

This is the authors' final, peer reviewed manuscript published in
European Journal of Public Health 23 (2013) 1101-1262 with the title:
Do smoking and fruit and vegetable intake mediate the association between socio-economic
status and plasma carotenoids?
<http://eurpub.oxfordjournals.org/content/early/2013/06/27/eurpub.ckt081.abstract>

Do smoking and fruit and vegetable intake mediate the association between socio-economic status and plasma carotenoids?

**Elisabeth Kvaavik^{1*}, Torunn H. Totland², Nasser Bastani², Marte K. Kjøllesdal², Grethe
S. Tell³ and Lene F. Andersen²**

¹ Norwegian Institute for Alcohol and Drug Research, Oslo, Norway

² Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway

³ Department of Public Health and Primary Health Care, University of Bergen, Norway

* Corresponding author: Norwegian Institute for Alcohol and Drug Research, P.b. 565 Sentrum, 0105 Oslo,
NORWAY. Tel: +47 22 34 04 00; E-mail: orwegian.institute.for.alcohol.and.drug.research, P.O. Box 565
Sentrum, N-0157 Oslo, Norway, Tel: +4740647383, Fax: +4722340401, e-mail: ek@sirus.no

Do smoking and fruit and vegetable intake mediate the association between socioeconomic status and plasma carotenoids?

Elisabeth Kvaavik^a

Torunn H. Totland^b

Nasser Bastani^b

Marte K. Kjøllesdal^b

Grethe S Tell^c

Lene Frost Andersen^b

^aNorwegian institute for alcohol and drug research, Oslo, Norway.

^bDepartment of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway.

^cDepartment of Public Health and Primary Health Care, University of Bergen, Norway.

Correspondence to: Elisabeth Kvaavik, Norwegian institute for alcohol and drug research, P.O. Box 565 Sentrum, N-0157 Oslo, Norway. Email: ek@sirus.no, Telephone: +4740647383, Telefax: +4722340401.

Conflicts of interest: none declared

Funding: The 2006 wave of the Oslo Youth Study was funded by the Norwegian Research Council.

Abstract

Background

The aim was to study whether the association between educational attainment and antioxidant status is mediated by smoking and fruit and vegetable intake.

Methods

Cross-sectional analyses of the Oslo Youth Study 2006 wave were carried out. Information about education, smoking habits and diet was collected by questionnaire for 261 subjects (142 women and 119 men aged 38 – 42 years). Blood samples, height and weight measurements were taken by the participants' General Practitioner. Blood were analysed for plasma carotenoids. Linear regression analyses were used to examine whether smoking and fruit and vegetable intake mediate the association between education and plasma carotenoids.

Results

Educational level was positively associated with β -cryptoxanthin, α -carotene and lutein/zeaxanthin, but not with total carotenoids, β -carotene or lycopene. Education was negatively associated with smoking and positively associated with fruit and vegetable intake. Smoking was negatively associated with β -cryptoxanthin and fruit and vegetable intake was positively associated with β -cryptoxanthin (adjusted for educational level). Moreover, cigarette consumption mediated the association between education and β -cryptoxanthin by 37 %, while fruit and vegetable intake mediated this association by 18 %. The total mediation effect was 55 %.

Conclusion

Smoking seemed to be more important as a mediator between education and plasma levels of β -cryptoxanthin than the intake of fruit and vegetables, but more studies are needed to establish the relative importance of smoking and diet as mediators of the association between education and antioxidant status.

Key words: education, smoking, fruit and vegetable intake, carotenoids

Introduction

Antioxidants are shown to be important in health and disease.^{1,2} Although much is known about the mechanisms underlying a person's antioxidant status, including dietary intakes and bioavailability, individual requirements, metabolism, expenditure and genetic factors,³⁻⁵ it is not fully understood which and how background variables affect antioxidant status.

As for other health related factors, socioeconomic status (SES), for which education is a common measure in health research,⁶ has been found to be of importance for antioxidant status.⁷⁻¹¹ However, only few studies have investigated the association between social status and carotenoids; a study from the United States found significantly lower serum carotenoids levels in neighbourhoods with high deprivation.¹⁰ Likewise, a study in an urban indigenous population in Australia showed a positive correlation between SES and plasma concentrations of several carotenoids.⁷ Findings from the CARDIA-study showed that total carotenoids decreased with decreasing SES,⁸ and results from the Scottish MIDSPAN study showed strong inverse associations between plasma carotenoids and adult markers of social deprivation,¹¹ while a study from Belgium found no association between social status and β -carotene-deficiency.⁹ Only one of these studies examined possible mechanisms through which SES may affect antioxidant status. The CARDIA study by Janicki-Deverts et al (2009)⁸ explored whether the proposed association between SES and carotenoids was mediated by smoking, alcohol consumption and depression, and found that these factors partly mediated the effect of SES on carotenoids.

