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Antioxidants from Diet and Supplements in Relation to Cardiovascular Disease

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ABSTRACT

Many epidemiological studies have reported an inverse association between fruit and vegetable consumption and cardiovascular diseases (CVD). Because fruits and vegetables are high in antioxidants they were hypothesized to be one the factors responsible for the protective mechanisms. However, studies focusing on single antioxidants such as vitamin C, vitamin E and beta-carotene have reported inconsistent results. In diet there is a wide range of substances present with antioxidant properties. Total Antioxidant Capacity is a concept aiming to measure the capacity from all present antioxidants in reducing reactive species by taking into account synergistic and antagonistic interactions. Multivitamin supplements are another source of antioxidants including vitamins and sometimes minerals usually in doses close to recommended daily allowances. A common belief is that multivitamin are good substitute for dietary derived nutrients and may help prevent CVD.

The aims with this thesis were to: 1) examine the validity and reproducibility of food-frequency questionnaire (FFQ)-based Total Antioxidant Capacity estimates. 2) examine whether Total Antioxidant Capacity of diet is associated with the risk of myocardial infarction and stroke among women and if the association is different between CVD-free women and women with CVD history at baseline. 3) examine whether multivitamin supplement use is associated with the risk of myocardial infarction and if the association is different between CVD-free women and women with CVD history at baseline. 4) examine whether multivitamin supplement use is associated with coronary heart disease (CHD) by quantitatively summarizing accumulated evidence with a meta-analytic approach.

The main contributors to Total Antioxidant Capacity of diet were fruits and vegetables (44%), whole grains (18%) and coffee (14%). The FFQ-based Total Antioxidant Capacity estimate, as measured with Oxygen Radical Absorbance Capacity (ORAC) assay, correlated with ORAC in whole plasma (r=0.29) and ORAC in the lipophilic part of plasma (r=0.32). Total Antioxidant Capacity of diet was inversely associated with the risk of myocardial infarction (HR in the highest quintile as compared to the lowest = 0.80, 95% CI: 0.67-0.97) and total stroke (HR in the highest quintile as compared to the lowest = 0.83, 95% CI: 0.70-0.99) among women who were CVD-free at baseline. Among women with CVD history Total Antioxidant Capacity of diet was not associated with myocardial infarction and total stroke. Multivitamin use was inversely associated with the risk of myocardial infarction only among CVD-free women and not among women with CVD history. The summarized evidence from 5 prospective cohort studies indicated a 21% (95% CI: 10-30%) decreased CHD risk among CHD/CVD-free study populations at baseline.

Taken together, these results suggest that Total Antioxidant Capacity may be of importance in primary prevention of myocardial infarction and stroke. Multivitamin supplement use may be of importance in primary prevention of CHD.

LIST OF PUBLICATIONS

I. **Susanne Rautiainen**, Mauro Serafini, Ralf Morgenstern, Ronald L Prior and Alicja Wolk. The validity and reproducibility of food-frequency questionnaire-based total antioxidant capacity estimates in Swedish women. *Am J Clin Nutr*. 2008 May;87(5):1247-53.

II. **Susanne Rautiainen**, Emily B. Levitan, Nicola Orsini, Agneta Åkesson, Ralf Morgenstern, Murray A Mittleman and Alicja Wolk. Total antioxidant capacity of diet and risk of myocardial infarction - a population-based prospective cohort of women. *Am J Med*. Accepted 2012 April.

III. **Susanne Rautiainen**, Agneta Åkesson, Emily B Levitan, Ralf Morgenstern, Murray A Mittleman and Alicja Wolk. Multivitamin use and the risk of myocardial infarction: a population-based cohort of Swedish women. *Am J Clin Nutr*. 2010 Nov;92(5):1251-6. Erratum in: *Am J Clin Nutr*. 2011 Mar;93(3):674.

IV. **Susanne Rautiainen**, Agneta Åkesson, Nicola Orsini and Alicja Wolk. Multivitamin use and risk of incident coronary heart disease – a meta-analysis of prospective cohort studies. Submitted.

V. **Susanne Rautiainen**, Susanna Larsson, Jarmo Virtamo, and Alicja Wolk. Total antioxidant capacity of diet and risk of stroke: a population-based prospective cohort of women. *Stroke*. 2012 Feb;43(2):335-40.

