International Journal of Epidemiology, 2018, 1947–1955
doi: 10.1093/ije/dyy220
Advance Access Publication Date: 22 October 2018
Original article

Diet

Dietary non-enzymatic antioxidant capacity and the risk of myocardial infarction: the Swedish National March Cohort

Essi Hantikainen,[1]* Alessandra Grotta,[2] Mauro Serafini,[3] Ylva Trolle Lagerros,[4,5] Olof Nyren,[2] Weimin Ye,[2] Luca Colarusso[1] and Rino Bellocco[1,2]

[1]Department of Statistics and Quantitative Methods, University of Milano Bicocca, Milan, Italy, [2]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, [3]Functional Food and Metabolic Stress Prevention Laboratory, Faculty of BioSciences and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy, [4]Department of Medicine, Clinical Epidemiology Unit T2, Karolinska University Hospital, Stockholm, Sweden and [5]Department of Medicine, Clinic of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital Huddinge, Stockholm, Sweden

*Corresponding author. Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Edificio U7, Via Bicocca degli Arcimboldi 8, 20126 Milan, Italy. E-mail: e.hantikainen@campus.unimib.it

Editorial decision 10 September 2018; Accepted 19 September 2018

Abstract

Background: Results from randomized trials of antioxidant supplementation have cast doubt on observational data linking diets high in antioxidants to a reduced risk of cardiovascular diseases. We hypothesized that supplementation of one or a few antioxidants might not simulate the complex actions of all antioxidants in the human diet. We therefore investigated the association between dietary Non Enzymatic Antioxidant Capacity (NEAC), reflecting the antioxidant potential of the whole diet, and the risk of myocardial infarction (MI).

Methods: In the Swedish National March Cohort, 34 543 men and women free from cardiovascular diseases and cancer were followed through record linkages from 1997 until 2010. NEAC was assessed with a validated food-frequency questionnaire at baseline. The distribution of NEAC was categorized into sex-specific quartiles. We fitted multivariable Cox proportional hazards regression models to estimate hazard ratios (HRs) with 95% confidence intervals (CIs).

Results: During a mean follow-up time of 12.7 years, we identified 1142 incident cases of MI. Successively higher quartiles (Qs) of dietary NEAC were accompanied by a monotonic trend of decreasing MI incidence, both for overall MI (HR Q4 vs Q1: 0.77; 95% CI: 0.61–0.96; p for trend = 0.008) and non-fatal MI (HR Q4 vs Q1: 0.72; 95% CI: 0.56–0.92; p for trend = 0.004). No such association was found for fatal MI.

Conclusions: A diet rich in antioxidants might protect from MI.

Key words: atherosclerosis, myocardial infarction, diet, antioxidants, non-enzymatic antioxidant capacity

Key Messages

- Clinical trials investigating antioxidant supplementation found no protection from cardiovascular diseases. However, antioxidant supplements might not simulate the complex actions of all antioxidants in the human diet.
- We investigated the association between dietary Non Enzymatic Antioxidant Capacity (NEAC), reflecting the antioxidant potential of the whole diet, and the risk of myocardial infarction in a large cohort of Swedish men and women.
- Higher dietary NEAC was associated with a lower risk of overall and non-fatal MI but not with fatal MI.

Introduction

Myocardial infarction (MI) is one of the leading causes of death in high-income countries.[1] Well-known risk factors are age, smoking, hypertension and obesity.[2] Epidemiological studies have focused on the potential role of dietary habits in modifying the risk of MI[3] and it has been suggested, that foods rich in antioxidants could play a protective role in the development of coronary diseases.[4,5] Biological mechanisms underlying this hypothesis are based on the role of endogenous and exogenous antioxidants in counterbalancing the effects of reactive oxygen and nitrogen species, which, in turn, are involved in the atherosclerotic process leading to heart disease.[6] Previous observational studies found a higher consumption of fruits and vegetables to be associated with reduced risk of cardiovascular diseases.[4] Similarly, inverse associations for single antioxidant vitamins from food or supplements were observed in some but not all observational studies.[3,7–9] However, clinical trials investigating single or multiple antioxidant supplements found no protection from cardiovascular diseases and in some studies supplement use was even harmful.[9,10]

The antioxidant mechanism is thought to be extremely complex, including an interactive mechanism between known and unknown antioxidants.[11] The Non Enzymatic Antioxidant Capacity (NEAC), also known as Total Antioxidant Capacity, is a marker of dietary antioxidant potential. It assesses overall antioxidant activity from foods and beverages by measuring how many moles of oxidants are neutralized by 1 liter of a specific food extract. This can be measured through different chemical assays, such as the Ferric-Reducing Antioxidant Potential (FRAP) and the Total Radical-Trapping Antioxidant Parameter (TRAP). We therefore postulated that NEAC provides more moderated information on the possibly protective role of antioxidants than does the intake of single antioxidant compounds.[11] To date, three studies have been conducted to investigate the association between dietary NEAC and myocardial infarction. In two large Swedish cohort studies of women, a baseline diet with high NEAC was associated with lower MI incidence.[12,13] Comparable results were reported from an Italian case–control study.[14] Moreover, similar inverse relationships have been reported for other cardiovascular outcomes, such as stroke and heart failure.[15–17]

Since the evidence is modest and data for men are limited, we prospectively investigated the relationship between NEAC of the whole diet at baseline and subsequent risk of MI in a large cohort of Swedish men and women.

