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Fruit and vegetable intake and serum cholesterol levels: a cross-sectional study in the Diet, Cancer and Health cohort

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SUMMARY
The aim of this study was to investigate, in a cross-sectional study, whether the intake of fruits and vegetables was associated with lower serum cholesterol levels. The groupings of interest for this study were apples, other fruits, and vegetables. A cross-sectional study was conducted among 42,972 men and women included in the Diet, Cancer and Health cohort (1993 – 1997). This population excluded persons with self-reported cardiovascular risk factors, in order to ensure a "heart-healthy" population. Among men, both apple and vegetable intake was found to be associated with significantly lower serum cholesterol levels. An increase in apple intake of 50 g d^{-1} was associated with a 0.04 mmol l^{-1} lower serum cholesterol level. Furthermore, analyses combining vegetable and alcohol intake showed lower serum cholesterol levels in men with a low alcohol intake (≤ 10 g d^{-1}). For women, the same pattern was seen for apples and vegetables, albeit to a lesser extent for apples. Analyses combining vegetable intake and waist circumference measurements showed that women with a smaller waist circumference had lower levels of serum cholesterol. Our findings thus provide some support for previous inverse associations found between higher apple intake and lower serum cholesterol levels.

A high intake of fruits and vegetables has been associated with a lower incidence of a variety of cancers and coronary heart disease (CHD; Dauchet *et al*., 2004; Joshipura *et al*., 2001; Liu *et al*., 2001; Riboli and Norat, 2003; Srinath and Katan, 2004; Willett, 1995) and, for CHD in particular, blood cholesterol is a risk factor. Two recent meta-analyses, including only cohort studies, reported a protective association between fruit and vegetable consumption and the risk of CHD (Dauchet *et al*., 2006; He *et al*., 2007) with relative risks ranging from 0.83 - 0.93 for the highest to the lowest fruit and vegetable intakes. A recent pooled case-control study, including data from 52 countries, found a 30% lower risk of myocardial infarction in those with the highest intakes of fruits or vegetables (Iqbal *et al*., 2008).

Fruits and vegetables are rich in anti-oxidant compounds such as phytochemicals, which are believed to protect against heart disease. However, the number and complexity of phytochemicals in most plant food groups is high, and determining which compound(s) are preventive, and which may even be potentially harmful, is a difficult task. Among the important groups of phytochemicals are the polyphenols. In Europe and the USA, apples are an important source of polyphenols. While fruits and vegetables, in general, are associated with protective effects, whole apple intake, and thus the intake of phytochemicals from apples in particular, is thought to be associated with health benefits. Several studies on rodents have shown a serum cholesterol-lowering effect of apple polyphenols (Aprikian *et al*., 2001; Bobek *et al*., 1990; Osada *et al*., 2006), as have two Japanese human intervention studies (Nagasako-Akazome *et al*., 2005; 2007). In a study on nearly 35,000 women in Iowa, it was found that the phytochemicals, catechin and epicatechin, from apple and tea were strongly inversely related to CHD mortality (Arts *et al*., 2001). It is believed that the beneficial effects of catechin and epicatechin are stronger among the more bio-available apple catechins. Finally, in a Finnish study, apple and onion intake was inversely related to coronary mortality in women (Knekt *et al*., 1996)

In this cross-sectional study, we investigated whether a relationship existed between serum cholesterol levels and the intake of three plant food groups: apples, other fruits, and vegetables, in the Danish Diet, Cancer and Health cohort. In addition to the above-mentioned rationale for investigating apples, this fruit contributes greatly to total fruit intake in Denmark. We also investigated the relationship between serum cholesterol levels and the intake of other fruits and vegetables, to

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examine the results with a broader perspective. Furthermore, we looked at the intake of the above-mentioned fruit and vegetable groups in association with three other possible risk factors for heart disease: smoking, alcohol intake, and waist circumference, in order to assess whether persons with higher or lower values of these factors had differential levels of cholesterol associated with their fruit and vegetable intake. We did this in order to assess the individual effects of these three factors.