RELATED PUBLICATIONS

Jin-jin Zheng**, Susanne Rautiainen**, Birgitta Ejdervik Lindblad, Ralf Morgenstern and Alicja Wolk. High-Dose Vitamin C and E Supplements, Low-Dose Multivitamins and the Risk of Age-Related Cataract: A Population-Based Prospective Cohort Study of Men. *Am j Epidemiol* . Accepted 2012 May.

Ann Burgaz, Lisa Byberg, **Susanne Rautiainen**, Nicola Orsini, Niclas Håkansson, Johan Arnlöv, Johan Sundström, Lars Lind L, Melhus H, Michaëlsson K and Wolk A. Confirmed hypertension and plasma 25(OH)D concentrations amongst elderly men. *J Intern Med*. 2011 Feb;269(2):211-8.

Jin-jin Zheng, **Susanne Rautiainen**, Ralf Morgenstern and Alicja Wolk. Relationship between plasma carotenoids, fruit and vegetable intake, and plasma extracellular superoxide dismutase activity in women: different in health and disease? *Antioxid Redox Signal*. 2011 Jan 1;14(1):9-14.

Susanne Rautiainen, Birgitta Ejdervik Lindblad, Ralf Morgenstern and Alicja Wolk. Vitamin C supplements and the risk of age-related cataract: a population-based prospective cohort study in women. *Am J Clin Nutr*. 2010 Feb;91(2):487-93.

CONTENTS

LIST OF ABBREVIATIONS

AAPH	2,2'-azobis(2-amidino-propane) dihydrochloride
BMI	Body Mass Index
CI	Confidence Interval
CVD	Cardiovascular Diseases
HAT	Hydrogen Atom Transfer
H_2O_2	Hydrogen peroxide
HR	Hazard ratio
FFQ	Food-Frequency Questionnaire
FRAP	Ferric Reducing Antioxidant Power
MI	Myocardial Infarction
O_2*	Superoxide
OH*	Hydroxyl radical
ORAC	Oxygen Radical Absorbance Capacity
ROO*	Peroxyl radical
RR	Relative Risk
SAT	Saturated fatty acid
SET	Single Electron Transfer
SMC	Swedish Mammography Cohort
SMC-C	Swedish Mammography Cohort - Clinical
SOD	Superoxide Dismutase
TAC	Total Antioxidant Capacity
TE	Trolox Equivalents
TPTZ	2,4,6-tri(2-pyridyl)-1,3,5-triazine
TRAP	Total Radical-Trapping Antioxidant Parameters
WHO	World Health Organization

1 BACKGROUND

1.1 OXIDATIVE AND ANTIOXIDATIVE SYSTEMS

1.1.1 Oxidative stress

Oxidative stress is a condition which is not clearly defined. Previously, it has been defined as an imbalance between antioxidants and pro-oxidants shifted towards excessive amounts of the latter. However, this old definition may be too simplified because the redox regulation system is more complex. In the textbook of Halliwell et al. the authors have redefined the oxidative stress as the "biochemical damage caused by attack of reactive species upon the constituents of living organism" (Halliwell et al. 2007). The authors state that oxidative stress can appear from different situations:

- Depletion of antioxidants as a results of gene mutation of antioxidant enzymes (endogenous antioxidants), low levels of dietary antioxidants due to poor diet, or a disease leading to malnutrition.
- Elevated production of reactive species as a consequence of high levels of oxygen, presence of toxins, or activation of biological systems producing reactive species.

There are many types of reactive species and they are usually referred to as free radicals, reactive oxygen species and reactive nitrogen species (Halliwell et al. 2007). Examples of reactive species are singlet oxygen, superoxide (O_2^{*}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^{*}). The defense system against oxidative stress consists of endogenous and exogenous (dietary) antioxidants (Serafini et al. 2004).

1.1.2 Endogenous antioxidants

The endogenous antioxidant defense system consists of a number of enzymes and other compounds. Much attention has been on the antioxidant enzymes such as superoxide dismutases (SOD), glutathione peroxidases, catalase and thioredoxin reductases (Forsberg et al. 2001). In **Figure 1.1,** a simplified picture of the interplay between oxidants and endogenous antioxidants is shown (Nordberg et al. 2001). The role of SOD enzymes is mainly to scavenge superoxide into the less but still reactive hydrogen peroxide. Hydrogen peroxide can then be removed by enzymes such as glutathione peroxidases (GPx) and catalase, as well as glutathione (GSH).