Materials and methods

Study cohort

The Swedish National March Cohort (SNMC)—a prospective cohort study designed to investigate associations between lifestyle factors and chronic diseases—was established in 1997 during a 4-day national fundraising event organized by the Swedish Cancer Society. The event took place in almost 3600 cities and villages around the country. Participants filled out a 36-page questionnaire with detailed questions about diet, physical activity, anthropometric measures, disease history and other background factors. In total, 43 880 subjects returned a completed questionnaire.

Follow-up was facilitated by the individually unique national registration numbers assigned to all Swedish residents. These permitted accurate tracking of health status through continuously updated nationwide databases. For the current analysis, we excluded individuals who provided an incorrect national registration number ($n = 11$), who were below the age of 18 years ($n = 1740$) and who emigrated ($n = 466$) or died ($n = 9$) before the beginning of the follow-up. We further excluded participants who were diagnosed with cancer (except non-melanoma skin cancer, ICD-10 code C44) ($n = 2673$) or had experienced a cardiovascular disease (ICD-10 codes: I00–I99) ($n = 4733$) before the beginning of the study. This was possible through exact linkages to the Swedish registries of population, migration, death, cancer and inpatient care, using the national registration numbers as identifiers. Further, to reduce exposure misclassification induced by self-reported information in the food-frequency questionnaire (FFQ), we excluded subjects who had extreme energy intake (± 3 SDs of the mean value of the ln-transformed energy intake) at baseline ($n = 470$),[12] leaving 34 543 subjects for the analyses. The study was approved by the Research Ethics Review

Committee at the Karolinska Institutet. All subjects provided informed consent.

Assessment of exposure and other variables

Participants were asked to fill in a validated 85-item semi-quantitative FFQ at baseline. Food-item-specific NEAC values were obtained from an Italian database developed by Pellegrini *et al.*,[18,19] where NEAC was assessed by the FRAP assay and expressed as mmol Fe^{2+} equivalents per 1-kg fresh weight of single foods. NEAC values were available for 66 out of the 85 items from the FFQ. These values were further multiplied with the portion size and consumption frequencies reported in the FFQ and added up to compute total NEAC of the daily diet. Vitamin and mineral supplements were not included in the NEAC assessment due to limited information on consumption frequency and duration. In addition, coffee consumption was not considered for exposure assessment, since it remains unclear whether the main contributors to the *in vitro* antioxidant capacity of coffee are absorbed efficiently and if the same antioxidant activity is exerted *in vivo*.[20]

The questionnaire further provided information on the following potential confounders: weight, height, educational level, alcohol consumption, smoking status, use of vitamin supplements, aspirin use, as well as self-reported history of diabetes, hyperlipoproteinemia and hypertension. Body mass index (BMI) was computed as weight divided by height squared (kg/m^2). Physical activity during a typical day was estimated using a validated Energy Expenditure Questionnaire (EEQ),[21,22] which allowed us to compute an estimate of metabolic equivalent of task (MET)-hours per day (METh/day). MET stands for metabolic energy turnover, with 1 MET corresponding to an energy expenditure of 1 kcal/kg body weight per hour.[23]

Follow-up and outcome

The cohort was followed from 1 October 1997 to 31 December 2010. When analysing incidence data, follow-up ended at time of first MI diagnosis, death, emigration or 31 December 2010, whichever occurred first. Incident cases of MI (ICD-10 code I21) were identified through the Inpatient and Causes of Death Register. We further distinguished between fatal and non-fatal MI by defining fatal cases as subjects who died within a period of 28 days after diagnosis and non-fatal cases as subjects who survived beyond this period.[24] Validation studies on the Inpatient Register have reported a high positive predictive value of at least 95% for MI.[25]

Statistical analyses

Daily dietary NEAC was adjusted for total energy intake according to the nutrient residual method.[26] The distribution of dietary NEAC was categorized into sex-specific quartiles (females Q1–Q4: <7.0, 7–9.2, 9.3–12.1, 12.2–46.5; males Q1–Q4: <6.3, 6.3–8.1, 8.2–10.6, 10.7–42.9). Cox proportional hazards regression models with age as underlying time scale were fitted to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) of MI incidence at different levels of dietary NEAC.

The first quartile of NEAC was considered as the reference value. Based on literature knowledge, we adjusted the multivariable model for the following potential confounders: sex, BMI (kg/m^2, continuous), total physical activity (METh/day, continuous), alcohol consumption (g/month, continuous), smoking status (never, former, current ≤15 cigarettes/day, current >15 cigarettes/day), level of education (<13 years, ≥13 years), history of diabetes (yes/no), aspirin use (yes/no), coffee consumption (cups/day; 0, 1–2, 3–4, ≥5), self-reported lipid disturbance (yes/no), self-reported hypertension (yes/no) and vitamin supplement use (yes/no). We further adjusted the model for energy intake (kcal/day, continuous) as suggested by the nutrient residual method.[26] Continuous variables were included in the model as standardized variables, with mean and standard deviation equal to 0 and 1, respectively.