There is substantial evidence for social inequality in health behaviours, with for instance poorer diet quality¹² and higher prevalence of smoking among lower SES groups,^{13,14} and both dietary intakes and smoking habits are shown to have an impact on an individual's

antioxidant status.^{4,5,15} However, by which mechanisms SES impact on antioxidant status is not known, and to our knowledge, only one study has investigated whether the association between SES and antioxidant status is mediated by smoking⁸ while none to date have explored whether intakes of fruit and vegetables may mediate this effect. Thus, which of these health behaviours that are the most important mediator in the association between educational level and antioxidant status has not been examined.

The main objective of the current study was to examine whether smoking and/or fruit and vegetable intake may mediate the association between socioeconomic status measured by educational level and single and total plasma carotenoids.

Methods

The Oslo Youth Study began in 1979 when 1016 primary- and secondary school students (mean age 13 years, range 11-15) attending six schools in socio-economically disparate neighbourhoods of Oslo were invited to participate in a health survey. The objective of the study was to obtain epidemiological data on risk factors for cardiovascular disease and cancer and to evaluate the effects of an intervention program which aimed at discouraging smoking initiation and improve physical activity and dietary habits (described in detail elsewhere^{16,17}). All those invited to participate in The Oslo Youth Study at baseline in 1979 or at the first follow-up in 1981 were invited to a new survey in 2006 (questionnaire and health examination, mean age 40 years). This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were initially approved by the Oslo City Health Authorities; subsequent surveys were approved by

the Norwegian Data Inspectorate and the Regional committees for medical and health research ethics (REC Central). Written informed consent was obtained from all subjects.

Subjects

With the exception of those who refused to participate at an earlier wave of the Oslo Youth Study (n=69), those who in 1979 or 1981 had been invited to participate in the study (N=1086) were eligible for participation in the present follow-up of the study (N=1017). Due to deaths (n=7), unknown addresses (n=177), and emigration (n=33) (in total 217 subjects), 800 men and women were invited to participate in 2006. Questionnaires were returned by 408 subjects of whom 280 completed the health survey. After exclusion of participants who reported to be pregnant (n=4) or to have anorexia nervosa (n=1) or with missing values of one or more of the included variables (education, smoking, dietary behaviours, plasma carotenoids, body weight and height and demographic factors, n=19), the study sample comprised of 261 subjects (119 men, 142 women, mean age 40.0 years; range 38.0 – 42.0). Overall participation rate among those invited in 2006 was 32.6 % (261/800).

Assessment of health behaviours, education, body mass index, diet and plasma carotenoids

Respondents were invited to attend a health examination survey at their primary care physician in addition to the questionnaire survey, see below.

The self-administered questionnaire contained enquiries regarding smoking habits, diet and education.

Cigarette smoking was assessed by the question “How often do you smoke cigarettes?” with five response alternatives; Never, More seldom than once a month, About 1-2 times a month, About 1-2 times weekly and Daily. For smokers, number of cigarettes was recorded; “If you smoke, how many cigarettes/pipes do you smoke daily?” with response categories Less than 1, 1-5, 6-10, 11-20 and 20+, that was given scores 1 to 5, respectively. Non-smokers were given value zero on this variable.

Educational attainment in 2006 was ascertained from the questionnaire, and was used as an indicator of socio-economic status. The response categories were: Elementary school (9 years), High school (10-11 years), High school/comprehensive school (12 years), College/University (1 to 4 years) and College/university (at least 4 years).

In the 2006 health examination, height and weight measurements (in light clothing and without shoes) and a blood sample were taken. Body mass index (BMI) was computed based on the usual formulae ($\text{weight}/(\text{height})^2 \text{ kg/m}^2$).

Venipuncture was done by the participants’ General Practitioners, and all blood samples were analysed for the plasma carotenoids lutein, zeaxanthin, lycopene, α - and β -carotene and β -cryptoxanthin at the University of Oslo. Plasma carotenoids were analysed as previously described in online supporting material by Karlsen et al.¹⁸ The plasma levels of lutein and zeaxanthin were summed and the combined measure was used in the analyses.