MATERIALS AND METHODS

The present study was based on data from the prospective Danish Diet, Cancer and Health (DCH) cohort, established between December 1993 - May 1997. A total of 57,053 men and women participated in the study and were included if they fulfilled the following criteria: aged between 50 – 64 years, born in Denmark, and not registered with any previous diagnosis in the Danish Cancer Registry. A detailed food frequency questionnaire (FFQ) and a lifestyle questionnaire were filled-in by each participant. Biological and anthropometric measurements were taken, including non-fasting blood samples for total cholesterol determination in the serum (Dyerberg *et al*., 1990) and measurements of waist circumference with a measuring tape, recorded to the nearest 0.5 cm. A thorough description of these data collection methods has been published elsewhere (Tjonneland *et al*., 2007).

Intake estimates of the fruits and vegetables used in the present analyses were based on the FFQ completed at study enrolment by participants. Daily intakes of specific foods and nutrients were calculated for each participant using the software programme FoodCalc by applying specifically developed, standardised recipes and portion sizes (Lauritsen, 2007). The groupings used for fruits were apple (all sorts of apple, but only fresh apple) and other fruit (consisting of oranges, mandarins, grapefruit, pears, bananas, nectarines/peaches, strawberries, kiwifruit, and melons). For vegetables, the grouping used was total vegetables (consisting of several types of cabbage, broccoli, cauliflower, carrots, peas, corn, leek, green beans, spinach, onions, and mushrooms). Fruit or vegetable juices were not included in any of the groupings.

Persons who, on the lifestyle questionnaire, indicated having had a cerebral or coronary thrombosis or having angina pectoris, increased blood pressure, or high serum cholesterol were excluded from the study in order to obtain a "heart-healthy" population. This was done in order to create a homogenous population with a diet that could be presumed to be stable in adult-life, despite heart disease or heart disease risk factors. Furthermore, persons were excluded from the study due to having an incomplete lifestyle questionnaire or missing information about potential confounders. In total, 14,081 persons were excluded, and 42,972 persons were ultimately included in this cross-sectional study, including 20,407 men and 22,565 women.

The associations between the plant food variables and serum cholesterol levels were based on multiple linear regression analyses. Variables were entered stepwise into the model. The first model described the crude association between the different plant food groups and serum cholesterol levels. In the second model, the different plant food groups were mutually adjusted (i.e., the groups were controlled for each other, and adjustment was made for non-intake of apples and other fruits). The remaining three models were performed as interaction analyses, to evaluate the effects of three well-established risk factors for heart disease, in association with fruit and vegetable intake, on serum cholesterol levels (Godsland *et al*., 1998; Jousilahti *et al*., 1996). Thus, the associations between serum cholesterol level and the fruit and vegetable groupings were analysed in combination with one of three variables: smoking status, alcohol consumption, and waist circumference. Smoking was included as never/ever smokers, and alcohol intake was evaluated based on an intake above or below 10 g d^{-1} (corresponding to approx. 1 unit of alcohol). For waist circumference, a cut-off value of 102 cm was used for men, while for women the corresponding value was 88 cm, based on differing risks according to sex (Han *et al*., 1995; Lean *et al*., 1995). All models were adjusted for age.

The linearity of the quantitative variables was evaluated graphically by linear splines, with three boundaries placed at quartiles among cases. Serum cholesterol level was evaluated graphically for a normal distribution. All quantitative variables (except alcohol and waist circumference) were entered linearly into the model (in increments of 50 g d^{-1} for the plant food groups), as this is biologically more plausible than the step functions corresponding to categorisation; furthermore, it increased the power of the analyses (Greenland, 1995).

The GLM procedure in SAS was used for statistical analyses on a TextPad platform (Release 8.2; SAS Institute Inc., Cary, NC, USA).