Ties were handled using the Breslow method.[27] The proportional hazards assumption was tested by using Schoenfeld's residuals. If the assumption was violated, we fitted stratified Cox regression models. We further investigated linear trends by creating a new categorical variable based on the median of each NEAC quartile and by implementing it as a continuous variable in the models. In addition, we investigated a dose–response relationship using restricted cubic splines with four knots based on the median of each NEAC quartile.

We conducted subgroup analyses after stratifying for sex, smoking status (never smokers/ever smokers), BMI (<25/≥25 kg/m^2), alcohol consumption [no (0 g/day)/yes (>0 g/day)], age (≤60/>60 years) and vitamin supplement use (yes/no) and further tested for effect modification on the additive scale using the relative excess risk due to interaction (RERI).[28] To do so, the exposure was dichotomized with the median as cut-off. We further investigated effect modification on the multiplicative scale by including the cross-product interaction term with the main effect terms for dietary NEAC and sex, smoking status (never, former, current ≤15 cigarettes/day, current >15 cigarettes/day), BMI (kg/m^2, continuous), alcohol consumption (g/month, continuous), age (years, continuous) and vitamin supplement use (yes/no), and by running likelihood ratio tests to compare nested models.

We repeated the main and sex-stratified analyses for fatal and non-fatal MI separately.

We performed a first sensitivity analysis by using the Total Radical-Trapping Antioxidant Parameter (TRAP)[18,19] assay expressed as mmol of Trolox per 1-kg fresh weight for the computation of daily dietary NEAC to compare different chemical assays. In addition, because we had no information on serum cholesterol levels, we adjusted the models for the intake of saturated, mono-unsaturated and poly-unsaturated fatty acids measured through the FFQ. We further repeated the analyses after excluding cases occurring during the first 3 years of follow-up to investigate a possible effect of reverse causation.

In a last sensitivity analysis, multiple imputation techniques were used to impute missing values for confounders. Percentages of missing values were equal to 10.3% for total physical activity, 7.7% for smoking status, 4.3% for BMI, 4.2% for history of hyperlipoproteinemia, 4.0% for aspirin use, 3.8% for history of diabetes, 3.2% for history of hypertension, 1.7% for coffee consumption, 1.3% for vitamin supplement use, 1.0% for level of education and 0.1% for alcohol drinking. Under the assumption of data missing at random,[29,30] we fitted multiple imputation models based on chained equations, and we used Rubin's rules[31] to estimate pooled effect estimates and standard errors.

All statistical analyses were performed with Stata version 15.1 (Stata Corporation, College Station, TX, USA). All reported *p*-values were two-sided.

Results

Demographic characteristics of the cohort are shown in Table 1. At baseline, subjects with high dietary NEAC were more likely to be older, better educated, to have a lower BMI and less likely to be smokers than subjects with low dietary NEAC. In our population, foods contributing most to total dietary NEAC were tea (26%), fruits (21%), vegetables (20%), grains (9%) and chocolate (5%).

During a mean follow-up time of 12.7 years (interquartile range: 0.0–13.2 years), we identified a total of 1142 cases of incident MI, of which 205 were fatal and 937 non-fatal. We found a monotonic inverse association between dietary NEAC and subsequent MI incidence in the age-adjusted model, with lowest incidence in the fourth quartile (HR: 0.75, 95% CI: 0.63–0.88, p for trend < 0.001) (Table 2). Since the proportional hazards assumption was violated for sex in the multivariate model, we had to fit a stratified Cox model. After adjustments for potential confounders, the risk among subjects in the fourth quartile of dietary NEAC was 23% lower (HR: 0.77, 95% CI: 0.61–0.96; p for trend $= 0.008$) compared with subjects in the first. The spline analysis did not reveal any departure from linearity (p-value $= 0.56$) (Figure 1). When we run separate Cox regression for men and women, we found similar HRs for men (HR: 0.78; 95% CI: 0.59–1.04; p for trend $= 0.056$) and women (HR: 0.73; 95% CI: 0.49–1.08; p for trend $= 0.040$), although we failed to confirm a statistically significant association in men.

Subgroup analyses further showed a slightly stronger association in non-obese subjects, in never smokers, in alcohol drinkers, in subjects below the age of 60 and in supplement non-users. However, formal tests for interaction were all non-significant, with p-values ranging from 0.49 to 0.80 on the multiplicative scale and p-values for all RERI > 0.14 on the additive scale.

When investigating associations of dietary NEAC with fatal and non-fatal MI separately, we found an inverse association only with non-fatal MI in the fully adjusted model with a HR for Q4 vs Q1 of 0.72 (95% CI: 0.56–0.92; p for trend $= 0.004$) (Tables 3 and 4). We did not detect any departure from linearity after conducting spline analyses (p-value $= 0.28$). When running the analyses for men and women separately, we detected an inverse association in women for non-fatal MI (HR Q4 vs Q1: 0.62, 95% CI: 0.41–0.96; p for trend $= 0.008$), whereas, for men, the effect remained again short of significance (HR: 0.75; 95% CI: 0.55–1.02; p for trend $= 0.065$).

