 2006). Each 6-week feeding phase was isocaloric, and matched for macronutrients and fibre, but diets provided either 10 or 25% of total energy as sucrose. All food was provided and body weights were maintained. Central augmentation pressure (a reflection of the outgoing and reflected pressure waves during systole), augmentation index corrected to a heart rate of 75 bpm (systemic arterial stiffness), time to reflectance (an indirect estimate of aortic pulse-wave velocity) and pulse wave velocity (a reflection of arterial stiffness) were not differentially influenced by the sucrose content of the diets.

Table 2.35 Markers of vascular function and dietary sucrose: RCT data

Author/ Result ID	Intervention groups	Completers / Allocated	Follow-up	p-value difference between groups	Outcome/ Assessment method	Result/ Outcome details	Result-specific follow-up	Weight change	Outcome Assessment Bias
(Black *et al.*, 2006) 16614	High sucrose diet	13/13	2.2 (SE 1.5)	NS	Aortic augmentation Systolic	(mm/Hg)	6 weeks	No change	unclear
	Low sucrose diet	13/13	3 (SE 1.7)					No change	
16615	High sucrose diet	13/13	0.8 (SE 3.9)	NS	Aortic augmentation Index Systolic	(%)	6 weeks	No change	unclear
	Low sucrose diet	13/13	-0.4 (SE 4.1)					No change	
17420	High sucrose diet	13/13	8.5 (SE 0.3)	NS	Brachial pulse-wave velocity	(m/s)	6 weeks	No change	unclear
	Low sucrose diet	13/13	8.4 (SE 0.4)					No change	
17427	High sucrose diet	13/13	171 (SE 7.0)	NS	Time to wave reflection	(m/s)	6 weeks	No change	unclear
	Low sucrose diet	13/13	167 (SE 8.0)					No change	

Markers of vascular function, dietary fibre and fibre isolates

Summary of cohort results

One publication reported results concerning dietary fibre and vascular function from a US cohort of premenopausal women (Park *et al.*, 2006). Coronary and aortic calcification scores (measured by electron beam CT scans) and carotid plaque index (measured by a carotid ultrasound scan) were used to assess postmenopausal vascular function and a dietary recall was employed to assess premenopausal diet. Baseline intake of dietary fibre was not associated with postmenopausal risk of aortic calcification or the carotid plaque index. However, when examining coronary calcification, there was clear evidence of a reduction in risk associated with increasing fibre density. The adjusted relative risk comparing the highest quartile of intake against the lowest was 0.49 (95% CI: 0.24, 0.98).

The Healthy Women study (Park *et al.*, 2006) adjusted for appropriate confounders such as age, HRT use and smoking.

Please interpret observational data with caution: With observational studies, especially in the field of diet and nutrition, there is substantial potential for biases.

Summary of RCT data

No trials provided data on vascular function and dietary fibre or fibre isolates.

Table 2.36 Markers of vascular function and dietary fibre: cohort study in adults

Result ID/ Reference/ Cohort Name	Country, Ethnicity, Inclusion criteria	Age range (mean) %Male	(Cases)/ Total	Follow Up (% loss)	Diet Assess-ment	Exposure	Outcome/ Assessment Details	Contrast (mean)	Exposure Units	RR (CI)	Adjustments
(Park *et al.*, 2006) 14520 Healthy Women Study	USA, Primarily White, Generally healthy, No hypertension, No medications which influence outcomes, Pre-menopausal	42-50 (48) %M 0	(261) /541	11 y (26)	Dietary recall	Fibre density (AOAC method)	Aortic calcification electron beam CT scans	Q4 vs Q1	g/1000 kcal	2 (0.84, 4.75)	Age, Alcohol, Blood glucose, Education, Energy intake, HRT, Parental CHD, Parental Stroke, Physical activity, Smoking, systolic blood pressure
14521 Healthy Women Study			(199) /541				Carotid plaque index electron beam CT scans	Q4 vs Q1	g/1000 kcal	1.71 (0.86, 3.4)	As above
14519 Healthy Women Study			(169) /541				Coronary calcification carotid ultrasound scan	Q4 vs Q1	g/1000 kcal	0.49 (0.24, 0.98)	As above

Markers of vascular function, whole grain foods

Summary of cohort results

Data were extracted from one publication from the Insulin Resistance Atherosclerosis Study (IRAS) (Mellen *et al.*, 2007). This study examined total whole grain and total refined grain foods using a food frequency questionnaire. Full details of this cohort are provided in Table 2.30.

