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The intake of flavonoids and carotid atherosclerosis: the Kuopio Ischaemic Heart Disease Risk Factor Study

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The role of flavonoids in CVD is still unclear. In this cross-sectional study we assessed the relation between the intakes of twenty-six flavonoids from five subclasses: flavonols, flavones, flavanones, flavan-3-ols and anthocyanidins, and the mean common carotid artery intima-media thickness (CCA-IMT). The study population consisted of 1380 middle-aged eastern Finnish men for whom the mean CCA-IMT examinations were carried out as a part of the prospective population-based Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). The mean intake of flavonoids was 128·5 (SD 206·7) mg/d and the mean CCA-IMT was 0·78 (SD 0·17) mm. In the lowest quartile of total flavonoid intake the non-adjusted mean CCA-IMT was 0·79 (SD 0·19) mm, while the mean CCA-IMT was 0·76 (SD 0·15) in the highest quartile ($P<0·001$). After adjustment for age, variables related to CCA-IMT measurement, history of atherosclerosis, smoking, BMI, diabetes, systolic blood pressure, serum HDL- and LDL-cholesterol, V_{O_2} max, and intakes of alcohol, SFA, folate, vitamins C and E, the total flavonoid intake was inversely associated with the mean CCA-IMT ($P=0·018$). Out of different flavonoid subclasses, flavan-3-ols were inversely associated with CCA-IMT ($P=0·025$) after statistical adjustment. There was a trend for an inverse association between intake of flavonols and mean CCA-IMT ($P=0·055$). We conclude that high intake of flavonoids is associated with decreased carotid atherosclerosis in middle-aged Finnish men.

Cross-sectional study: Atherosclerosis: Common carotid artery intima-media thickness: Flavonoids: Kuopio Ischaemic Heart Disease Risk Factor Study

Increasing evidence suggests that high consumption of fruit and vegetables decreases the risk of chronic diseases such as CVD[1,2]. Flavonoids, a large group of polyphenolic compounds (>5000 identified) abundant in vegetables, fruits and berries, may be responsible for the health-promoting effects of these plant foods[3]. To date several epidemiological studies on flavonoid intake and the risk of CVD have been published[4–7] and the results suggest that flavonoids may protect against CVD.

Out of tens of subclasses of flavonoids, five subclasses (anthocyanidins, flavonols, flavones, flavan-3-ols, and flavanones) have been estimated to contribute significantly to the daily intake and thus have potential effects on health[18]. Previous epidemiological studies have, however, concentrated mainly on two subclasses, flavonols and flavones [4,5,7–11,13,15–17], while the role of other subclasses have been evaluated only in a few studies[8,9,15,19].

Studying the role of those flavonoid subclasses which are considered relevant to the daily intake has been difficult because of incomplete databases. Efforts to update databases are constantly made; for example in 2003 the United States Department of Agriculture published a new food composition data which contained five subclasses: flavones, flavonols, flavan-3-ols, flavanones and anthocyanidins, a total of twenty-six flavonoids.

Our aim was to use the updated flavonoid database to investigate the associations between the intakes of the most commonly consumed flavonoids and the mean common carotid artery intima-media thickness (CCA-IMT) in Finnish men in a cross-sectional study.

Materials and methods

Study population

The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) is an ongoing population-based study designed to investigate risk factors for CVD, atherosclerosis and related outcomes in middle-aged men from eastern Finland[20]. The study was approved by the Research Ethics Committee, Hospital District of Northern Savo. A total of 2682 participants (82·9 % of those eligible), aged 42, 48, 54, or 60 years, was enrolled in the study between March 1984 and December 1989. All study subjects gave their written informed consent. CCA-IMT measurements were set up on December 1987 and thereafter the measurements were done

Abbreviations: CCA-IMT, common carotid artery intima-media thickness; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study.

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for 1380 men. Complete data for the present cross-sectional analysis were available for 1380 subjects.