After conducting sensitivity analyses by using the TRAP assay for the assessment of dietary NEAC, by further adjusting the models for saturated, mono-unsaturated and poly-unsaturated fatty acids, by excluding cases occurring during the first 3 years of follow-up and by using imputed missing values in the model, estimates remained essentially the same (results not shown).

Discussion

Our results indicate that the higher the dietary NEAC was at baseline, the lower was the incidence of total and non-fatal MI during follow-up in this cohort of 34 543 Swedish men and women. No association was found for fatal MI.

To the best of our knowledge, only three previous studies have investigated the association between dietary NEAC and MI.[12,14] The Swedish Mammography Cohort study analysed a sample of 38 984 women aged 49–83 years and found a 20% risk reduction for total (HR: 0.80, 95% CI: 0.67–0.97; p for trend $= 0.02$) and a 22% risk reduction for non-fatal MI (HR: 0.78; 95% CI: 0.65–0.95) when comparing the highest with the lowest quintile of dietary NEAC. No association was found for fatal MI (HR: 0.76; 95% CI: 0.48–1.20).[12] Similarly, in the Women's Lifestyle and Health cohort, a cohort consisting of 45 882 women aged 30–49 years, a 40% (HR: 0.60;

Table 1. Baseline characteristics of participants in the Swedish National March Cohort by quartiles of dietary NEAC[a]

Sex-specific quartiles of NEAC	All sample	Q1	Q2	Q3	Q4
Females		<7.0	7.0–9.2	9.3–12.1	12.2–46.5
Males		<6.3	6.3–8.1	8.2–10.6	10.7–42.9
Characteristics	$n = 34\,543$	$n = 8636$	$n = 8636$	$n = 8636$	$n = 8635$
Age (years) mean (SD)	49.4 (15.8)	47.6 (16.1)	49.0 (16.0)	49.8 (15.8)	51.4 (15.2)
Female, %	65.7	65.7	65.7	65.7	65.7
BMI (kg/m^2), mean (SD)	24.5 (3.5)	24.8 (3.6)	24.6 (3.5)	24.4 (3.5)	24.2 (3.3)
Total physical activity (METh/day), mean (SD)	39.5 (12.8)	39.7 (13.6)	39.8 (12.9)	39.5 (12.4)	39.1 (12.0)
Alcohol (grams/month), mean (SD)	306.8 (619.5)	263.9 (411.4)	304.3 (494.9)	317.8 (486.8)	341.1 (938.5)
Energy intake (kcal/day), mean (SD)	2042.9 (538.9)	1992.2 (539.9)	2089.1 (534.3)	2093.6 (540.4)	1996.9 (529.1)
Tea (cups/day), mean, SD	1.0 (1.2)	0.0 (0.2)	0.4 (0.5)	0.9 (0.7)	2.3 (1.4)
Coffee (cups/day), mean, SD	2.9 (1.8)	3.4 (1.9)	3.1 (1.8)	2.8 (1.7)	2.2 (1.7)
Diabetes (yes), %	2.0	1.9	2.0	2.1	2.1
Lipid disturbance (yes), %	2.3	2.2	2.3	2.5	2.3
Aspirin use (yes), %	63.7	63.4	64.2	64.3	62.9
High blood pressure (yes), %	9.9	9.0	9.9	9.8	10.8
Vitamin and supplement use (yes), %	49.6	44.8	48.4	51.2	53.9
Education (≥13 years), %	29.4	22.0	26.1	30.8	38.8
Smoking %					
Never	65.0	61.7	64.5	65.9	67.9
Former	26.9	26.7	26.9	27.2	26.8
Current (≤15 cigarettes/day)	7.6	10.7	8.1	6.6	5.0
Current (>15 cigarettes/day)	0.5	0.9	0.5	0.4	0.4

[a]NEAC was assessed by a validated food-frequency questionnaire and estimated through the Ferric-Reducing Antioxidant Power (FRAP) assay, expressed in mmol Fe^{2+} equivalents/day.

MET, metabolic equivalent of task.

Table 2. Hazard ratios (HRs) with 95% confidence intervals (CIs) for dietary Non Enzymatic Antioxidant Capacity (NEAC)[a] in relation to the risk of overall myocardial infarction, Swedish National March Cohort, 1997–2010

Quartiles of dietary Non Enzymatic Antioxidant Capacity (NEAC)[a]					
	Q1	Q2	Q3	Q4	*p* for trend
No. of cases[b]	292	301	271	278	
Person-years	109 819.07	109 687.52	109 767.58	109 304.98	
Incidence rate[c] (per 100 000 person-years)	532.46	490.41	415.58	428.95	
Total sample					
Age-adjusted HR (95% CI)	1.0 (reference)	0.92 (0.79–1.09)	0.80 (0.68–0.94)	0.75 (0.63–0.88)	0.000
Multivariable HR (95% CI)[d]	1.0 (reference)	0.99 (0.80–1.22)	0.80 (0.65–1.00)	0.77 (0.61–0.96)	0.008
Women					
No. of cases	122	120	108	106	
Age-adjusted HR (95% CI)	1.0 (reference)	0.91 (0.80–1.17)	0.81 (0.62–1.04)	0.74 (0.57–0.96)	0.019
Multivariable HR (95% CI)[d]	1.0 (reference)	1.09 (0.78–1.54)	0.82 (0.57–1.18)	0.73 (0.49–1.08)	0.040
Men					
No. of cases	170	181	163	172	
Age-adjusted HR (95% CI)	1.0 (reference)	0.92 (0.75–1.14)	0.75 (0.60–0.93)	0.69 (0.56–0.86)	0.000
Multivariable HR (95% CI)[d]	1.0 (reference)	0.95 (0.73–1.24)	0.78 (0.59–1.02)	0.78 (0.59–1.04)	0.056