TABLE I
Baseline characteristics for the Diet, Cancer and Health cohort, by men and women, for selected demographic and lifestyle factors as well as plant food intake

	Men (n = 20,407)		Women (n = 22,565)	
Variable†	Median (5 – 95%)	Fraction (%)	Median (5 – 95%)	Fraction (%)
Age (years)	55 (50 – 64)		55 (50 – 64)	
Serum cholesterol (mmol l^{-1})	5.8 (4.3 – 7.8)		6.1 (4.5 – 8.2)	
Waist circumference (cm)	94 (81 – 112)		79 (67 – 101)	
Apples (g d^{-1})	53.6 (2.1 – 312.5)		53.6 (2.1 – 312.5)	
Other fruits (g d^{-1})	56.4 (10.2 – 232.0)		99.7 (17.2 – 332.4)	
Vegetables (g d^{-1})	149.5 (46.5 – 328.4)		167.9 (50.4 – 372.7)	
Alcohol intake (g d^{-1})	19.2 (1.8 – 79.1)		9.5 (0.4 – 41.1)	
Alcohol abstainers (%)		1.7		2.6
"Ever smokers" (%)		73.5		56.2

†Values are expressed as medians (5th and 95th percentiles) or as fractions (%) within each category.

TABLE II
Effect (in difference in mmol l^{-1} serum cholesterol) of the intake of 50 g d^{-1} of the different plant food groups on total serum cholesterol levels in 20,407 men in the Diet, Cancer and Health cohort[‡]

	Plant food variable (g d^{-1})		
Model used	Apples (50) Slope (95% CI)	Other fruits (50) Slope (95% CI)	Vegetables (50) Slope (95% CI)
Model 1[∫]	–0.04* (–0.05, –0.03)	–0.03* (–0.04, –0.02)	–0.04* (–0.05, –0.03)
Model 2[§]	–0.03* (–0.04, –0.02)	–0.007 (–0.02, 0.003)	–0.03* (–0.04, –0.02)
Model 3[¶]– smoking status			
"Never"	–0.05* (–0.07, –0.04)	–0.03* (–0.04, –0.01)	–0.05* (–0.06, –0.04)
"Ever"	–0.02* (–0.03, –0.009)	–0.0004 (–0.01, 0.01)	–0.02* (–0.03, –0.01)
P-value	0.48	0.30	0.03
Model 4[†] – alcohol intake			
≤ 10 g d–1	–0.07* (–0.08, –0.05)	–0.04* (–0.06, –0.03)	–0.07* (–0.08, –0.06)
> 10 g d–1	–0.01* (–0.02, –0.002)	0.01 (–0.001, 0.02)	–0.02* (–0.03, –0.01)
P-value	0.12	0.15	0.0006
Model 5[#] – waist circumference			
≤ 102 cm	–0.04* (–0.05, –0.03)	–0.01* (–0.02, –0.001)	–0.04* (–0.04, –0.03)
> 102 cm	–0.02 (–0.03, 0.0004)	0.006 (–0.009, 0.02)	–0.01* (–0.03, –0.002)
P-value	0.48	0.18	0.73

[‡]*P* < 0.05 is indicated by *, and 95% confidence intervals (CI) are presented. All models were age-adjusted.
[∫]Model 1; Crude effect, age–adjusted.
[§]Model 2; Mutual and age-adjusted effect.
[¶]Model 3; Combined effect of plant food intake and smoking status.
[†]Model 4; Combined effect of plant food intake and alcohol intake.
[#]Model 5; Combined effect of plant food intake and waist circumference.

RESULTS

Table I shows the characteristics of the study population. The median age at entry into the cohort was 55 years for both men and women (range 50 – 64 years). In general, women had a higher intake of fruits and vegetables than men, except for the median apple intake, which was equal for both sexes. Women also had a slightly higher median level of serum cholesterol. There were more smokers among the men, and men had a higher alcohol intake.

