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Serum amyloid A-related inflammation is lowered by increased fruit and vegetable intake, while high-sensitive C-reactive protein, IL-6 and E-selectin remain unresponsive

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Abstract

The present study assessed whether increased fruit and vegetable (F&V) intake reduced the concentrations of the inflammatory marker serum amyloid A (SAA) in serum, HDL_2 and HDL_3 and whether the latter reduction influenced any of the functional properties of these HDL subfractions. The present study utilised samples from two previous studies: (1) the FAVRIT (Fruit and Vegetable Randomised Intervention Trial) study – hypertensive subjects (systolic blood pressure (BP) range 140–190 mmHg; diastolic BP range 90–110 mmHg) were randomised to receive a 1-, 3- or 6-portion F&V/d intervention for 8 weeks, and (2) the ADIT (Ageing and Dietary Intervention Trial) study – older subjects (65–85 years) were randomised to receive a 2- or 5-portion F&V/d intervention for 16 weeks. HDL_2 and HDL_3 were isolated by rapid ultracentrifugation. Measurements included the following: serum high-sensitive C-reactive protein (hsCRP) by an immunoturbidimetric assay; serum IL-6 and E-selectin and serum-, HDL_2- and HDL_3-SAA by ELISA procedures; serum-, HDL_2- and HDL_3-cholesterol ester transfer protein (CETP) activity by a fluorometric assay. Although the concentrations of hsCRP, IL-6 and E-selectin were unaffected by increasing F&V intake in both studies ($P>0{\cdot}05$ for all comparisons), those of SAA in HDL_3 decreased in the FAVRIT cohort ($P=0{\cdot}049$) and those in HDL_2 and HDL_3 decreased in the ADIT cohort ($P=0{\cdot}035$ and $0{\cdot}032$), which was accompanied by a decrease in the activity of CETP in HDL_3 in the FAVRIT cohort ($P=0{\cdot}010$) and in HDL_2 in the ADIT cohort ($P=0{\cdot}030$). These results indicate that SAA responds to increased F&V intake, while other inflammatory markers remain unresponsive, and this leads to changes in HDL_2 and HDL_3, which may influence their antiatherogenic potential. Overall, the present study provides tangible evidence of the effectiveness of increased F&V intake, which may be of use to health policy makers and the general public.

Key words: Fruit and vegetables: Serum amyloid A: Inflammation: HDL

CVD is currently the leading cause of illness and death in developed countries, and over the past decade, it has become apparent that chronic inflammation plays a major role in its development[1,2]. C-reactive protein (CRP) is a commonly measured marker of cardiovascular risk, and elevated concentrations are associated with CVD, in both cross-sectional[3] and many longitudinal studies[4–6]. However, a direct causal link between CRP and the development of CVD has not been identified. On the other hand, serum amyloid A (SAA), another marker of inflammation, is produced acutely by the liver and chronically by hypertrophic adipocytes and its expression is regulated by inflammation-associated cytokines and activated monocytes and macrophages[7–10], and it may have a causal role in the development of CVD. This concept is based on the fact that one of the major proatherogenic properties of SAA is its rapid association with HDL, especially with HDL_3, in the circulation, which results in the production of dysfunctional HDL[11]. Normally, the major antiatherogenic role of HDL is its involvement in reverse cholesterol transport. However, when HDL is associated with SAA, this and other antiatherogenic properties may be attenuated or lost[12,13]. Dysfunctional HDL binds to proteoglycans on the vascular wall, favouring their retention and subsequent modification by the vascular matrix, which has an important role in the formation of macrophage foam cells. The presence of SAA within HDL

Abbreviations: ADIT, Ageing and Dietary Intervention Trial; CETP, cholesteryl ester transfer protein; CRP, C-reactive protein; F&V, fruit and vegetables; FAVRIT, Fruit and Vegetable Randomised Intervention Trial; hsCRP, high-sensitive C-reactive protein; SAA, serum amyloid A.