[a]NEAC was assessed by a validated food-frequency questionnaire and estimated through the Ferric-Reducing Antioxidant Power (FRAP) assay, expressed in mmol Fe^{2+} equivalents/day.

[b]Numbers refer to observations included in the age- and sex-adjusted models.

[c]Age-adjusted incident rates.

[d]Adjusted for age, education (<13 years, ≥13 years), smoking [no, former, current (≤15 cigarettes/day; >15 cigarettes/day)], total alcohol intake (g/month), coffee (0, 1–2, 3–4, ≥5 cups/day), diabetes (yes, no), BMI (kg/m^2), total energy intake (kcal/day), vitamin and mineral supplements use (yes, no), total physical activity (METh/day), lipid disturbance (yes, no), high blood pressure (yes, no) and aspirin use (yes, no) (stratified for sex).

95% CI; 0.45–0.81; p for trend < 0.001) lower risk was found for women in the highest quintile of dietary NEAC.[13] Moreover, a case–control study involving 760 cases and 682 controls performed by Rossi *et al.*[14] found that dietary NEAC was inversely and dose-dependently related with the risk of acute non-fatal MI, with an estimated OR equal to 0.41 (95% CI: 0.27–0.63), comparing the fifth with the first quintile of dietary NEAC. This inverse association was stronger in women and in subjects aged 60 years or older. Our results are consistent with these findings, supporting an inverse association for total and non-fatal MI.

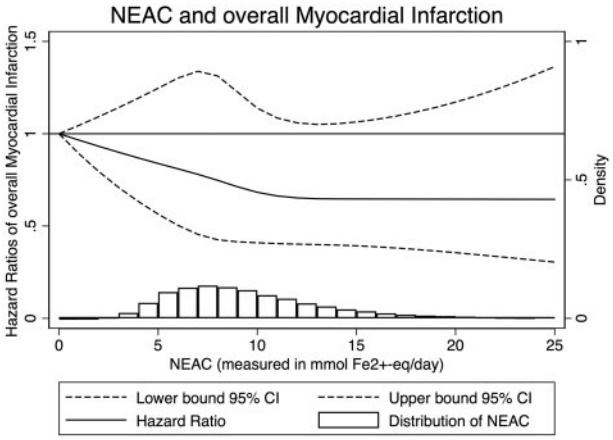

Figure 1. Multivariable-adjusted restricted cubic spline curve for the relation between dietary Non Enzymatic Antioxidant Capacity (NEAC), measured in mmol Fe^{2+} equivalents per day, and the risk of overall myocardial infarction. Adjustments were made for age, (sex), education (<13 years, ≥13 years), smoking [no, former, current (≤15 cigarettes/day; >15 cigarettes/day)], total alcohol intake (g/month), coffee (0, 1–2, 3–4, ≥5 cups/day), diabetes (yes, no), BMI (<18.5, 18.5–25, 25–30, ≥30 kg/m^2), total energy intake (kcal/day), vitamin and mineral supplements use (yes, no), total physical activity (METh/day), lipid disturbance (yes, no), high blood pressure (yes, no) and aspirin use (yes, no).

The role of antioxidants in relation to cardiovascular outcomes is widely recognized and research on this topic is growing.[32,33] However, the hypothesis of a dose–response relationship between dietary antioxidants and risk of cardiovascular events has been questioned due to inconsistent results from observational studies and in particular due to findings of several randomized–controlled trials, which observed an increased risk among individuals who were allocated to high doses of antioxidant supplementation[6,9] exceeding a regular intake through plant-based foods.[34] Additionally, several meta-analyses of randomized clinical trials with large numbers of patients have shown that antioxidant supplementation with vitamins is associated neither with MI nor with other major cardiovascular events.[10,35,36] Based on these findings, it has been suggested that supplements taken alone may not have the same health benefits as a fruit and vegetable rich diet. It has further been argued that isolated antioxidant compounds lose their bioactivity and might not exert the same effect as the compound

Table 3. Hazard ratios (HRs) with 95% confidence intervals (CIs) for dietary Non Enzymatic Antioxidant Capacity (NEAC)[a] in relation to the risk of non-fatal myocardial infarction, Swedish National March Cohort, 1997–2010