Measurements

The subjects came to give blood samples between 08.00 and 10.00 hours. They were instructed to abstain from ingesting alcohol for 3 d and from smoking and eating for 12 h. After the subject had rested in the supine position for 30 min, blood was drawn with Terumo Venoject (Leuven, Belgium) vacuum tubes. No tourniquet was used. The main serum lipoprotein fractions, LDL-cholesterol and HDL-cholesterol (Kone Instruments, Espoo, Finland), and TAG (Boehringer Mannheim, Mannheim, Germany) were determined from fresh serum samples using combined ultracentrifugation and precipitation. V_{O_2} max was measured as previously described[21]. Diabetes was assessed by previous diagnosis or fasting blood glucose concentration $\geq 6{\cdot}7$ mmol/l. BMI was computed as the ratio of weight to the square of height (kg/m^2). Resting systolic blood pressure was measured in the morning by two trained nurses with a random-zero Hg sphygmomanometer (Hawksley, Lancing, UK). The measuring protocol included, after supine rest of 5 min, three measurements in supine, one on standing and two in sitting position with 5-min intervals. The mean of all six measurements was used as the systolic blood pressure. The number of cigarettes, cigars, and pipefuls of tobacco currently smoked daily, duration of regular smoking in years, alcohol consumption, history of myocardial infarction, angina pectoris, and medication were recorded with a self-administered questionnaire, which was checked by an interviewer. Repeated interviews to obtain medical history of CHD were conducted by a physician. The family history of CHD was defined as positive if the father, mother, sister, or brother of the subject had a history of CHD. A subject was defined as a smoker if he had ever smoked on a regular basis and had smoked cigarettes, cigars, or pipe within the past 30 d.

Assessment of common carotid artery intima-media thickness

CCA-IMT was assessed by high-resolution B-mode ultrasonography of the right and left CCA at the distal end, proximal to the carotid bulb. The ultrasound equipment (Biosound Phase 2; Biosound Inc, Indianapolis, USA) was equipped with a high-resolution probe. Images were focused on the posterior wall of the right and left CCA and were recorded on videotape for image analysis. The ultrasonographic examinations were carried out by well-trained ultrasound technicians and were performed after the subjects had rested in a supine position for 15 min. IMT measurements were made through computerized analysis of the videotaped ultrasound images with PROSOUND software (University of Southern California, Los Angeles, USA). This software uses an edge-detection algorithm, specifically designed for use with ultrasound imaging, that allows automatic detection, tracking, and recording of the intima-lumen and media-adventitia interfaces, estimated at approximately 100 points, in both the right and left CCA in a 1·0–1·5 cm section[22]. Mean IMT was computed as the mean of approximately 100 IMT measurements in the right CCA and another 100 measurements in the left CCA. A separate study concerning the intra- and inter-observer variability of IMT measurements was carried out three times with 1-week intervals in ten randomly chosen middle-aged men who had participated in the KIHD. For four observers the between-observer CV was 10·5 % for both the right and left CCA. The correlation coefficients ranged from 0·90 to 0·99. The intra-observer variability (reproducibility) was described by the difference between the first and the third measurement by each observer. The mean absolute difference was 0·087 mm, which is 8·1 % of the mean of all measurements23.

Assessment of nutrient intake

The consumption of foods was assessed at the time of blood sampling at the study baseline with an instructed 4-d food recording by household measures. The instructions were given and the completed food records were checked by a nutritionist. The intakes of nutrients were estimated using the NUTRICA® version 2.5 software (Social Insurance Institution, Turku, Finland). The intakes of nutrients were energy adjusted by the residual method[24]. The residuals were standardized by the mean nutrient intake of a subject consuming 10 MJ/d, the approximate average total energy intake in this study population. The measurement of total, subclass and individual flavonoid intake was mainly based on the United States Department of Agriculture flavonoid database (http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.html). The database includes a total of twenty-six flavonoids from five subclasses: flavonols (quercetin, kaempferol, myricetin, isorhamnetin), flavones (luteolin, apigenin), flavanones (hesperitin, naringenin, eriodictyol), flavan-3-ols ((+)-catechin, (+)-gallocatechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin-3-gallate, (−)-epigallocatechin-3-gallate, theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, theaflavin-3,3′-digallate, thearubigins) and anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin). The United States Department of Agriculture database is incomplete for anthocyanidin-rich berries which are commonly consumed in Finland. Therefore additional anthocyanidin data for those Finnish berries were derived from the work conducted by Kähkönen and colleagues[24,25].