Participants completed a validated quantitative food frequency questionnaire (FFQ) designed to assess usual diet during the past year.¹⁹ The questionnaire included 180 food items grouped together according to the typical Norwegian diet and meal pattern. Questions were

phrased to assess the usual intake, and both frequencies (10 response alternatives) and amounts (ranging between four and 14 response alternatives dependent on food item) were reported by the participants. We estimated daily intake (in grams) of total *fruit and vegetables* (potatoes were excluded). The FFQs were scanned and the image files transferred into data files using Cardiff Teleform 2006 software. The data files were checked for completeness and daily intakes of fruit and vegetables were computed using the food database AE-07 and KBS software system (KBS, version 4-9, 2008) developed at the Department of Nutrition, University of Oslo, Norway. The food database AE-07 is based on the 2006 edition of the Norwegian food composition table (www.norwegianfoodcomp.no).

Statistical Analyses

Effect modification by sex was tested by linear regression analyses, but as there were little evidence of an effect modification, analyses were run for men and women combined. Single- and two-mediation analyses were adjusted for sex, age, BMI and fasting status, and assumptions for the mediation analysis were met. Linear regression analyses were used in the single- and two- mediation models which were carried out for the total effect of education on plasma carotenoids represented by the c-path (Figure 1). The a_1 -path represented the relationship between education and smoking as one mediator (M_1) and the a_2 -path represented the relationship between education and fruit and vegetables as a second mediator (M_2). The relationships between each of the mediators M_1 and M_2 and plasma carotenoids were represented by the b-paths (which are adjusted for the independent variable, i.e. education in the present study). In two-mediation analyses, the c' -path represented the relationship between education and plasma carotenoids when adjusted for the mediators M_1 and M_2 .^{20,21} Results for single-mediation analyses are not shown. The mediated effect (a_1 -path* b_1 -path + a_2 -path* b_2 -path) with 95% confidence intervals and statistical significance was obtained from

bootstrap samples (1000), calculated with Andrew F. Hayes' macro INDIRECT generated for SPSS to assess and calculate indirect effects in multiple mediator models (<http://www.afhayes.com>). Bias corrected bootstrap analyses were performed as recommended to increase the power of the analyses^{21,22} by obtaining statistical power estimates of 0.8.²² Full or complete mediation is present when the total effect (the c-path) is significant, the direct effect (the c'-path) is non-significant and either a_1b_1 or a_2b_2 or both are significant, while partly or incomplete mediation is present when the direct effect is also significant. Inconsistent mediation is present when neither total nor direct effect is significant and either a_1b_1 or a_2b_2 or both are significant.²⁰ T-tests and chi-square statistics were used to compare men and women, and those participating in 1981 (substituted with data from 1979 if missing) and in 2006 with 2006-drop-outs (from the physical health examination). The Statistical Package for Social Sciences (version SPSS 19.0) was used in all analyses.

Results

The attrition analyses revealed significant differences between 2006 participants and non-participants with regard to sex, age, smoking status, and parental education. 2006 participants were more likely to be female (62.6 % versus 42.7 %, $P < 0.001$ (Chi square)), to be older (14.7 versus 14.4 years, $P < 0.01$ (T-test)), less likely to be a weekly smoker (11.3 % versus 16.8 %, $P < 0.05$ (Chi square)) and more likely to have parents with higher education (at least 12 years, high school/comprehensive school or college/university) (52.5 % versus 41.6 %, $P < 0.01$ (Chi square)) in 1981 (or 1979) compared to 2006-drop-outs. There were no significant differences in body mass index or intake of fruit and vegetables in 1981/1979 by participation in the 2006 health examination.

Study participants are described in table 1 with regard to antioxidants, education, smoking and fruit and vegetable intake as well as the confounders sex, age, BMI and fasting status. Total carotenoid level was 1.649 $\mu\text{mol/L}$. The proportion of non-smoking participants were 71%, and the average intake of fruit and vegetables were 452 grams per day.

The associations between education, cigarette consumption, fruit and vegetable intake and carotenoids are presented in table 2 in two-mediation models. Results from single-mediation models did not change noticeably when both mediators were included in two-mediation models, and therefore only results from the two-mediation models are presented. Education was associated with β -cryptoxanthin, α -carotene, and with lutein and zeaxanthin combined (c-path, which are identical for single- and multi-mediation models), but not with total carotenoids, β -carotene or lycopene.

In the a-paths (that is the associations between the independent variable and the mediators, and identical for all end points and single- and two mediation models) the association between education and cigarette consumption and between education and fruit and vegetable intake were significant; education was negatively associated with smoking and positively associated with fruit and vegetable intake.