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fractions also decreases the efflux of cholesterol from lipid-laden macrophages[14]. In addition, HDL-associated enzymes may be influenced by the presence of SAA. For example, the activity of cholesteryl ester transfer protein (CETP) may be increased[15], which leads to altered lipoprotein remodelling[16] and may contribute to an atherogenic phenotype. In support of this, we have found that increased lycopene intake leads to a reduction in the concentrations of SAA and a concomitant decrease in the activity of CETP[17].

Therefore, considering the above-mentioned findings, it is likely that factors that reduce systemic concentrations of SAA and, therefore, its association with HDL may reduce the burden of CVD risk. One such factor that may mediate this response is increased fruit and vegetable (F&V) intake. Meta-analyses of prospective cohort studies have suggested an association between increased F&V intake and reduced CVD risk[18] and stroke[19]. However, although these observational studies have demonstrated a significant inverse relationship between high F&V intake and CVD risk, no biological marker that responds to increased F&V intake has been identified. For example, intervention studies examining the inflammatory molecule CRP have reported conflicting results[20–22], while observational studies have rarely shown an association between increased F&V intake and reduced CRP concentrations after adjustment for possible confounding factors[23–25]. In fact, both the FAVRIT (Fruit and Vegetable Randomised Intervention Trial) and ADIT (Ageing and Dietary Intervention Trial) studies have found that CRP does not respond to increased F&V intake[26–28]. However, we suggest that SAA may respond to inflammatory changes brought about by increased F&V intake, based on the concept that SAA has been documented as a more sensitive marker of inflammatory changes than CRP[29,30] and on the fact that we have found that SAA responds to increased intake of foods rich in lycopene, while CRP is unresponsive[17].

Therefore, the present study was carried out to investigate whether (1) SAA responded to increased F&V intake, while other inflammatory markers were unresponsive, and (2) increased F&V intake lowered HDL-associated inflammation and thereby influenced the antiatherogenic properties of HDL. This was achieved by utilising samples from two previous F&V intervention studies[26–28] and was not part of the original analyses.

Materials and methods

Study groups

The present study was carried out using samples from two previous Food Standards Agency-funded studies. The first study examined the effect of increased F&V intake in a group of subjects with mild hypertension (the FAVRIT study). The second study examined the effect of increased F&V intake in an older population (the ADIT study).

Fruit and Vegetable Randomised Intervention Trial study design. Details of the FAVRIT study have been published previously[26,27]. In brief, 112 participants aged between 40 and 65 years, with brachial blood pressure in the range of 140–190 mmHg (systolic) and 90–110 mmHg (diastolic), were recruited from medical outpatient clinics and through local press release. Exclusion criteria were diabetes mellitus, an acute coronary ischaemic attack within the past 3 months, dietary requirements, food sensitivities or vegetarian/vegan diet by choice, oral anticoagulation therapy, BMI $>35\,kg/m^2$, excessive alcohol consumption (defined as >221 g/week in men and 166 g/week in women), fasting TAG concentration >4 mmol/l, or pregnancy/lactation. Suitable participants gave written informed consent and were put on a 4-week washout phase, during which F&V consumption was limited to 1 portion/d. After the washout phase, the participants were randomised to one of three groups, consuming 1, 3 or 6 portions of F&V daily for 8 weeks. They were asked to maintain other aspects of their lifestyle. Fasting blood samples were collected before and after the intervention period and were separated appropriately for the proposed assays and stored at −75°C until analysis. This study was approved by the Research Ethics Committee of Queen's University Belfast.

Ageing and Dietary Intervention Trial study design. Details of the ADIT study have been published elsewhere[28]. In brief, eighty-two free-living, healthy older participants (aged 65–85 years) with low F&V intake (≤2 portions/d) were recruited. Exclusion criteria were consumption of special diets, use of nutritional supplements or medications known to affect the variables being assessed, excessive alcohol consumption (>221 g/week in men or >166 g/week in women), BMI $>35\,kg/m^2$, history of diabetes or dementia, inability to provide informed consent, any other problems that would prevent adherence to a high-F&V diet, or a recent infection (<3 weeks since the completion of any antibiotic course or symptoms of viral illness). Following acquisition of written informed consent, the participants were randomised to one of two arms – either to increase F&V consumption to at least 5 portions/d or to follow their normal diet (therefore consuming ≤2 portions/d) for 16 weeks. They were asked to maintain other aspects of their lifestyle. Fasting blood samples were collected at baseline and week 16, separated appropriately for the proposed assays and stored at −75°C until analysis. A total of eighty subjects were included in the final analysis: thirty-nine in the ≤2 portions/d group and forty-one in the 5 portions/d group.