Statistics

The data are expressed as means with their standard deviation. Correlations between the intakes of flavonoids and other risk factors with CCA-IMT were estimated with Pearson correlation coefficients. The heterogeneity of the means of baseline variables between the quartiles of total flavonoid intake was tested by using ANOVA and frequency distribution of the categorical variables between quartiles of total flavonoid intake was compared by the χ^2 test. Baseline risk factors used as covariates in the ANOVA included age and technical covariates (examination years and baseline zooming depth given separately for right and left side), history of atherosclerosis, smoking, BMI, diabetes, systolic blood pressure, serum HDL- and LDL- cholesterol, V_{O_2} max, and intakes of alcohol, SFA (% energy), and energy adjusted intakes of folate, vitamin C and E. All statistical tests were two-tailed. Data were analysed using SPSS for Windows version 11.5 statistical software (SPSS Inc., Chicago, IL, USA).

Results

The mean intake of flavonoids was 128·5 (SD 206·7) mg/d and each subclass contributed to the total intake as follows: flavan-3-ols 84 % (107·7 mg/d); flavonols 7 % (9·1 mg/d); anthocyanidins 6 % (7·5 mg/d); flavanones 3 % (3·9 mg/d); and flavones < 1 % (0·3 mg/d). The mean CCA-IMT at baseline was 0·78 (SD 0·17) mm. Men who consumed more flavonoids had lower CCA-IMT thickness, were less likely to be a smoker, had lower intakes of alcohol, total fat, and SFA, but had higher intakes of folate, fibre, and vitamins C and E (Table 1).

In the lowest quartile of total flavonoid intake (< 12·5 mg/d) the mean CCA-IMT was 0·79 (SD 0·19) mm, while the mean CCA-IMT was 0·76 (SD 0·15) in the highest quartile of intake (> 166·3 mg/d;Table 2). In the covariance analysis after statistical adjustment as described in Methods and materials, total flavonoid intake was inversely associated with the mean CCA-IMT (P=0·018). Out of five subclasses, flavan-3-ols were significantly inversely associated with CCA-IMT (P=0·025) after identical statistical adjustment. There was also a trend for an inverse association between intake of flavonols and mean CCA-IMT (P=0·055, after statistical adjustment). Other subclasses of flavonoids; flavones (P=0·505), flavanones (P=0·875) and anthocyanidins (P=0·577) were not associated with the mean CCA-IMT. The cut-off points for flavonoid quartiles and linear trends across these quartiles are presented in Table 2.

Discussion

The aim of our study was to study the role of the most commonly consumed flavonoids in carotid atherosclerosis in middle-aged Finnish men in a cross-sectional study. The main finding of our study was that the high total intake of flavonoids, a sum of twenty-six compounds, was associated with decreased mean CCA-IMT. In a further analysis, out of five flavonoid subclasses significant inverse association was found for flavan-3-ols and a non-significant trend for flavonols. The associations found were strong and were not attenuated by extensive adjustment for IMT and CVD risk factors. For the other subclasses flavones, flavanones and anthocyanidins, no associations were found.

The main drawback of our study was the cross-sectional setting which does not enable evaluation of temporality. In addition, in the KIHD the dietary intake of flavonoids was assessed using 4-d food recording before the study visits. The intake of flavonoids may vary between different seasons, being highest in summer when the consumption of vegetables is highest. Seasonal variation may have caused some misclassification of subjects and may have caused underestimation in the relation between flavonoid intake and CCA-IMT.

The lack of association for three subclasses is most likely explained by the fact that altogether the intake of those three subclasses was < 10 % of the total intake and thus the association could be too weak to be detected. On the other hand, flavonoids differ in chemical structure and properties thus in theory different compounds could have different effects on human health[3].