Cigarette smoking was negatively associated with β -cryptoxanthin in single- (not shown) and two-mediator models (b-paths) and fruit and vegetable intake was positively associated with β -cryptoxanthin in single- (not shown) and two-mediator models (b-paths). Cigarette consumption fully mediated the association between education and β -cryptoxanthin in the two-mediation model. There were no significant associations between cigarette consumption or fruit and vegetable intake and total carotenoids, α - or β -carotene, lutein/zeaxanthin or lycopene in single- (not shown) or two-mediation models.

Discussion

Findings

Education was positively associated with plasma β -cryptoxanthin, α -carotene and lutein/zeaxanthin as well as with cigarette consumption and fruit and vegetable intake. Cigarette consumption seemed to be more important as a mediator between education and plasma levels of β -cryptoxanthin than the intake of fruit and vegetables.

Other studies

The positive association found between educational level and β -cryptoxanthin, α -carotene and lutein/zeaxanthin in the current study is partly supported by previous research; only few of the earlier studies have, however, included β -cryptoxanthin as an end-point,^{7,10} while more studies have included α -carotene.^{7,10,11} The two identified studies including β -cryptoxanthin have conflicting results regarding the influence of education and SES on this specific carotenoid; one study⁷ did not find any association between SES and β -cryptoxanthin, while the other one¹⁰ found that higher SES predicted higher levels of β -cryptoxanthin. In line with our finding, three previous studies found that different measures of SES, including educational level, were positively associated with α -carotene.^{7,10,11} However, unlike our findings that education was not associated with β -carotene, lycopene and total carotenoids, previous research have reported that higher SES predicts higher levels of antioxidants.⁷⁻¹¹

Education was strongly associated with smoking and fruit and vegetable intake in the current study, with a negative association with smoking and a positive association with fruit and vegetables. These results are in line with previous research.¹²⁻¹⁴

Consistent with other studies, smoking was negatively associated with β -cryptoxanthin in the current study.²³⁻²⁵ Previous research has been equivocal regarding the association between α -carotene and smoking; in the present study, smoking was non-significantly and negatively associated with α -carotene. Other studies report both negative^{25,26} and no associations.^{23,24} In the current study we did not find a significant negative association between smoking and β -carotene and zeaxanthin-plus-lutein as previously reported, while the lack of an association between smoking and lycopene in the current study is supported by numerous previous studies.²³⁻²⁵ Previous research examining the association between smoking and carotenoid status has mostly contrasted current daily smoking against non-smoking, while in the present study number of cigarettes smoked daily was applied as the independent variable. This might explain some of the inconsistencies between this and earlier research.

The only statistically significant association between fruit and vegetable intake and antioxidants found in the current study was for β -cryptoxanthin, with higher intakes associated with higher levels of β -cryptoxanthin. This is in agreement with earlier research.^{27,28} However, previous research has demonstrated a strong association between dietary intakes of antioxidants and antioxidant level in plasma/serum, which we did not find here.^{9,27-29}

Explanations

The inconsistencies between the current study and studies from other countries might be ascribed to differences in age, ethnicity, socioeconomic status, unadjusted confounders, for example other health behaviours, and methodological issues. The participants' age range in the current study was narrow, originally they were all from the capital of Norway and due to a high drop-out rate, selection bias might have occurred, which may contribute to low

variability in socioeconomic status and other health behaviours. Further, different measures of socioeconomic status and different methods used to examine dietary habits, smoking and carotenoid status can not necessarily be used interchangeably.

Epidemiological studies have shown that cigarette smokers consume fewer fruits and vegetables than non-smokers.^{25,30,31} A weak and non-significant negative correlation was observed between smoking and intake of fruit and vegetables in the present study (Spearman's $\rho = -0.14$, $P = 0.020$). However, results from previous studies indicate that smoking is associated with antioxidant status independent of dietary intakes.¹⁵ The mediating effect of smoking on the association between education and anti-oxidant status in the present study is likely to be beyond the effect of different dietary habits because including intake of fruit and vegetables in the analyses did not change the effect of smoking. A French study found that plasma β -carotene was lower in smokers compared to non-smokers, and that the association did not change after adjustment for dietary intakes.³¹

How cigarette smoking leads to lower levels of antioxidants is not fully understood.