The study was approved by the Office for Research Ethics Committees Northern Ireland (ORECNI) and was registered in ClinicalTrials.gov (no. NCT00858728).

Specific dietary advice was given to all participants of the FAVRIT and ADIT studies helping ensure a similar energy and macronutrient intake from their normal diet, while weekly contact encouraged compliance. Compliance was monitored using diet history, interview and laboratory assessment of micronutrient status (data not shown).

Serum analysis

Measurement of serum carotenoid concentrations. The serum concentrations of carotenoids were measured by HPLC, as described by Craft[31].

Measurement of high-sensitive C-reactive protein, IL-6 and E-selectin concentrations. The concentrations of high-sensitive C-reactive protein (hsCRP) were measured in the primary analyses[27,28] by an immunoturbidimetric assay (Randox), using an ILab-600 biochemical analyser (Instrumentation Laboratories), and their values are reported in comparison with SAA values. The serum concentrations of IL-6 and E-selectin were measured using ELISA procedures, as per the manufacturer's instructions (product no.: HS600B and DSLE00; Randox).

Isolation of HDL_2 and HDL_3 from serum. HDL_2 and HDL_3 were isolated from serum by rapid ultracentrifugation, according to the method of McPherson *et al.*[32]. This method is a three-step procedure: crude HDL was isolated by 2 h rapid ultracentrifugation, which allows crude HDL to sediment at the bottom of the ultracentrifuge tube. This crude HDL was then subfractionated into HDL_2 and HDL_3 by two 2 h sequential rapid flotation ultracentrifugation procedures, with total isolation time being 6 h. HDL_2 and HDL_3 were stored at −75°C until the analyses described below were carried out. HDL subfractions are stable when frozen at −75°C for up to 1 year following their isolation from serum (data not shown).

Serum, HDL_2 and HDL_3 analyses

Determination of total protein concentration. The concentration of protein in HDL_2 and HDL_3 was determined spectrophotometrically, as described by McEneny *et al.*[33].

Determination of serum amyloid A concentrations. The concentrations of SAA in serum, HDL_2 and HDL_3 were determined using an ELISA procedure (KHA0011; Invitrogen Life Technologies), as per the manufacturer's instructions. This commercially available ELISA recognises the SAA isoforms 1 and 2. The concentrations of serum-SAA, HDL_2-SAA and HDL_3-SAA are expressed as μg/l.

Measurement of cholesteryl ester transfer protein activity. The activity of CETP in serum, HDL_2 and HDL_3 was measured using a commercially available fluorometric assay, as per the manufacturer's instructions (RB-CETP; Roar Biomedical, Inc.). The activity of CETP was compared with that of a known concentration of CETP; therefore, it is expressed as μmol/l in serum and as μmol/mg protein in HDL_2 and HDL_3. The values of CETP were standardised to total protein concentration in HDL_2 and HDL_3 to obtain an estimation of the activity of this enzyme within an individual HDL particle.

Statistical analyses

Normally distributed continuous variables are summarised as means and standard deviations. Skewed variables were logarithmically transformed for parametric analysis, and these are summarised as geometric means and interquartile ranges.

Between-group comparisons of change in each outcome variable were made using one-way ANOVA for the FAVRIT study. Because the intervention involved increasing numbers of portions of F&V, a test for linear trend across the groups was used. Between-group comparisons of change in each outcome variable were made using independent-samples *t* tests for the ADIT study. Within-group analyses were conducted using paired-samples *t* tests for both the FAVRIT and ADIT studies. Associations between outcome variables were tested using Pearson's correlation coefficients. All tests were two-tailed, and a P value $<0{\cdot}05$ was considered statistically significant. The analyses were carried out using the software SPSS (version 17.0.1; SPSS, Inc.).