Quartiles of dietary Non Enzymatic Antioxidant Capacity (NEAC)[a]					
	Q1	Q2	Q3	Q4	p for trend
No. of cases[b]	249	241	221	226	
Person-years	109 819.07	109 687.52	109 767.58	109 304.98	
Incidence rate[c] (per 100 000 person-years)	435.82	366.09	322.79	309.28	
Total sample					
Age-adjusted HR (95% CI)	1.0 (reference)	0.87 (0.73–1.04)	0.76 (0.64–0.92)	0.72 (0.60–0.86)	0.000
Multivariable HR (95% CI)[d]	1.0 (reference)	0.94 (0.75–1.19)	0.77 (0.61–0.98)	0.72 (0.56–0.92)	0.004
Women					
No. of cases	109	104	82	89	
Age-adjusted HR (95% CI)	1.0 (reference)	0.88 (0.67–1.15)	0.69 (0.52–0.92)	0.70 (0.53–0.93)	0.008
Multivariable HR (95% CI)[d]	1.0 (reference)	1.06 (0.74–1.51)	0.68 (0.46–1.02)	0.62 (0.41–0.96)	0.008
Men					
No. of cases	140	137	139	137	
Age-adjusted HR (95% CI)	1.0 (reference)	0.85 (0.67–1.08)	0.77 (0.61–0.98)	0.68 (0.53–0.86)	0.001
Multivariable HR (95% CI)[d]	1.0 (reference)	0.88 (0.65–1.18)	0.80 (0.59–1.07)	0.75 (0.55–1.02)	0.065

[a]NEAC was assessed by a validated food-frequency questionnaire and estimated through the Ferric-Reducing Antioxidant Power (FRAP) assay, expressed in mmol Fe^{2+} equivalents/day.

[b]Numbers refer to observations included in the age- and sex-adjusted models.

[c]Age-adjusted incident rates.

[d]Adjusted for age, education (<13 years, ≥13 years), smoking [no, former, current (≤15 cigarettes/day; >15 cigarettes/day)], total alcohol intake (g/month), coffee (0, 1–2, 3–4, ≥5 cups/day), diabetes (yes, no), BMI (kg/m^2), total energy intake (kcal/day), vitamin and mineral supplements use (yes, no), total physical activity (METh/day), lipid disturbance (yes, no), high blood pressure (yes, no) and aspirin use (yes, no) (stratified for sex).

Table 4. Hazard ratios (HRs) with 95% confidence intervals (CIs) for dietary Non Enzymatic Antioxidant Capacity (NEAC)[a] in relation to the risk of fatal myocardial infarction, Swedish National March Cohort, 1997–2010

Quartiles of dietary Non Enzymatic Antioxidant Capacity (NEAC)[a]					
	Q1	Q2	Q3	Q4	*p* for trend
No. of cases[b]	43	60	50	52	
Person-years	109 819.07	109 687.52	109 767.58	109 304.98	
Incidence rate[c] (per 100 000 person-years)	96.64	124.32	92.80	120.67	
Total sample					
Age-adjusted HR (95% CI)	1.0 (reference)	1.23 (0.83–1.83)	0.99 (0.66–1.49)	0.91 (0.61–1.37)	0.342
Multivariable HR (95% CI)[d]	1.0 (reference)	1.27 (0.77–2.11)	0.95 (0.55–1.63)	1.10 (0.63–1.91)	0.980
Women					
No. of cases	13	16	26	17	
Age-adjusted HR (95% CI)	1.0 (reference)	1.13 (0.55–2.36)	1.79 (0.92–3.49)	1.07 (0.52–2.21)	0.800
Multivariable HR (95% CI)[d]	1.0 (reference)	1.68 (0.62–4.55)	2.29 (0.87–6.03)	1.90 (0.68–5.28)	0.265
Men					
No. of cases	30	44	24	35	
Age-adjusted HR (95% CI)	1.0 (reference)	1.24 (0.78–1.98)	0.62 (0.36–1.06)	0.77 (0.47–1.25)	0.077
Multivariable HR (95% CI)[d]	1.0 (reference)	1.17 (0.65–2.12)	0.57 (0.29–1.15)	0.86 (0.44–1.66)	0.353

[a]EAC was assessed by a validated food-frequency questionnaire and estimated through the Ferric-Reducing Antioxidant Power (FRAP) assay, expressed in mmol Fe^{2+} equivalents/day.

[b]Numbers refer to observations included in the age- and sex-adjusted models.

[c]Age-adjusted incident rates.

[d]Adjusted for age, education (<13 years, ≥13 years), smoking [no, former, current (≤15 cigarettes/day; >15 cigarettes/day)], total alcohol intake (g/month), coffee (0, 1–2, 3–4, ≥5 cups/day), diabetes (yes, no), BMI (kg/m^2), total energy intake (kcal/day), vitamin and mineral supplements use (yes, no), total physical activity (METh/day), lipid disturbance (yes, no), high blood pressure (yes, no) and aspirin use (yes, no) (stratified for sex).

would do when consumed through whole foods.[37] In addition, the mechanism behind the antioxidant network is extremely complex and exogenous antioxidants come not only from vitamins, but also other compounds with antioxidant properties, such as phytochemicals.[38] NEAC may be a more relevant measure to assess total antioxidant activity from diet because it reflects the antioxidant potential of all redox ingredients in foods. It further considers the synergistic interactions between antioxidants, where especially the interplay between phytochemicals has been suggested to exert potent antioxidant activity.[37] Indeed, foods with the highest contribution to total dietary NEAC in our cohort, such as tea, fruits and vegetables, have been associated with reduced risk of cardiovascular diseases.[4,38–42] To further study the complex mechanisms between antioxidants, it seems indicated to carry out observational prospective studies with even tighter control for known and suspected confounding factors.