Reasonable theories are that cigarette smoking could result in increased metabolic turnover because of increased oxidative stress, or that smoking could decrease micronutrient absorption.¹⁵ Exposure to cigarette smoke has been observed to degrade α -tocopherol, β -carotene, cryptoxanthin, lutein/zeaxanthin, and lycopene in human plasma.³³ This can explain our finding of a significant inverse association between smoking and α -tocopherol and β -cryptoxanthin. That the other investigated antioxidants did not show a significant association with smoking may be due to lack of statistical power to detect significant associations. Alberg (2002)¹⁵ concluded in a review of the literature on the influence of smoking on circulating antioxidants that the effect of cigarette smoking was weaker for nonprovitamin A carotenoids

as zeaxanthin-plus-lutein and lycopene than for the provitamin A carotenoids α - and β -carotene and β -cryptoxanthin. Our findings are consistent with this.

Strengths and weaknesses – methodological issues

The food frequency questionnaire used to obtain dietary data has been validated among Norwegian men and found to adequately rank people with regard to energy intake and most nutrients.¹⁹ Furthermore, due to that the intake of only selected nutritional supplements were assessed by the questionnaire combined with a high degree of missing on these questions, intake of such supplements could not be calculated. This may have influenced the results in the current study. Previous research indicates that intake of supplements is lower among smokers than others.³⁴⁻³⁶ If this holds true also in the present sample, the mediating effect of smoking may have been somewhat overestimated. Thus, this is a possible confounding variable which we are not able to control for, as data are not available.

The participants in the Oslo Youth Study were included through schools in different socio-economic areas in Oslo, indicating that they are fairly representative of the source population. The presence of missing data raises obvious concerns regarding selection bias, but although the attrition rate was rather high, it was comparable to that reported in previous longitudinal studies with similar follow-up periods.^{37,38} However, a small analytical sample in 2006 may have diminished the chances of detecting significant associations between smoking, fruit and vegetable-intake and anti-oxidants. Furthermore, that the participants in the present study were more likely to be female, older and have parents with higher education and less often be smokers in 1979/81, may indicate that the present sample has a healthier lifestyle than the initial sample.^{12,39,40} This should, however, not influence the associations between education, AA-status and intake of fruit and vegetables/smoking.

Cross-sectional data were used to conduct mediation analysis and therefore conclusions on causality cannot be made. Normally, mediation analyses require longitudinal data in order to be able to discern the causes and effects of several variables. However, when exploring the associations between education, intake of fruit and vegetables, smoking and antioxidant status, the associations are likely to be in the suggested direction. Also, educational level measured in the 1999-wave of the current study was highly correlated with educational level in 2006 (results not shown), indicating that education in 2006 represents education at an earlier point in time. However, investigating the mediation of an associated variable on an already established relationship between two other variables may imply where emphasis should be made in further analyses based on causality. Reverse causality cannot be completely ruled out due to the cross sectional nature of the study.

Using cross-sectional data only means that changes over time cannot be detected. As measurements were based on self-reported data, the answers may be biased as well as social desirable.

Conclusion

Both cigarette consumption and fruit and vegetable intake mediated the association between education and plasma levels of β -cryptoxanthin, of which smoking seemed to be the more important mediator. There was no evidence of a mediating effect for other single or for total carotenoids in plasma.

Acknowledgements The 2006 wave of the Oslo Youth Study was funded by the Norwegian Research Council.

Conflicts of interest: none declared

Ethical information: This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were initially approved by the Oslo City Health Authorities; subsequent surveys were approved by the Norwegian Data Inspectorate and the Regional committees for medical and health research ethics (REC Central). Written informed consent was obtained from all subjects.

Authors' contributions: EK and LFA generated the idea for the present manuscript which was developed by all co-authors, and wrote the first draft around analyses conducted by EK, THT and MKK. GST designed and conducted the baseline and early follow-up surveys for the Oslo Youth Study and contributed substantially to data interpretation. NB analysed the blood samples and contributed to the interpretation of the results and data analyses. All authors have contributed to critical revision of the manuscript, and have read and approved of the final manuscript.

Key points:

- It is not fully understood which background variables are of importance for a person's carotenoid status, and how these background variables affect carotenoid status.
- In this study, smoking seemed to be somewhat more important than fruit and vegetable intake in mediating the association between education and plasma levels of β -cryptoxanthin.
- As our findings suggest that education may affect carotenoid status by means of dietary intakes and of cigarette smoking, interventions aiming at reducing the social inequality in health might benefit from including actions to improve diet as well as programs for

smoking cessation.