Results

Subject characteristics

The baseline characteristics of the FAVRIT cohort have been described previously[26,27]; however, in brief, the following baseline characteristics were similar among the groups randomised to receive a 1-, 3- or 6-portion F&V/d intervention: age (52·4 (SD 7·9) *v.* 56·1 (SD 8·4) *v.* 53·7 (SD 7·1) years); BMI (29·7 (SD 4·4) *v.* 28·2 (SD 3·2) *v.* 28·8 (SD 3·3) kg/m^2); blood pressure (systolic: 139·4 (SD 15·0) *v.* 144·6 (SD 18·1) *v.* 145·3 (SD 15·7) mmHg; diastolic: 82·0 (SD 11·9) *v.* 81·1 (SD 11·1) *v.* 86·3 (SD 11·0) mmHg); antihypertensive medication use; lipid-lowering therapy ($P>0{\cdot}05$ for all comparisons). Following intervention and assessment by dietary recall, F&V intake was found to increase across the three groups (pre *v.* post: 0·9–1·1, 1·1–3·2 and 1·1–5·6 portions/d in the 1-, 3- and 6-portion groups, respectively; $P<0{\cdot}001$ for linear trend), which was accompanied by an increase in serum lutein ($P<0{\cdot}05$) and β-cryptoxanthin ($P<0{\cdot}001$) concentrations, while the increase in zeaxanthin and vitamin C concentrations approached significance ($P=0{\cdot}09$ and 0·06, respectively). In addition, BMI remained unaltered following the 1-, 3- and 6-portion F&V/d interventions ($P>0{\cdot}05$ for all comparisons). All the above results have been reported in detail in McCall *et al.*[26,27].

The baseline characteristics of the ADIT cohort have also been described previously[28]; however, in brief, the following baseline characteristics were similar between the 2- and 5-portion groups : age (71·1 (SD 5·0) *v.* 70·9 (SD 5·0) years); BMI (28·1 (SD 4·5) *v.* 28·5 (SD 10·9) kg/m^2); blood pressure (systolic: 150·5 (SD 24·4) *v.* 152·9 (SD 20·9) mmHg; diastolic: 84·1 (SD 10·9) *v.* 87·0 (SD 10·9) mmHg); antihypertensive medication use; lipid-lowering therapy ($P>0{\cdot}05$ for all comparisons). Following intervention and assessment by dietary recall, F&V intake was found to increase across the two groups (from 1·4 to 1·8 and 1·4 to 6·0 portions/d in the 2- and 5-portion groups, respectively; $P<0{\cdot}001$), which was accompanied by an increase in serum lutein ($P<0{\cdot}05$), β-cryptoxanthin ($P<0{\cdot}01$), lycopene ($P<0{\cdot}05$), zeaxanthin ($P<0{\cdot}001$) and vitamin C ($P<0{\cdot}001$) concentrations. In addition, BMI remained unaltered following the 2- and 5-portion F&V/d interventions ($P>0{\cdot}05$ for both comparisons). All the above results have been reported in detail in Gibson *et al.*[28].

Serum analysis

High-sensitive C-reactive protein, IL-6 and E-selectin concentrations. The concentrations of hsCRP in the FAVRIT and ADIT cohorts have been reported previously[27,28]; however,

Table 1. Pre- and post-fruit and vegetable (F&V) intervention serum amyloid A (SAA) concentrations in the FAVRIT (Fruit and Vegetable Randomised Intervention Trial) study

(Geometric mean values and interquartile ranges (IQR))

	Pre (week 0)		Post (week 8)			
SAA (μg/l)	Geometric mean	IQR	Geometric mean	IQR	*P**	*P*†
1 portion F&V/d (*n* 33)						
Serum	14 001	7175–21 824	14 986	7914–22 213	0·221	0·070
HDL_2	851	319–1492	937	362–1494	0·226	0·130
HDL_3	10 642	10 204–10 772	10 769	10 163–11 135	0·156	0·049
3 portions F&V/d (*n* 39)						
Serum	13 005	7036–32 112	12 304	6995–40 250	0·918	
HDL_2	933	296–1386	705	204–1047	0·101	
HDL_3	10 799	10 238–11 247	10 579	10 236–10 856	0·068	
6 portions F&V/d (*n* 40)						
Serum	13 873	3940–26 301	10 309	4455–27 528	0·088	
HDL_2	995	288–1220	628	259–933	0·038	
HDL_3	10 689	10 231–10 847	10 428	10 181–10 593	0·041	

* *P* value within the groups pre- *v.* post-intervention.
† *P* value for trend across the groups.

the concentrations of hsCRP, IL-6 and E-selectin were unaffected by increasing F&V intake in both studies ($P>0·05$ for all comparisons; data not shown).