Table II and Table III show the relationship between the intake of fruits and vegetables and serum cholesterol levels for men (Table II) and women (Table III). When entered as a single variable in the regression model, all three groups of plant foods were inversely related to serum cholesterol level, and these relationships were statistically significant (Model 1). An increase in apple intake of 50 g d^{-1} was associated with a 0.04 mmol l^{-1} lower serum cholesterol level in men and a 0.03 mmol l^{-1} lower serum cholesterol in women. An increase of 50 g d^{-1} in the intake of other fruits was associated with a 0.03 mmol l^{-1} lower serum cholesterol level in men, and a 0.02 mmol l^{-1} lower serum cholesterol level in women. For vegetable intake, a 0.04 mmol l^{-1} lower serum cholesterol was seen per 50 g d^{-1} intake for both men and women. All these results were statistically significant, by confidence intervals and *P*-values ($P < 0.05$). When each of the plant food variables were mutually adjusted, the association with serum cholesterol level became slightly weaker, but remained statistically significant, except for the intake of "other fruits" (Model 2).

In Model 3, we evaluated the potential associations between fruits and vegetables and serum cholesterol levels in relation to smoking status. For men, lower serum

TABLE III
Effect (in difference in mmol l^{-1} serum cholesterol) of the intake of 50 g d^{-1} of the different plant food groups on total serum cholesterol levels in 22,565 women in the Diet, Cancer and Health cohort[‡]

	Plant food variable (g d^{-1})		
Model used	Apples (50) Slope (95% CI)	Other fruits (50) Slope (95% CI)	Vegetables (50) Slope (95% CI)
Model 1[∫]	–0.03* (–0.03, –0.02)	–0.02* (–0.03, –0.01)	–0.04* (–0.04, –0.03)
Model 2[§]	–0.01* (–0.02, –0.005)	–0.007 (–0.01, 0.0008)	–0.03* (–0.04, –0.03)
Model 3[¶] – smoking status			
"Never"	–0.03* (–0.04, –0.01)	–0.02* (–0.03, –0.008)	–0.04* (–0.05, –0.03)
"Ever"	–0.003 (–0.01, 0.01)	0.001 (–0.007, 0.010)	–0.03* (–0.03, –0.02)
P-value	0.30	0.38	0.13
Model 4[†] – alcohol intake			
≤ 10 g d^{-1}	–0.01* (–0.03, –0.004)	–0.002 (–0.010, 0.006)	–0.03* (–0.04, –0.02)
> 10 g d^{-1}	–0.01* (–0.03, –0.002)	–0.02* (–0.02, –0.006)	–0.04* (–0.05, –0.03)
P-value	0.05[‡]	0.08	0.82
Model 5[#] – waist circumference			
≤ 88 cm	–0.03* (–0.04, –0.02)	–0.02* (–0.02, –0.008)	–0.04* (–0.05, –0.03)
> 88 cm	0.03* (0.02, 0.05)	0.03* (0.02, 0.04)	0.003 (–0.007, 0.01)
P-value	0.39	0.90	0.02

[‡]*P* < 0.05 is indicated by *, and 95% confidence intervals (CI) are presented. All models were age-adjusted.
[∫]Model 1; Crude effect, age-adjusted.
[§]Model 2; Mutual and age-adjusted effect.
[¶]Model 3; Combined effect of plant food intake and smoking status.
[†]Model 4; Combined effect of plant food intake and alcohol intake.
[#]Model 5; Combined effect of plant food intake and waist circumference.
[‡]The estimates presented are statistically significantly different, but have been rounded-off to the nearest whole number. The values were –0.015 for ≤ 10 g d^{-1} and –0.014 for > 10 g d^{-1}.

cholesterol levels were seen in the "never smokers" per 50 g d^{-1} higher apple intake (0.05 mmol l^{-1} *vs.* 0.02 mmol l^{-1} in the "ever smokers"), but the interaction was not statistically significant (*P* for interaction, P_{int} = 0.48). The same was true for "other fruits". However, vegetable intake was associated with significantly lower serum cholesterol levels in the "never smokers". Lower serum cholesterol levels (0.05 mmol l^{-1} per 50 g d^{-1} higher vegetable intake) was seen in the "never smokers" *vs*. 0.02 mmol l^{-1} in the "ever smokers" (P_{int} = 0.03). For women, no significant associations were seen.