Beta coefficients in the Insulin Resistance Atherosclerosis Study (Mellen *et al.*, 2007) suggest a decrease in both common carotid and internal carotid internal medial thickness and thickness progression with each additional serving of whole grain foods per day. These associations persisted with further adjustment for nutrients associated with whole grains, such as B vitamins and dietary fibre. Adjustment for a 'healthy dietary pattern score' also did not attenuate the association with whole grain consumption, which may indicate an independent association with atherosclerotic progression. Internal carotid artery intimal medial thickness and internal carotid artery intimal medial thickness progression, were weakly associated and not associated respectively with whole grain consumption.

This study adjusted for important covariates, including age, hypertension mediation, physical activity and statin use, and provides some evidence of benefit with regard to coronary artery atherosclerosis in association with increasing consumption of whole grain foods.

Please interpret observational data with caution: With observational studies, especially in the field of diet and nutrition, there is substantial potential for biases.

Summary of RCT data

No trials provided data concerning wholegrains and vascular function.

Table 2.37 Markers of vascular function and whole grain foods: cohort study in adults

Result ID/ Reference/ Cohort Name	Country, Ethnicity, Inclusion criteria	Age range (mean) %Male	(Cases) / Total	Follow Up (% loss)	Diet Assess-ment	Exposure	Outcome/ Assessment Details	Exposure Units	Beta coefficient	p trend	Adjustments
(Mellen *et al.*, 2007) 14591 Insulin Resistance Atherosclerosis Study	USA, Multi-ethnic, Impaired glucose tolerance, Normal glucose tolerance	40-69 (55) %M 44	1178	5 years (19)	FFQ (114)	Whole grains. Whole-grains including, dark bread (whole-wheat, rye, pumpernickel, other high-fibre breads), High-fibre bran or granola cereals, shredded wheat, cooked cereals (oatmeal, cream of wheat, grits)	Common carotid artery intimal medial thickness High-resolution B-mode carotid ultrasonography	1 serving/day	-0.043 (0.013)	0.005	Age, Energy Intake, Ethnicity, diabetes mellitus type 2, hypertension, physical activity, gender, Smoking, Statin use, hypertension medication, diabetes medication, Visit
14598 Insulin Resistance Atherosclerosis Study							Common carotid artery intimal medial thickness *progression* High-resolution B-mode carotid ultrasonography	1 serving/day	-0.019 (0.011)	0.09	As above
14599 Insulin Resistance Atherosclerosis Study							Internal carotid artery intimal medial thickness High-resolution B-mode carotid ultrasonography	1 serving/day	-0.049 (0.023)	0.05	As above
14600 Insulin Resistance Atherosclerosis Study							Internal carotid artery intimal medial thickness *progression* High-resolution B-mode carotid ultrasonography	1 serving/day	-0.013 (0.014)	0.35	As above

Markers of vascular function and glycaemic index

No cohort studies reported results concerning glycaemic index and markers of vascular function.

Summary of RCT data

One trial provided data on the impact of high and low glycaemic index (GI) diets on arterial compliance as reflected in pulse wave velocity, which is a reflection of arterial stiffness (Philippou *et al.*, 2009). This UK study of middle-aged men with at least one coronary heart disease risk factor, randomised participants to 6 months of healthy eating and weight loss advice (in participants with BMI >25kg/m^2) including either high or low GI foods. This generated a difference in GI between the groups of 12 GI units. Glycaemic load was also higher in the high GI group compared to the low GI group (175 vs. 114), but there were no other differences in dietary composition between the two groups. Both groups lost weight as planned. However, there was no difference between the diet groups in pulse wave velocity at the 6 month follow-up.

Table 2.38 Markers of vascular function and glycaemic index: RCT data

Author/ Result ID	Inter-vention groups	Comp-leters/ Allo-cated	Base-line	Follow-up	Within group Δ from baseline	p-value within arm Δ from baseline	p-value difference between groups	Outcome/ Assessment method	Result/ Outcome details	Result-specific follow-up	Weight change	Outcome Assess-ment Bias
(Philippou *et al.*, 2009) 14667	High GI	14/28	9.9	9.4	-0.3 (CI -0.6, 0.5)	NS		Pulse wave velocity	Carotid-femoral (ms)	6 months	Decrease	unclear
	Low GI	18/28	10.3	9.7	-0.4 (CI -1.4, 0)	<0.01	NS				Decrease	

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