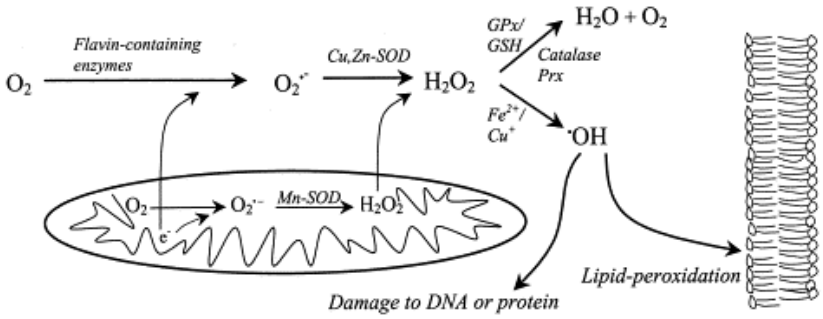


Figure 1.1. Simplified nonstoichiometric scheme of oxidative and antioxidative systems in cells (Nordberg et al. 2001)

In the presence of metals e.g. iron or cupper hydrogen peroxide can be converted into hydroxyl radical, a very reactive molecule. This is called the Fenton reaction (Jomova et al. 2011).

1.1.3 Exogenous Antioxidants

Derived from diet

The interest in antioxidants in relation to health has arisen from many epidemiological studies reporting inverse association between fruit and vegetable intake and cardiovascular diseases (CVD) (Dauchet et al. 2009). Because fruits and vegetables are high in antioxidants they were hypothesized to be one of the factors responsible for the protective mechanisms (Prior 2003) . This hypothesis led to a wide range of studies focusing mainly on antioxidant vitamins such as vitamin C and E as well the beta-carotene. These studies were not that convincing. Especially clinical trials were not providing evidence of protective effects on CVD risk from supplementation of vitamin C, vitamin E and beta-carotene since high doses had little effect (Rapola et al. 1997; Yusuf et al. 2000; Lee et al. 2005; Lonn et al. 2005; Sesso et al. 2008) and even harmful effects were reported (Bjelakovic et al. 2008). Moreover, a randomized clinical trial on a low-dose antioxidant supplement (including 120 mg ascorbic acid, 30 mg vitamin E, 6 mg beta carotene, 100 µg selenium, and 20 mg zinc) did not observe any association with ischemic CVD (Hercberg et al. 2004). The results from clinical trials were disappointing and many researchers lost their trust in the antioxidant hypothesis.

However, there is wide range of phytochemicals besides vitamin C, vitamin E and beta-carotene with antioxidant properties such as other carotenoids and polyphenols present in diet (Dauchet et al. 2009). These phytochemicals are usually present in doses much lower than those used in supplementation trials and are working synergistically or antagonistically with each other.

Derived from dietary supplements

The use of dietary supplements has substantially increased during the past decade, and in Sweden and the United States nearly 50% of people report use of any kind of supplements (Messerer et al. 2001; Millen et al. 2004). Multivitamin and mineral supplements are the most frequently used preparations (Messerer et al. 2001; Radimer et al. 2004) and a common belief is that they ensure an adequate nutrient intake not only to prevent deficiency but also CVD (Neuhouser et al. 1999). Multivitamins contain a wide range of nutrients such as antioxidant vitamins (Fairfield et al. 2002; Thomson et al. 2007), B-vitamins (Fairfield et al. 2002; McNulty et al. 2008) and some supplements include also minerals such as magnesium and selenium (Brigelius-Flohe et al. 2003; Bo et al. 2008), which all have been inversely related to CVD.

1.1.4 Total Antioxidant Capacity

Total Antioxidant Capacity is a concept aiming to measure the total antioxidant defense system in reducing reactive species by taking synergistic and antagonistic interactions between compounds into account. The magnitude of the antioxidant capacity is affected by several parameters such as antioxidant activity, oxidizing substrate, red-ox interactions, red-ox potential and physiological stress (Serafini et al. 2004; Prior et al. 2005). There are several assays available to capture Total Antioxidant Capacity of a food item or biological sample. In these assays, a reactive specie is introduced to the sample and antioxidant activity is measured with spectrophotometry or fluorescence.