Limitations of our study should be considered when interpreting the results. First, this was an observational study. We cannot confidently rule out unmeasured or residual confounding, in particular by elements of a generally healthy lifestyle that were not captured by our data on diet, education, smoking and physical-activity habits. Second, dietary intake was self-reported and assessed only once, hindering us from capturing changes in dietary habits. However, because of the prospective design, reverse causation is essentially eliminated and the potential exposure misclassification due to self-report is most likely non-differential. This would bias estimates of association towards the null, leading to under-estimation of any positive or inverse relationships. Third, we were not able to include vitamin and mineral supplements in the exposure assessment. Therefore, we adjusted for supplement use and additionally fitted stratified models to yield unbiased results for supplement non-users. Finally, the generalizability cannot be taken for granted. Subjects participated on a voluntary basis during a fundraising event for cancer research, which makes our cohort prone to a potential healthy volunteer bias. Further, the composition of diets, including the distribution of NEAC values, may vary considerably between populations. Therefore, the strength of the association might vary from study to study, but the biological mechanism is likely to be the same in most or all populations.

The present study has several strengths. First, it has a prospective design. Second, we used a validated instrument to assess total food consumption in a detailed way. In addition, the assessment of dietary NEAC through an FFQ has been evaluated previously and was suggested to be a useful tool in epidemiological studies.[43–45] Third, essentially complete follow-up through record linkages to well-managed registers with nationwide coverage and mandatory reporting from health care seems to vouch for an almost negligible misclassification of the outcome. Fourth, the size of our

cohort was large and included both men and women aged 18–94 years, allowing us to study differential associations in subgroups defined by age and sex.

In conclusion, the findings of this large prospective study, with careful control for confounding, contradict the summary findings of several randomized intervention trials of antioxidant supplementation. Our findings suggest that a diet rich in antioxidants might protect against MI.

Funding

This work was supported by ICA AB, Telefonaktiebolaget LM Ericsson, the Swedish Cancer Society (Grant CAN 2012/591) and the regional agreement on medical training and clinical research between Stockholm County Council and Karolinska Institutet (to Y.T.L.). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Acknowledgements

We want to thank Statistics Sweden for scanning the questionnaires. Furthermore, we thank the Swedish Cancer Society and volunteers who worked with the National March. Y.T.L. and O.N. designed the research; Y.T.L., E.H., A.G., L.C., R.B., W.Y. and O.N. conducted the research; E.H. and A.G. analysed the data; E.H. and A.G. drafted the paper; Y.T.L, E.H., A.G., L.C., R.B., W.Y. and O.N. revised the manuscript. E.H. and A.G. had primary responsibility for the final content. All authors read and approved the final manuscript.

Conflict of interest: None declared.

References

1. Roth GA, Huffman MD, Moran AE *et al*. Global and regional patterns in cardiovascular mortality from 1990 to 2013. *Circulation* 2015;**132**:1667–78.
2. Yusuf S, Hawken S, Ounpuu S *et al*. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004;**364**:937–52.
3. Klipstein-Grobusch K, Geleijnse JM, den Breeijen JH *et al*. Dietary antioxidants and risk of myocardial infarction in the elderly: the Rotterdam Study. *Am J Clin Nutr* 1999;**69**:261–66.
4. Dauchet L, Amouyel P, Hercberg S, Dallongeville J. Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. *J Nutr* 2006;**136**:2588–93.
5. Wang S, Melnyk JP, Tsao R, Marcone MF. How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health. *Food Res Int* 2011;**44**:14–22.
6. Tribble DL, Committee FN. Antioxidant consumption and risk of coronary heart disease: emphasis on vitamin C, vitamin E, and β-carotene: a statement for healthcare professionals from the American Heart Association. *Circulation* 1999;**99**:591–95.
7. Gaziano JM, Manson JE, Branch LG, Colditz GA, Willett WC, Buring JE. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. *Ann Epidemiol* 1995;**5**:255–60.
8. Tavani A, Negri E, D'Avanzo B, La Vecchia C. Beta-carotene intake and risk of nonfatal acute myocardial infarction in women. *Eur J Epidemiol* 1997;**13**:631–37.
9. Willcox BJ, Curb JD, Rodriguez BL. Antioxidants in cardiovascular health and disease: key lessons from epidemiologic studies. *Am J Cardiol* 2008;**101**:75D–86D.
10. Ye Y, Li J, Yuan Z. Effect of antioxidant vitamin supplementation on cardiovascular outcomes: a meta-analysis of randomized controlled trials. *PLoS One* 2013;**8**:e56803.
11. Serafini M, Del Rio D. Understanding the association between dietary antioxidants, redox status and disease: is the Total Antioxidant Capacity the right tool? *Redox Rep* 2004;**9**:145–52.
12. Rautiainen S, Levitan EB, Orsini N *et al*. Total antioxidant capacity from diet and risk of myocardial infarction: a prospective cohort of women. *Am J Med* 2012;**125**:974–80.
13. Hantikainen E, Lof M, Grotta A *et al*. Dietary non enzymatic antioxidant capacity and the risk of myocardial infarction in the Swedish women's lifestyle and health cohort. *Eur J Epidemiol* 2018;**33**:213–21.
14. Rossi M, Praud D, Monzio Compagnoni M *et al*. Dietary non-enzymatic antioxidant capacity and the risk of myocardial infarction: a case-control study in Italy. *Nutr Metab Cardiovasc Dis* 2014;**24**:1246–51.
15. Rautiainen S, Larsson S, Virtamo J, Wolk A. Total antioxidant capacity of diet and risk of stroke: a population-based prospective cohort of women. *Stroke* 2012;**43**:335–40.
16. Colarusso L, Serafini M, Lagerros YT *et al*. Dietary antioxidant capacity and risk for stroke in a prospective cohort study of Swedish men and women. *Nutrition* 2017;**33**:234–39.
17. Rautiainen S, Levitan EB, Mittleman MA, Wolk A. Total antioxidant capacity of diet and risk of heart failure: a population-based prospective cohort of women. *Am J Med* 2013;**126**:494–500.
18. Pellegrini N, Serafini M, Colombi B *et al*. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J Nutr* 2003;**133**:2812–19.
19. Pellegrini N, Serafini M, Salvatore S, Del Rio D, Bianchi M, Brighenti F. Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different in vitro assays. *Mol Nutr Food Res* 2006;**50**:1030–38.
20. Morales FJ, Somoza V, Fogliano V. Physiological relevance of dietary melanoidins. *Amino Acids* 2012;**42**:1097–109.
21. Lagerros YT, Mucci LA, Bellocco R, Nyrén O, Bälter O, Bälter KA. Validity and reliability of self-reported total energy expenditure using a novel instrument. *Eur J Epidemiol* 2006;**21**:227–36.
22. Lagerros YT, Bellocco R, Adami H-O, Nyrén O. Measures of physical activity and their correlates: the Swedish National March Cohort. *Eur J Epidemiol* 2009;**24**:161–69.
23. Ainsworth BE, Haskell WL, Leon AS *et al*. Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* 1993;**25**:71–80.
24. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project: registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation* 1994;**90**:583–612.