References

1. Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. *Mol Aspects Med* 2005;26: 459-516.
2. Voutilainen S, Nurmi T, Mursu J, Rissanen TH. Carotenoids and cardiovascular health. *Am J Clin Nutr* 2006;83: 1265-71.
3. Borel P, Moussa M, Reboul E, et al. Human plasma levels of vitamin E and carotenoids are associated with genetic polymorphisms in genes involved in lipid metabolism. *J Nutr* 2007;137: 2653-9.
4. Galan P, Viteri FE, Bertrais S, et al. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur J Clin Nutr* 2005;59: 1181-90.
5. Maiani G, Caston MJ, Catasta G, et al. Carotenoids: actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Mol Nutr Food Res* 2009;53 Suppl 2: S194-218.
6. Galobardes B, Lynch J, Smith GD. Measuring socioeconomic position in health research. *Br Med Bull* 2007;81-82: 21-37.
7. Hodge A, Cunningham J, Maple-Brown L, Dunbar T, O'Dea K. Plasma carotenoids are associated with socioeconomic status in an urban Indigenous population: an observational study. *BMC Public Health* 2011;11: 76.
8. Janicki-Deverts D, Cohen S, Matthews KA, Gross MD, Jacobs DR, Jr. Socioeconomic status, antioxidant micronutrients, and correlates of oxidative damage: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Psychosom Med* 2009;71: 541-8.
9. Pincemail J, Vanbelle S, Degruene F, et al. Lifestyle Behaviours and Plasma Vitamin C and beta-Carotene Levels from the ELAN Population (Liege, Belgium). *J Nutr Metab* 2011;2011: 494370.

10. Stimpson JP, Nash AC, Ju H, Eschbach K. Neighborhood Deprivation is associated with lower levels of serum carotenoids among adults participating in the Third National Health and Nutrition Examination Survey. *J Am Diet Assoc* 2007;107: 1895-902.
11. Talwar D, McConnachie A, Welsh P, et al. Which circulating antioxidant vitamins are confounded by socioeconomic deprivation? The MIDSPAN family study. *PLoS One* 2010;5: e11312.
12. Darmon N, Drewnowski A. Does social class predict diet quality? *Am J Clin Nutr* 2008;87: 1107-17.
13. Cavelaars AE, Kunst AE, Geurts JJ, et al. Educational differences in smoking: international comparison. *BMJ* 2000;320: 1102-7.
14. Lund KE, Lund M. [Smoking and social inequality in Norway 1998-2000]. *Tidsskr Nor Laegeforen* 2005;125: 560-3.
15. Alberg A. The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients. *Toxicology* 2002;180: 121-37.
16. Klepp KI, Tell GS, Vellar OD. Ten-year follow-up of the Oslo Youth Study Smoking Prevention Program. *Prev Med* 1993;22: 453-62.
17. Tell G, Vellar O. Noncommunicable Disease Risk Factor Intervention in Norwegian Adolescents: the Oslo Youth Study. In: Hetzel B, Berenson G, editors. *Cardiovascular risk factors in childhood: epidemiology and prevention*. Amsterdam: Elsevier, 1987.
18. Karlsen A, Retterstol L, Laake P, et al. Anthocyanins inhibit nuclear factor-kappaB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. *J Nutr* 2007;137: 1951-4.
19. Andersen LF, Solvoll K, Johansson LR, Salminen I, Aro A, Drevon CA. Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. *Am J Epidemiol* 1999;150: 75-87.

20. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. *Annu Rev Psychol* 2007;58: 593-614.
21. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods* 2008;40: 879-91.
22. Fritz MS, MacKinnon DP. Required sample size to detect the mediated effect. *Psychol Sci* 2007;18: 233-239.
23. Dietrich M, Block G, Norkus EP, et al. Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase gamma-tocopherol in vivo after adjustment for dietary antioxidant intakes. *Am J Clin Nutr* 2003;77: 160-6.
24. Suzuki K, Ito Y, Ochiai J, et al. The relationship between smoking habits and serum levels of 8-OHdG, oxidized LDL antibodies, Mn-SOD and carotenoids in rural Japanese residents. *J Epidemiol* 2003;13: 29-37.
25. Wei W, Kim Y, Boudreau N. Association of smoking with serum and dietary levels of antioxidants in adults: NHANES III, 1988-1994. *Am J Public Health* 2001;91: 258-64.
26. Rust P, Lehner P, Elmadfa I. Relationship between dietary intake, antioxidant status and smoking habits in female Austrian smokers. *Eur J Nutr* 2001;40: 78-83.
27. Ito Y, Shima Y, Ochiai J, et al. Effects of the consumption of cigarettes, alcohol and foods on serum concentrations of carotenoids, retinol and tocopherols in healthy inhabitants living in a rural area of Hokkaido. *Nippon Eiseigaku Zasshi* 1991;46: 874-82.
28. Kitamura Y, Tanaka K, Kiyohara C, et al. Relationship of alcohol use, physical activity and dietary habits with serum carotenoids, retinol and alpha-tocopherol among male Japanese smokers. *Int J Epidemiol* 1997;26: 307-14.
29. Faure H, Preziosi P, Roussel AM, et al. Factors influencing blood concentration of retinol, alpha-tocopherol, vitamin C, and beta-carotene in the French participants of the SU.VI.MAX trial. *Eur J Clin Nutr* 2006;60: 706-17.