Serum, HDL_2 and HDL_3 analyses

Serum amyloid A concentrations in the Fruit and Vegetable Randomised Intervention Trial. Between-group analyses showed that although the concentrations of serum-SAA and HDL_2-SAA decreased as F&V intake increased, the decrease was not significant ($P=0·070$ and 0·130, respectively, for linear trend), while those of HDL_3-SAA decreased significantly as F&V intake increased ($P<0·05$ for linear trend) (Table 1). Within-group analyses showed that the concentrations of serum-SAA, HDL_2-SAA and HDL_3-SAA were unaffected by the 1-portion F&V/d intervention ($P>0·05$ for all comparisons), and a similar trend was observed for the concentrations of serum-SAA and HDL_2-SAA following the 3-portion F&V/d intervention ($P>0·05$ for both comparisons), although those of HDL_3-SAA appeared to decrease, which was unfortunately not significant ($P=0·068$). However, following the 6-portion F&V/d intervention, the concentrations of SAA in serum, HDL_2 and HDL_3 decreased; although this decrease was not significant in serum, it was significant in HDL_2 and HDL_3 ($P=0·088$, 0·038 and 0·041, respectively).

Serum amyloid A concentrations in the Ageing and Dietary Intervention Trial. Between-group analyses of the 2- *v.* 5-portion groups showed that as F&V intake increased, the concentrations of SAA in serum were unaffected ($P>0·050$), while those in HDL_2 and HDL_3 decreased ($P=0·035$ and 0·032, respectively) (Table 2). In addition, within-group analyses showed that the concentrations of serum-SAA, HDL_2-SAA and HDL_3-SAA were unaffected by the 2-portion F&V/d intervention ($P>0·05$ for all comparisons). However, following the 5-portion F&V/d intervention, the concentrations of SAA in serum tended to decrease ($P=0·05$), but decreased significantly in HDL_2 and HDL_3 ($P=0·001$ and 0·040, respectively).

Cholesteryl ester transfer protein activity in the Fruit and Vegetable Randomised Intervention Trial. Between-group analyses showed that the activity of CETP in HDL_3 decreased as F&V intake increased ($P<0·050$ for linear trend) (Table 3). In addition, within-group analyses showed that following the 1- and 3-portion F&V/d interventions, the activity of CETP in serum, HDL_2 and HDL_3 remained unaltered. However, following the 6-portion F&V/d intervention, although the activity of CETP was unaffected in serum ($P>0·050$), it decreased in

Table 2. Pre- and post-fruit and vegetable (F&V) intervention serum amyloid A (SAA) concentrations in the ADIT (Ageing and Dietary Intervention Trial) study

(Geometric mean values and interquartile ranges (IQR))

	Pre (week 0)		Post (week 16)			
SAA (μg/l)	Geometric mean	IQR	Geometric mean	IQR	*P**	*P*†
2 portions F&V/d (*n* 39)						
Serum	17 098	11 094–34 229	16 350	8047–32 345	0·411	0·310
HDL_2	1732	656–1790	1794	625–2662	0·815	0·035
HDL_3	17 505	9138–26 216	17 333	9510–25 592	0·914	0·032
5 portions F&V/d (*n* 41)						
Serum	18 907	11 312–37 556	14 698	7321–33 415	0·050	
HDL_2	1863	744–2564	1333	490–1829	0·001	
HDL_3	17 784	6067–26 244	12 727	4247–20 825	0·040	

* *P* value within the groups pre- *v.* post-intervention.
† *P* value for difference between the 2- and 5-portion groups.