IMT has been shown to be an independent predictor of CVD[26,27]. To our knowledge the role of flavonoids in CCA-IMT has not been previously studied, but in general, our results support the previous findings suggesting that high intake of flavonoids may decrease the risk of CVD. The role

Table 1. Characteristics of the 1380 study subjects and according to the quartiles of energy-adjusted flavonoid intake
(Mean values with standard deviations)

			Quartiles of flavonoid intake (mg/d)								
	Total population		1 (lowest)		2		3		4 (highest)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P*
Flavonoid intake (not energy adjusted;mg/d)	128·5	207·7	3·6	1·8	18·2	7·2	85·9	37·5	406·4	253·7	< 0·001
Flavonoid intake (energy adjusted;mg/d)	128·5	206·7	0·0	16·4	26·4	8·8	89·8	35·6	404·9	250·3	< 0·001
Age (years)	52·4	6·4	51·6	6·3	53·6	6·3	52·4	6·3	52·0	6·6	< 0·001
BMI (kg/m^2)	26·6	3·5	26·5	3·7	26·7	3·6	26·8	3·4	26·6	3·5	0·739
Systolic blood pressure (mmHg)	132	17	132	16	133	17	131	17	133	16	0·426
Serum LDL cholesterol (mmol/l)	3·94	0·96	3·98	0·90	4·00	1·06	3·80	1·03	3·97	0·84	0·020
Serum HDL cholesterol (mmol/l)	1·29	0·30	1·31	0·28	1·29	0·32	1·30	0·30	1·27	0·29	0·291
Serum TAG (mmol/l)	1·42	0·86	1·33	0·73	1·46	0·94	1·47	0·91	1·44	0·84	0·097
Maximal oxygen uptake (ml/kgper min)	30·6	7·4	31·5	7·3	29·6	7·2	30·9	7·4	30·5	7·4	0·006
Smokers (%)	39·6		52·5		44·1		35·4		26·7		< 0·001
Nutrient intake†											
Total fat (g/d)	99·2	17·2	101·7	21·4	101·4	15·8	96·5	15·2	97·4	14·9	< 0·001
SFA (g/d)	45·2	11·5	46·6	14·3	46·9	10·5	43·8	10·0	43·7	10·3	< 0·001
Alcohol (g/d)	11·8	20·2	14·0	26·9	11·3	18·6	11·8	16·7	10·1	16·6	0·087
Folate (μg/d)	256	61	234	59	247	54	269	61	276	62	< 0·001
Fibre (g/d)	25·4	8·9	24·0	9·6	25·0	6·8	26·5	7·7	26·2	7·4	< 0·001
Vitamin C (mg/d)	71·2	51·0	51·8	34·3	65·8	45·1	86·4	55·3	80·9	58·5	< 0·001
Vitamin E (mg/d)	9·2	2·5	8·8	2·8	8·9	2·4	9·4	2·3	9·5	2·6	< 0·001
CCA-IMT (mm)	0·78	0·17	0·79	0·19	0·81	0·18	0·77	0·16	0·76	0·15	< 0·001

CCA-IMT, common carotid artery intima-media thickness.
*P value from ANOVA (continuous variables) or P value from χ^2 test (discrete variables).
† Intakes of nutrients are energy adjusted.

Table 2. Common carotid artery intima-media thickness (CCA-IMT) of the 1380 study subjects according to the energy-adjusted quartiles of flavonoid intake

(Mean values with standard deviations)

	Quartiles of flavonoid intake (mg/d)								
	1 (lowest)		2		3		4 (highest)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P**
Total flavonoid intake (mg/d)†	<12·5		12·5–43·7		>43·7–166·3		>166·3		
CCA-IMT (mm)	0·79	0·19	0·81	0·18	0·77	0·16	0·76	0·15	0·018
Flavonol intake (mg/d)	<3·7		3·7–6·6		>6·6–11·9		>11·9		
CCA-IMT (mm)	0·81	0·20	0·79	0·17	0·77	0·16	0·76	0·15	0·055
Flavone intake (mg/d)	0·0		>0·0–0·1		>0·1–0·3		>0·3		
CCA-IMT (mm)	0·76	0·17	0·80	0·19	0·79	0·17	0·77	0·16	0·505
Flavanones intake (mg/d)	0·0		>0·0–0·1		>0·1–0·5		>0·5		
CCA-IMT (mm)	0·76	0·17	0·79	0·17	0·80	0·19	0·77	0·16	0·875
Flavan-3-ol intake (mg/d)	<0·2		0·2–20·3		>20·3–140·6		>140·6		
CCA-IMT (mm)	0·78	0·17	0·81	0·19	0·78	0·17	0·76	0·15	0·025
Anthocyanidin intake (mg/d)	0·0		>0·0–1·3		>1·3–5·6		>5·6		
CCA-IMT (mm)	0·78	0·17	0·78	0·17	0·79	0·17	0·78	0·18	0·577