Assays for measurement of Total Antioxidant Capacity

Because the antioxidant defense system is very complex and constitutes several different reaction mechanisms, there is no single assay that will give accurate results (Serafini et al. 2004; Prior et al. 2005). The mechanism of an assay system will depend on factors such as antioxidant structure and properties, solubility and system solvent (Prior et al. 2005). The assays measuring Total Antioxidant Capacity are based on two major mechanisms called Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET). Methods based on HAT measure an antioxidant's capacity to quench reactive species by hydrogen donation, which is proposed to be a typical action of antioxidants. Methods based on SET mechanisms measure an antioxidant's ability to reduce any compound through delivery of an electron. Both HAT and SET mechanisms appear in a sample and the balance between these two reactions is determined by the structure of the present antioxidants and pH. **Table 1.1** lists examples of available assays for measurement of Total Antioxidant Capacity.

Table 1.1. Examples of assays measuring Total Antioxidant Capacity (Prior et al. 2005).

Assay	Simplicity	Biological relevance	Mechanism	Lipophilic and hydrophilic TAC
ORAC	++	+++	HAT	+++
TRAP	---	+++	HAT	--
FRAP	+++	--	SET	---
TEAC	+	-	SET	+++
LDL oxidation	-	+++	HAT	---

+, ++, +++; desirable to highly desired characteristic. -, - -, - - -; less desirable to highly undesirable. TAC: Total Antioxidant Capacity; ORAC: Oxygen Radical Absorbance Capacity; TRAP: Total Radical-trapping Antioxidant Parameters; FRAP: Ferric Reducing Antioxidant Power; TEAC (Trolox Equivalent Antioxidant Capacity); HAT: Hydrogen Atom Transfer; SET: Single Electron Transfer

Examples of assays based on HAT are the Oxygen Radical Absorbance Capacity (ORAC), Total Radical-trapping Antioxidant Parameters (TRAP) and LDL-oxidation. Assays based on SET are Ferric Reducing Antioxidant Power (FRAP), and the Trolox Equivalent Antioxidant Capacity (TEAC) assay (Prior et al. 2005). Total Antioxidant Capacity is often expressed as Trolox Equivalents TE/l or TE/g. Trolox is a vitamin E analogue and is used as the standard for determination of Total Antioxidant Capacity of a sample (Huang et al. 2005).

At the First International Congress on Antioxidant Methods, researchers discussed how to resolve the complex of problems that have arisen from the wide range of methods used in analyzing antioxidants (Prior et al. 2005). The ORAC assay was considered to be the method that meets most of the important requirements when analyzing Total Antioxidant Capacity. The ORAC assay measures an antioxidant's ability to inhibit peroxyl radical induced oxidation which is a classical radical chain breaking antioxidant activity.

Total Antioxidant Capacity in foods

There are several food databases constructed with Total Antioxidant Capacity values including a wide range of food items that are commonly consumed. These databases are based on the ORAC (Prior et al. 2003; Sanchez-Moreno et al. 2003; Wu et al. 2004), TRAP (Pellegrini et al. 2003; Pellegrini et al. 2006), FRAP (Halvorsen et al. 2006; Carlsen et al. 2010) or TEAC assay (Pellegrini et al. 2003; Pellegrini et al. 2006). The most extensive available food database is based on the FRAP assay (Carlsen et al. 2010).

The method most frequently used in nutritional epidemiology to study dietary exposures is the food-frequency questionnaire (FFQ) and it is designed to reflect the usual intake over a longer period of time. Intake of antioxidants can be estimated by the use of FFQs by summarizing different food items with known Total Antioxidant Capacity values. However, nutrient estimates obtained with FFQs are measured with varying degree of measurement error because the assessment relies on participant's ability to accurately recall usual frequency of each food consumed over the designated time period (Spiegelman et al. 2005). Furthermore, before FFQ-based Total Antioxidant Capacity estimates can be used as a reliable estimate for assessing dietary antioxidant intake in epidemiological studies it needs to be validated by comparison with a more objective measure.