Table 3. Pre- and post-fruit and vegetable (F&V) intervention cholesteryl ester transfer protein (CETP) activity in the FAVRIT (Fruit and Vegetable Randomised Intervention Trial) study
(Mean values and standard deviations; geometric mean values and interquartile ranges (IQR))

	Pre (week 0)		Post (week 8)			
CETP	Mean	SD	Mean	SD	*P**	*P*†
1 portion F&V/d (*n* 33)						
Serum (μmol/l)	288	50	281	48	0·866	0·530
HDL_2 (μmol/mg protein)					0·784	0·140
Geometric mean	1·61		1·54			
IQR	1·10–2·41		1·02–2·55			
HDL_3 (μmol/mg protein)	0·065	0·012	0·066	0·009	0·205	0·010
3 portions F&V/d (*n* 39)						
Serum (μmol/l)	299	54	290	58	0·330	
HDL_2 (μmol/mg protein)					0·443	
Geometric mean	1·66		1·78			
IQR	0·93–2·30		1·19–2·33			
HDL_3 (μmol/mg protein)	0·068	0·012	0·069	0·014	0·804	
6 portions F&V/d (*n* 40)						
Serum (μmol/l)	293	56	290	55	0·274	
HDL_2 (μmol/mg protein)					0·052	
Geometric mean	1·79		1·53			
IQR	1·05–2·99		1·21–2·21			
HDL_3 (μmol/mg protein)	0·077	0·017	0·070	0·010	0·015	

* *P* value within the groups pre- *v.* post-intervention.
† *P* value for trend across the groups.

HDL_2 and HDL_3; although this was not significant in HDL_2 ($P=0·052$), it was significant in HDL_3 ($P<0·050$).

Cholesteryl ester transfer protein activity in the Ageing and Dietary Intervention Trial. Between-group analyses of the 2- *v.* 5-portion groups showed that the activity of CETP in serum ($P>0·050$) and HDL_3 ($P>0·050$) was unaffected (Table 4). However, the activity of CETP in HDL_2 decreased ($P>0·050$). Within-group analyses showed that the activity of CETP in serum, HDL_2 and HDL_3 was unaffected following the 2-portion F&V/d intervention ($P>0·05$ for all comparisons). However, following the 5-portion F&V/d intervention, the activity of CETP decreased in HDL_2 ($P<0·001$), but was unaffected in serum and HDL_3 ($P>0·050$ for both comparisons).

Correlations between self-reported changes in fruit and vegetable intake, serum antioxidants and serum and HDL_2 and HDL_3 analyses

Only a few significant correlations were found between the findings from the secondary analysis carried out in the present study and those from the original studies[26–28] were that:

In the FAVRIT cohort, changes in self-reported F&V intake were negatively correlated with changes in HDL_3-CETP activity ($r -0·281$, $P=0·016$), while changes in serum vitamin C concentrations were negatively correlated with changes in HDL_3-SAA concentrations ($r -0·290$, $P=0·013$).

In the ADIT cohort, changes in self-reported F&V intake were negatively correlated with changes in HDL_3-SAA concentrations ($r -0·383$, $P=0·001$) and serum- and HDL_2-CETP activity ($r -0·228$, $P=0·047$; $r -0·252$, $P=0·035$, respectively). In addition, changes in serum zeaxanthin and β-cryptoxanthin concentrations were negatively correlated with changes in HDL_2-SAA concentrations ($r -0·257$, $P=0·037$; $r -0·270$, $P=0·024$, respectively), while changes in serum lycopene concentrations were negatively correlated with changes in HDL_3-SAA concentrations ($r -0·320$, $P=0·008$).

Discussion

The present study was designed based on the knowledge that F&V consumption has been found to be beneficial to CVD health in observational epidemiological studies. However, direct trial evidence of the effect of F&V consumption on a biological marker that is related to changes in CVD health is limited. Although the ability of increased F&V intake to influence CRP concentrations has been widely studied, its usefulness as a marker of inflammatory changes is disputed[34]. On the other hand, SAA may have a causal role in the development of CVD[35], which we suggest may be due to its association with HDL, as this reduces the antiatherogenic capabilities of this lipoprotein[11].