* Adjusted *P* value from ANOVA. Adjusted for age, examination years, baseline zooming depth given separately for right and left side, history of atherosclerosis, smoking, BMI, diabetes, systolic blood pressure, serum HDL- and LDL-cholesterol, V_{O_2}max, and intakes of alcohol, SFA (% energy), and energy adjusted intakes of folate, vitamins C and E.

† Intakes of flavonoids are energy adjusted.

of flavonoids has been evaluated in several prospective cohort studies and half of these studies have found flavonoids to be associated with significantly decreased risk of CVD[4–17,19]. In a meta-analysis based on the data from seven cohorts, the high intake of flavonol subclass was found to be associated with modest 20 % decreased CHD mortality[28].

The studies assessing the role of flavonoids in CVD have, however, included mainly only two subclasses, flavonols and flavones, and the role of other subclasses has been studied much less, or not at all. It has been estimated that out of eleven to twenty-six identified subclasses of flavonoids at least five subclasses (flavonols, flavones, flavanones, flavan-3-ols and anthocyanidins), a total of twenty to thirty individual compounds, may contribute significantly to the daily intake and thus also to CVD health[18]. Only a few cohort studies have assessed the role of flavanones[15,19] flavan-3-ols[8,9,19] or anthocyanidins19 in CVD. Recent study evaluated the role of seven subclasses[19] and found flavanones, anthocyanidins, and flavonoid-rich food to be associated with total, CHD, and CVD mortality.

The mechanism(s) by which flavonoids decrease the risk of CVD was not assessed in this study, but flavonoids have several properties which may provide protection against CVD. First, flavonoids and flavonoid-rich foods have been reported to improve endothelial function probably by increasing NO production[29,30]. Second, flavonoids possess strong antioxidant properties *in vitro* and in addition in animal studies flavonoids have decreased oxidative stress and inhibited the progression of atherosclerosis[31,32]. The evidence, however, *in vivo* is conflicting and the effects of oxidative stress in human subjects still remains under debate[33]. In addition, some studies have suggested that flavonoids may have beneficial effects, for example on blood pressure[29], platelet function and inflammation34.

Alternatively, it has been suggested that the high intake of flavonoids could merely be an overall marker of healthy lifestyle rather than a causative factor. In our study subjects with high intake were less likely to smoke, had lower intakes of total fat, and SFA, but higher intakes folate, fibre, and vitamins C and E. Similar finding have also been reported in previous epidemiological studies, and therefore the possibility that the protection is at least partly the result of residual confounding cannot be ruled out.

The total intake of flavonoids (129 mg/d) was much higher than reported previously for a Finnish population (from 8·0 to 24·2 mg/d)[7,12,13] and was mainly because the intake of flavan-3-ols has not been included in the previous studies. In our study flavan-3-ols were the main contributor (almost 90 %) to the daily intake, while the intake of other flavonoids was relatively small. Because of limitations in our computer software used to calculate the intakes of nutrients, we were not able to calculate the food sources of flavonoids, but black tea was most likely the main source of flavan-3-ols. Even though the intake was very high compared with previous calculations, the actual intake of some of the subclasses is probably still higher.

Further studies are still needed to evaluate the role of flavonoids in CVD, especially in strokes. In addition to flavonoids, other phenolic compounds such as simple phenols may also play a role in human health. It has been estimated that simple compounds may account for as much as one-third of the total daily intake of phenolic compounds35 and therefore, the databases should be updated concerning simple phenolics.

We conclude that in our cross-sectional study the high intake flavonoids is associated with decreased carotid atherosclerosis in a population-based sample of middle-aged Finnish men.

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