Total Antioxidant Capacity in blood

Blood has been considered as the best biological system for measuring Total Antioxidant Capacity, because it has a central role in transport of nutrients and redistribution of antioxidants in the body (Serafini et al. 2004). However, it may be questioned how well the Total Antioxidant Capacity assays are capturing the true network in blood. Some assays are very sensitive to certain compounds present in blood e.g. the FRAP assay is highly correlated with uric acid (Natella et al. 2002; Moura-Nunes et al. 2009).

Dietary supplements

The dietary supplement industry has adapted the Total Antioxidant Capacity concept by labeling their product's antioxidant content. There is very little evidence supporting a hypothesis that dietary supplements are a good substitute for diet high in antioxidant content. Moreover, today it is unknown whether intake of dietary supplements is contributing to Total Antioxidant Capacity in blood. In one randomized clinical trial a multivitamin supplement was not associated with any significant increase in ORAC in whole plasma (McKay et al. 2000).

1.2 CARDIOVASCULAR DISEASES

1.2.1 Atherosclerosis

Atherosclerosis is a condition with a long lag time from onset to clinical manifestation (Napoli et al. 2006). The early onset of atherosclerosis is characterized by fatty streaks which occur when oxidized low-density lipoproteins (ox-LDL) initiate inflammation. Fatty streaks can further develop into atherosclerotic plaques, induced by smooth muscle cell migration together with monocytes and macrophage propagation.

Calcification of atherosclerotic plaques cause fibrous plaques which if rupture can cause myocardial infarction or stroke (Madamanchi et al. 2005; Napoli et al. 2006).

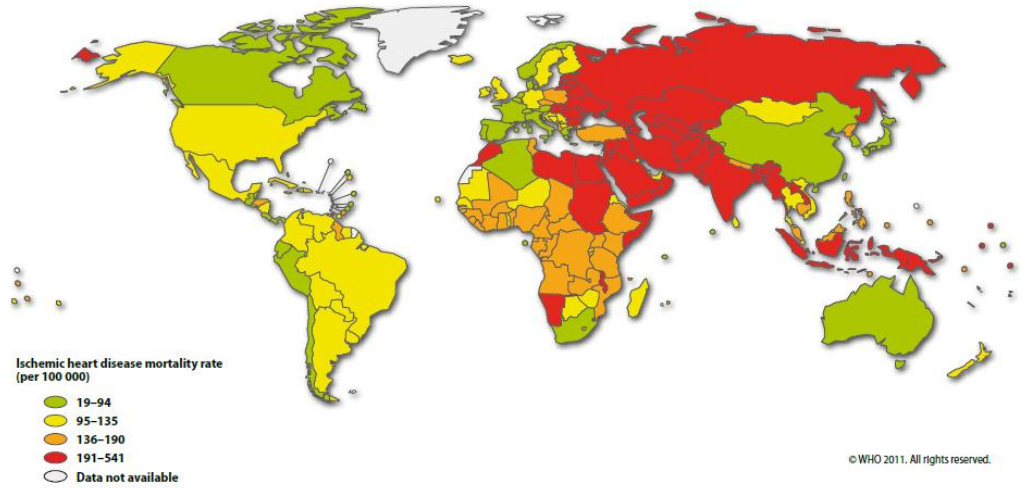
1.2.2 Myocardial infarction

The heart is a muscle that unlike other muscles never rest. The heart is responsible for all cardiovascular system functions by pumping around the blood to allow continuous exchange between the peripheral tissues and the blood stream. The heart requires a constant supply of oxygen and nutrients through arteries which is maintained by the coronary circulation. Atherosclerotic plaques can clot blood vessels supplying the heart, causing myocardial infarction (Thygesen et al. 2007; WHO 2011).

Descriptive Epidemiology

In **Figure 1.2** the world distribution of mortality rates of coronary heart disease are shown (WHO 2011). Myocardial infarction is one of the leading causes of death worldwide (Kim et al. 2011; Socialstyrelsen 2011). The diagnosis of myocardial infarction and the etiology may differ between women and men. The incidence rate is higher among men than among women but has during the past decade decreased among men. The incidence rate among women in Sweden has been stable over time, however, during the last years a decrease in rate is observed. **(Figure 1.3)** (Socialstyrelsen 2011). The age-standardized fatality rate within 28 days from myocardial infarction has dramatically decreased over the past decades but the fatility rate is yet still somewhat higher among women (32%) than in men (29%) (Socialstyrelsen 2011). This difference may be due to different roles of risk factors, delay in event diagnosis and treatment.