Fruit and vegetable intervention and serum-, HDL_2- and HDL_3-serum amyloid A concentrations

In the primary analyses of the FAVRIT and ADIT cohorts, subject compliance was confirmed by food diaries and by appropriate changes in serum antioxidants, relative to F&V interventions[27,28]. However, these primary analyses were unable to detect any changes in hsCRP concentrations following increased F&V intake[27,28]. Therefore, the present study investigated whether a change in F&V intake was accompanied by changes in SAA concentrations, especially as SAA may be a more sensitive marker of inflammatory changes than other

Table 4. Pre- and post-fruit and vegetable (F&V) intervention cholesteryl ester transfer protein (CETP) activity in the ADIT (Ageing and Dietary Intervention Trial) study

(Mean values and standard deviations; geometric mean values and interquartile ranges (IQR))

	Pre (week 0)		Post (week 16)			
CETP	Geometric mean	IQR	Geometric mean	IQR	*P**	*P*†
2 portions F&V/d (*n* 39)						
Serum (μmol/l)					0·10	0·12
Mean	466		484			
SD	175		193			
HDL_2 (μmol/mg protein)	1·13	0·74–1·62	1·12	0·73–1·93	0·84	0·03
HDL_3 (μmol/mg protein)	0·080	0·059–0·107	0·082	0·063–0·106	0·52	0·40
5 portions F&V/d (*n* 41)						
Serum (μmol/l)					0·64	
Mean	511		514			
SD	159		152			
HDL_2 (μmol/mg protein)	1·19	0·77–1·78	0·99	0·66–1·52	0·001	
HDL_3 (μmol/mg protein)	0·073	0·060–0·086	0·069	0·056–0·085	0·56	

* *P* value within the groups pre- *v.* post-intervention.
† *P* value for difference between the 2- and 5-portion groups.

acute-phase reactants[29,30] and also responds to changes brought about by diet[17,25]. However, within the context of a F&V intervention trial, changes in SAA concentrations have not been examined. Subsequently, the results of the present study demonstrate for the first time that SAA does respond to increased F&V intake in hypertensive and elderly populations. This was particularly apparent in the 16-week ADIT study, where both self-reported F&V intake and changes in serum lycopene concentrations were negatively correlated with changes in HDL_3-SAA concentrations (r −0·383, P=0·001; r −0·320, P=0·008, respectively), and the effect observed on lycopene concentrations confirms our previous findings[17]. Furthermore, the concentrations of both zeaxanthin and β-cryptoxanthin were negatively correlated with HDL_2-SAA concentrations (r −0·257, P=0·037; r −0·270, P=0·024, respectively). In addition, the between-group analyses showed that SAA concentrations in HDL_2 and HDL_3 decreased as F&V intake increased (P=0·035 and 0·032, respectively). In the 8-week FAVRIT study, the only significant finding related to SAA was a decrease in HDL_3-SAA concentrations observed in the between-group analyses (P=0·049), which was also negatively correlated with changes in serum vitamin C concentrations. Overall, we suggest that the small disparity between the two studies may be due to (1) study duration, i.e. 8 weeks' duration of the FAVRIT study may be an insufficient time frame to fully detect the effects of an increase in F&V intake, compared with the 16 weeks' duration of the ADIT study; (2) the age difference between the cohorts of both studies, especially as the older subjects in the ADIT study had higher baseline SAA concentrations in serum, HDL_2 and HDL_3 than their younger counterparts in the FAVRIT study (although this was not examined statistically); this indicates that the older ADIT subjects may have had a greater capacity to respond to increased F&V intake; or (3) an increase in the average intake of F&V to 6 portions/d in the ADIT cohort and of 5·6 portions/d in the FAVRIT cohort, which may also have influenced the results (although this was not examined statistically). However, these suggestions need to be investigated further.