Alcohol intake was also evaluated as a potential intermediate factor (Model 4). Lower serum cholesterol levels were seen in men with a low alcohol intake *vs*. men with a higher alcohol intake in association with apple intake (–0.07 mmol l^{-1} *vs*. -0.01 mmol l^{-1}), but the test for interaction was non-significant (P_{int} = 0.12). For "other fruits", no significant difference was seen. Vegetable intake was associated with lower serum cholesterol in men with a lower alcohol intake (–0.07 mmol l^{-1} for ≤ 10 g d^{-1} *vs*. –0.02 for > 10 g d^{-1}; P_{int} = 0.0006). For women, no significant associations were seen.

Finally, an evaluation of waist circumference was performed (Model 5). Although men with smaller waists seemed to have lower serum cholesterol levels, both in association with apple intake and with vegetable intake, the interactions were non-significant. For women, a smaller waist circumference was associated with lower serum cholesterol levels for vegetable intake. Women with a waist circumference ≤ 88 cm had 0.04 mmol l^{-1} lower serum cholesterol levels per 50 g d^{-1} of vegetable intake, whereas women with waist circumference > 88 cm had slightly higher levels of serum cholesterol levels (0.003 mmol l^{-1} per 50 g d^{-1} of vegetable intake). The intake of apples or "other fruits" was not associated with any significant differences.

DISCUSSION

In this cross-sectional study, which included 42,972 persons between the ages of 50 - 64, we found a weak negative association between apple intake and serum cholesterol levels in both men and women. Likewise, vegetable intake was associated with lower serum cholesterol levels, in particular in association with smoking status and alcohol intake in men, and with waist circumference in women. The strengths of this study include the high number of participants and the separate analyses of apples and other fruit, making it possible to examine any specific role for apple in total fruit intake. This was possible because of data from the detailed and validated dietary questionnaire. Furthermore, limiting the study population to a "heart-healthy" population increased comparability. The cross-sectional design was a limitation of this study, and longitudinal research with multiple cholesterol measurements may result in better evidence. Furthermore, serum cholesterol was measured in non-fasting blood samples, which could potentially have affected the cholesterol levels measured. Finally, mechanisms to explain the possible associations between food intake and cardiovascular risk factors are complicated, and residual confounding may have affected the results, despite attempts to overcome this issue *via* interaction analyses.

Our findings on apple intake and serum cholesterol levels are consistent with a study in which apple polyphenols decreased total serum and liver cholesterol levels in rats (Aprikian *et al*., 2002). Furthermore, a double-blind, placebo-controlled intervention study on 48 healthy Japanese men and women found a significant decrease of 10.2 mg 100 ml^{-1} [approx. 0.26 mmol l^{-1}; using a conversion factor of 38.7 (Furberg *et al*., 2005)] in serum cholesterol when a high dose (1,500 mg apple polyphenols d^{-1}) was ingested (Nagasako-Akazome *et al*., 2005). There are several possible biological mechanisms behind the postulated cholesterol-lowering effects of apples, one is that apple polyphenols can bind to cholesterol and bile acids, and increase their excretion in the faeces. This results in decreased cholesterol micelle formation and, therefore, in decreased lipid uptake from the intestine to the blood (Nagasako-Akazome *et al*., 2005).

This study supports other research indicating that apples may have cholesterol-lowering properties and, therefore, possibly a role in the prevention of heart disease when related to risk involving total cholesterol levels. Apple intake, and not the intake of other fruits, was associated with lower serum cholesterol levels in this study. Therefore, apples have a potentially important role in the cholesterol-lowering effects of daily fruit intake. Furthermore, vegetables also showed a favourable effect on serum cholesterol levels. Further research is needed to provide more evidence that will make it possible to understand the mechanisms behind these effects, which may improve the clinical relevance of the somewhat weak associations found thus far. In particular, the possible associations seen in this study should be looked at in a prospective, experimental design. It would also be of benefit to look at sub-fractions of serum cholesterol rather than at total cholesterol.

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