Men



Women

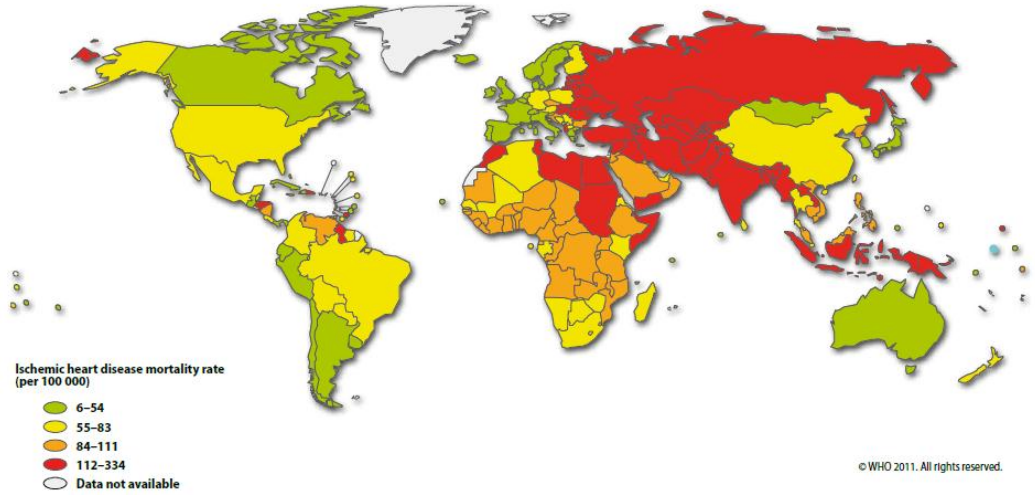


Figure 1.2. Age- and sex-adjusted mortality rates of ischemic heart disease per 100,000 among men and women (WHO 2011).

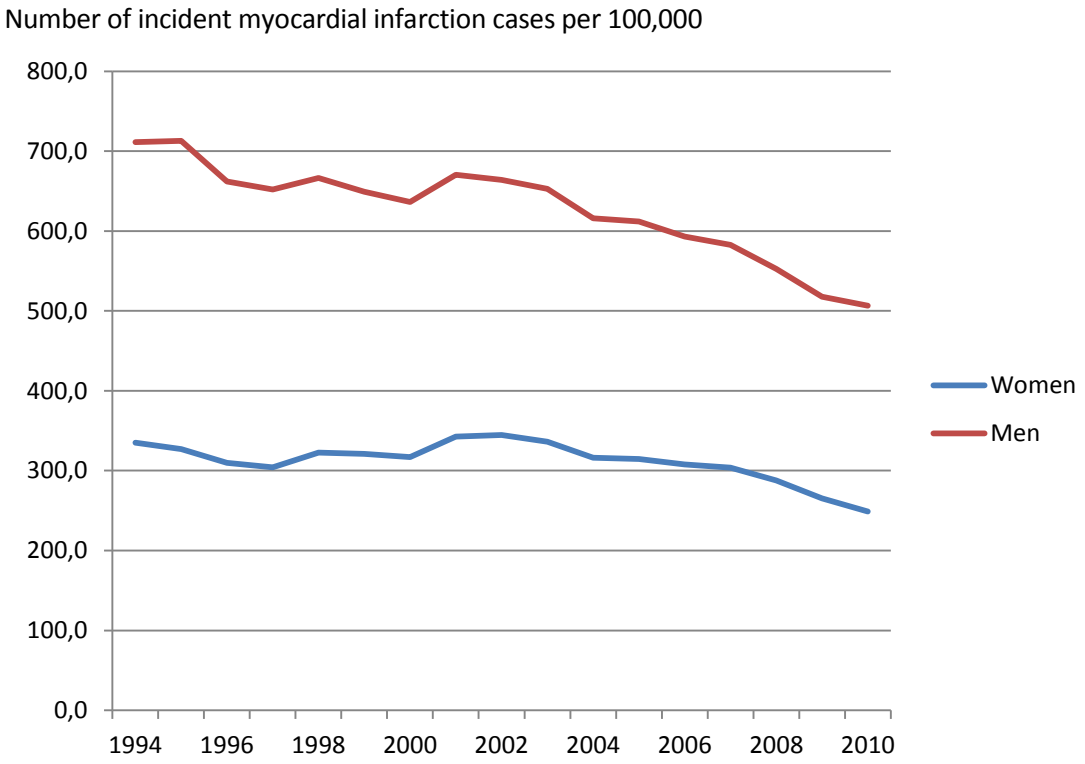


Figure 1.3. Trends in incidence rates of myocardial infarction per 100,000 in Sweden between 1994 and 2010. Rates are adjusted to the age-distribution in Sweden 2010 (Socialstyrelsen 2012).