30. Dallongeville J, Marecaux N, Fruchart JC, Amouyel P. Cigarette smoking is associated with unhealthy patterns of nutrient intake: a meta-analysis. *J Nutr* 1998;128: 1450-7.
31. Ma J, Hampl JS, Betts NM. Antioxidant intakes and smoking status: data from the continuing survey of food intakes by individuals 1994-1996. *Am J Clin Nutr* 2000;71: 774-80.
32. Marangon K, Herbeth B, Lecomte E, et al. Diet, antioxidant status, and smoking habits in French men. *Am J Clin Nutr* 1998;67: 231-9.
33. Handelman GJ, Packer L, Cross CE. Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. *Am J Clin Nutr* 1996;63: 559-65.
34. Block G, Cox C, Madans J, Schreiber GB, Licitra L, Melia N. Vitamin supplement use, by demographic characteristics. *Am J Epidemiol* 1988;127: 297-309.
35. Slesinski MJ, Subar AF, Kahle LL. Trends in use of vitamin and mineral supplements in the United State: the 1987 and 1992 National Health Interview Surveys. *J Am Diet Assoc* 1995;95: 921-3.
36. Zondervan KT, Ocke MC, Smit HA, Seidell JC. Do dietary and supplementary intakes of antioxidants differ with smoking status? *Int J Epidemiol* 1996;25: 70-9.
37. Barnekow-Bergkvist M, Hedberg G, Janlert U, Jansson E. Adolescent determinants of cardiovascular risk factors in adult men and women. *Scand J Public Health* 2001;29: 208-17.
38. Myers L, Coughlin SS, Webber LS, Srinivasan SR, Berenson GS. Prediction of adult cardiovascular multifactorial risk status from childhood risk factor levels. The Bogalusa Heart Study. *Am J Epidemiol* 1995;142: 918-24.
39. Johansson L, Andersen LF. Who eats 5 a day?: intake of fruits and vegetables among Norwegians in relation to gender and lifestyle. *J Am Diet Assoc* 1998;98: 689-91.

40. Prattala R, Paalanen L, Grinberga D, Helasoja V, Kasmel A, Petkeviciene J. Gender differences in the consumption of meat, fruit and vegetables are similar in Finland and the Baltic countries. *Eur J Public Health* 2007;17: 520-5.

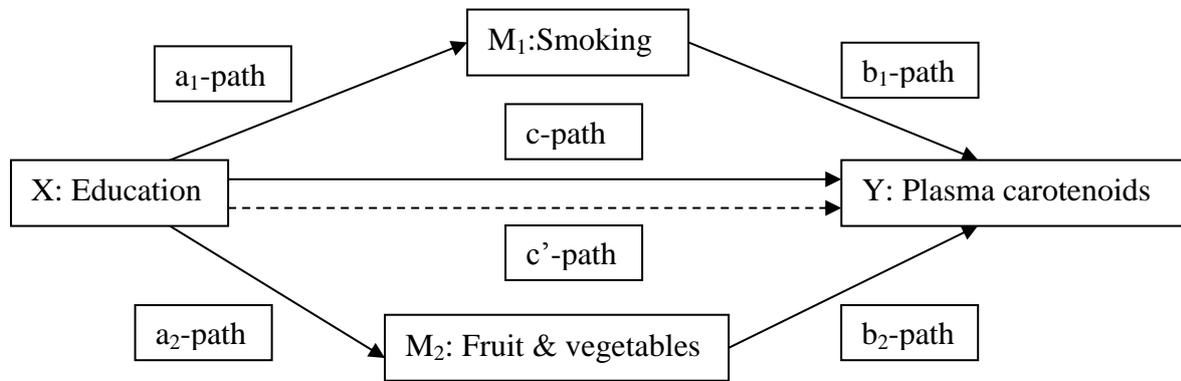


Figure 1. Mediation (two-mediator model) of the association between parental education and plasma carotenoids by smoking and fruit and vegetable intake.