The beneficial effects exerted by increased F&V intake on SAA, but not on the other markers assessed, namely hsCRP, IL-6 and E-selectin, may be explained by the fact that SAA, as well as being expressed by the liver[36], is also expressed in and released from hypertrophic adipocytes[37,38]. This may be particularly relevant, as both cohorts were, on average, overweight, verging on obese[26,28], and would have higher levels of hypertrophic adipocytes. In addition, these cells, as well as being responsible for the chronic release of SAA, are one of the main storage sites of lipid-soluble antioxidants[39–41], which, in the case of tocopherol, lycopene, lutein and β-cryptoxanthin, have been shown to limit the release of pro-inflammatory cytokines and chemokines from these cells[42–44]. Therefore, as lutein, lycopene, β-cryptoxanthin and zeaxanthin concentrations increased to varying degrees in the cohorts of both studies[26,28], we can deduce that this would increase their incorporation into adipocytes, where they may limit the release of SAA, similar to their ability to limit the release of cytokines and chemokines. This concept was further supported by the fact that BMI was unaltered in the cohorts of both studies, indicating that increased F&V intake may have influenced adipocyte function. However, this proposed mechanism needs to be confirmed through further investigations.

Fruit and vegetable intervention and serum-, HDL_2- and HDL_3-cholesteryl ester transfer protein activity

CETP is essential for the normal metabolic functioning of HDL, although it has been suggested that when HDL is associated with SAA, its activity may be altered to a proatherogenic phenotype[11,15]. This can reduce the ability of HDL to participate in reverse cholesterol transport[45,46]. Therefore, the reduction in the activity of CETP in HDL3 in the FAVRIT cohort and in HDL2 in the ADIT cohort and also the negative correlation of this reduction with self-reported F&V intake demonstrate an anti-atherogenic property of increasing F&V intake and/or decreasing SAA concentrations.

F&V intake may have exerted this effect through one or several mechanisms. First, as CETP is also released by adipocytes[47], its expression may have been down-regulated by the increase in lipid-soluble antioxidant concentrations, similar to that suggested for SAA. However, to date, only vitamin E has been investigated in this context, with contradictory findings. In one study, no effect was observed[48], while in another study, vitamin E was found to inhibit the activity of CETP, although the latter study was conducted in hamsters[49]. Second, the increased F&V intake may have reduced the expression of CETP in the liver, thereby lowering the levels available to associate with HDL. Third, increased F&V intake may, via its ability to lower SAA concentrations within HDL fractions, have altered the conformation of HDL and/or CETP, thus reducing their interaction[50]. Unfortunately, as a mass assay was not carried out in the present study, it is difficult to confirm whether the amount of CETP was reduced or remained the same, but its activity was found to be reduced. However, regardless of the mechanism, the present study is the first to show that increased F&V intake leads to reduced CETP activity, which may enhance the antiatherogenic properties of this lipoprotein, although this concept needs to be investigated further.

Conclusions

Overall, by carrying out a further analysis on samples from the FAVRIT and ADIT studies in the present study, we have shown that increased F&V intake (≥5 portions/d) augments serum, HDL_2 and HDL_3 antioxidant concentrations and have also shown for the first time that such a dietary pattern lowers the concentrations of the inflammatory marker SAA in HDL_2 and HDL_3, indicating that this marker may be sensitive to changes in F&V intake in hypertensive and older populations. In addition, we have shown that by decreasing the association of SAA with HDL and by reducing the activity of HDL-associated CETP, increased intake of F&V may enhance the antiatherogenic properties of HDL_2 and HDL_3. These results highlight a dual antioxidant/anti-inflammatory impact of such a dietary pattern, which would probably affect cardiovascular health.

Overall, the results of the present study provide tangible evidence of the effectiveness of increasing F&V intake, which is an encouraging endorsement of the '5-a-day' public health message and may be of use to health policy makers.

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The authors' contributions are as follows: J. M. designed the study; N. N. conducted the study; J. V. W. and I. S. Y. designed the FAVRIT and ADIT studies, which generated the samples for the present study; C. E. N. and D. O. M. conducted the FAVRIT and ADIT studies; D. M. and D. E. recruited the FAVRIT and ADIT subjects; J. M., J. V. W. and N. N. wrote the article; J. M. had primary responsibility for the final content. All authors read and approved the final manuscript.

None of the authors has any conflicts of interest to declare.

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