25. National Board of Health and Welfare. *Värdering av diagnoskvaliten för akut hjärtinfarkt i patientregistret 1987 och 1995 [Evaluation of the Validity of the Diagnosis of Acute Myocardial Infarction in the Patient Registry 1987 and 1995]*. Stockholm: National Board of Health and Welfare, Center of Epidemiology, 2000.
26. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997;**65**: 1220S–28S; discussion 9S–31S.
27. Breslow N. Covariance analysis of censored survival data. *Biometrics* 1974;**30**:89–99.
28. Knol MJ, VanderWeele TJ, Groenwold RH, Klungel OH, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. *Eur J Epidemiol* 2011;**26**:433–38.
29. Rubin DB. Inference and missing data. *Biometrika* 1976;**63**: 581–90.
30. van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. *Stat Med* 1999;**18**:681–94.
31. Royston P. Multiple imputation of missing values: update. *Stata J* 2005;**5**:188–201.
32. Salvayre R, Negre-Salvayre A, Camare C. Oxidative theory of atherosclerosis and antioxidants. *Biochimie* 2016;**125**:281–96.
33. Bastide N, Dartois L, Dyevre V *et al*. Dietary antioxidant capacity and all-cause and cause-specific mortality in the E3N/EPIC cohort study. *Eur J Nutr* 2017;**56**:1233–43.
34. Institute of Medicine. *Food and Nutrition Board. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington, DC: National Academy Press, 2000.
35. Myung S-K, Ju W, Cho B *et al*. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. *BMJ* 2013;**346**:f10.
36. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* 2003;**361**:2017–23.
37. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* 2003;**78**:517S–20S.
38. Prior RL, Cao GH. Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. *Hortscience* 2000;**35**:588–92.
39. Hooper L, Kroon PA, Rimm EB *et al*. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2008;**88**:38–50.
40. Geleijnse JM, Launer LJ, van der Kuip DAM, Hofman A, Witteman JCM. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. *Am J Clin Nutr* 2002;**75**:880–86.
41. Liu SM, Stampfer MJ, Hu FB *et al*. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am J Clin Nutr* 1999;**70**:412–19.
42. McKeown NM, Meigs JB, Liu S, Wilson PW, Jacques PF. Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. *Am J Clin Nutr* 2002;**76**:390–98.
43. Rautiainen S, Serafini M, Morgenstern R, Prior RL, Wolk A. The validity and reproducibility of food-frequency questionnaire-based total antioxidant capacity estimates in Swedish women. *Am J Clin Nutr* 2008;**87**:1247–53.
44. Pellegrini N, Salvatore S, Valtuenña S *et al*. Development and validation of a food frequency questionnaire for the assessment of dietary total antioxidant capacity. *J Nutr* 2007;**137**:93.
45. Puchau B, Zulet MA, de Echavarri AG, Hermsdorff HHM, Martinez JA. Dietary total antioxidant capacity is negatively associated with some metabolic syndrome features in healthy young adults. *Nutrition* 2010;**26**:534–41.