Table 1 Characteristics of study participants. The Oslo Youth Study follow-up 2006

Background variables	
Sex, n (%) women	142 (54.4)
Age, years, mean (SD)	40.0 (0.9)
BMI, mean (SD)	25.2 (4.3)
Fasting, n (%)	154 (59.0)
Carotenoids (µmol/L), mean (SD)	
Total carotenoids	1.649 (0.808)
β-cryptoxanthin	0.167 (0.135)
α-carotene	0.119 (0.115)
β-carotene	0.504 (0.397)
Lutein and zeaxanthin	0.205 (0.102)
Lycopene	0.654 (0.390)
Education, n (%)	
Elementary/secondary school (≤9 years)	11 (4.2)
High school (10-11 years)	34 (13.0)
High/comprehensive school (12 years)	67 (25.7)
College/university (1-4 years)	90 (34.5)
College/university (≥ 4 years)	59 (22.6)
Smoking (cigarettes per day), n (%)	
Non-smokers	185 (70.9)
< 1	14 (5.4)
1-5	10 (3.8)
6-10	17 (6.5)
11-20	31 (11.9)
≥20	4 (1.5)
Fruit and vegetable intake, grams/day, mean (SD)	
	452 (271)

Table 2 Mediation effects of fruit and vegetable intake and smoking in the relation between education (X) and single and total plasma carotenoid level (Y) among 261 men and women. Two-mediator model (number of bootstrap samples for bias corrected bootstrap confidence intervals: 1000). The Oslo Youth Study follow-up 2006

	c-path (SE)¹	c'-path (SE)¹	a-path (SE)	b-path (SE)	a*b (SE)	95 % CI of a*b	a*b/c
Total carotenoids							
Education	0.0492 (0.0460)	0.0255 (0.0507)					
Smoking ²			-0.4955 (0.0836)***	-0.0145 (0.0344)	0.0072 (0.0199)	-0.0390, 0.0430	-
F&V intake ³			66.6435 (12.2815)***	0.0002 (0.0002)	0.0166 (0.0134)	-0.0099, 0.0439	-
β-cryptoxanthin							
Education	0.0230 (0.0075)**	0.0103 (0.0081)					
Smoking			-0.4955 (0.0836)***	-0.0171 (0.0055)**	0.0085 (0.0026)	0.0042, 0.0150	37.0
F&V intake			66.6435 (12.2815)***	0.0001 (0.0000)*	0.0042 (0.0022)	0.0007, 0.0099	18.3
Total					0.0127 (0.0035)	0.0067, 0.0209	55.2
α-carotene							
Education	0.0204 (0.0063)***	0.0168 (0.0070)*					
Smoking			-0.4955 (0.0836)***	-0.0053 (0.0047)	0.0026 (0.0020)	-0.0009, 0.0068	-
F&V intake			66.6435 (12.2815)***	0.0000 (0.0000)	0.0009 (0.0015)	-0.0018, 0.0042	-
β-carotene							
Education	0.0171 (0.0227)	0.0058					

(0.0250)						
Smoking		-0.4955 (0.0836)***	-0.0181 (0.0170)	0.0090 (0.0094)	-0.0084, 0.0296	-
F&V intake		66.6435 (12.2815)***	0.0000 (0.0001)	0.0023 (0.0060)	-0.0095, 0.0154	-
Lutein and zeaxanthin						
Education	0.0132 (0.0058)*	0.0087 (0.0063)				
Smoking		-0.4955 (0.0836)***	-0.0036 (0.0043)	0.0018 (0.0023)	-0.0025, 0.0072	-
F&V intake		66.6435 (12.2815)***	0.0000 (0.0000)	0.0027 (0.0021)	-0.0006, 0.0077	-
Lycopene						
Education	-0.0244 (0.0224)	-0.0162 (0.0245)				
Smoking		-0.4955 (0.0836)***	0.0296 (0.0167)	-0.0147 (0.0102)	-0.0398, 0.0031	-
F&V intake		66.6435 (12.2815)***	0.0001 (0.0001)	0.0064 (0.0066)	-0.0044, 0.0225	-

¹All paths are adjusted for sex, age, BMI and fasting status. ²Cigarettes per day had six categories; 0, <1, 1-5, 6-10, 11-20, >20 cigarettes per day, was entered as a continuous variable with values 0 to 5. ³F&V intake= daily intake of fruit and vegetables. *P<0.05, **P<0.01, ***P<0.001.