Potential dietary risk factors

A healthy dietary pattern characterized by high consumptions of fruits, vegetables, whole grains, and nuts has been associated with reduced risk of coronary heart disease (Akesson et al. 2007; Hu 2009). Moreover, a low-risk lifestyle defined as not smoking, not being obese, exercise >30 minutes/day, a healthy dietary pattern, and a moderate intake of alcohol has been associated with reduced risk of sudden cardiac death (Chiuve et al. 2011). Studies also indicate that different types of fats and carbohydrates are more important than total amounts with regard to coronary heart disease risk (Hu 2009; Astrup et al. 2011). Several constituents besides those with antioxidant properties have been suggested to be responsible for the protective effects such as, dietary fiber, folate, vitamin B12, vitamin B6, calcium, magnesium, and polyunsaturated fatty acids (Hu 2009; Mozaffarian et al. 2011).

Fruit and vegetable consumption has been inversely associated with coronary heart disease risk (Mente et al. 2009). In a systematic review, authors concluded that fruit and vegetable consumption met three of four criteria of causation score based on the Bradford Hill guidelines (Mente et al. 2009).

Coffee consumption has in moderate amounts been inversely associated with coronary heart disease in a meta-analysis of 21 prospective cohort studies (Wu et al. 2009). In the Swedish Mammography Cohort, coffee was non-significantly inversely associated with coronary heart disease (Rosner et al. 2007). Coffee is high in polyphenols e.g. chlorogenic acid and caffeic acid which have antioxidant properties (Ranheim et al. 2005).

Tea consumption in association with coronary heart disease risk was evaluated in a meta-analysis of prospective cohort studies. Green tea consumption was inversely related to coronary heart disease risk, however, only a few studies were included in this summary estimate. The summary estimates did not support a protective role of black tea consumption (Wang et al. 2011).

Whole grain consumption has been inversely associated with coronary heart disease risk (Mente et al. 2009). In a meta-analysis, authors concluded that whole grain consumption met three of four criteria of causation score based on the Bradford Hill guidelines (Mente et al. 2009). Whole grain are high in fiber which have been inversely associated with ischemic CVD in Swedish women (Wallstrom et al. 2012). In an American study, fibers from grains were associated with reduced CVD mortality among both women and men (Park et al. 2011).

Fish consumption has been inversely associated with coronary heart disease risk. In a meta-analysis, the inverse association between fish consumption and coronary heart disease risk met three of four criteria of causation score based on the Bradford Hill guidelines (Mente et al. 2009). Omega-3 fatty acids in fish have been hypothesized to be responsible for the protective mechanisms. Omega-3 fatty acid supplementation was not associated with secondary vascular events in a randomized clinical trial (Galan et al. 2010). In a recent meta-analysis of secondary prevention randomized clinical trials, the author concluded that there is insufficient evidence that omega-3 fatty acid supplementation is reducing CVD mortality (Kwak et al. 2012).

Meat consumption was non-significantly associated with increased coronary heart disease risk in a meta-analysis. However, this association only met one of four criteria of causation score based on the Bradford Hill guidelines (Mente et al. 2009). In a recent study of two American prospective cohort studies of women and men, both unprocessed and processed red meat was associated with a statistical significant increased CVD mortality (Pan et al. 2012).

Non-dietary risk factors

Lipid profile has been a major target in coronary heart disease prevention. Low levels of low-density lipoproteins (LDL) and high levels of high-density-lipoproteins (HDL) have been suggested to be favorable in inhibiting the atherosclerotic process. Lipid-

lowering therapies e.g. statins have been successful in the prevention of coronary heart disease (Schaefer 2011).

Hypertension is a well-established risk factor for coronary heart disease (Reaven et al. 1996). Hypertension decreases coronary blood flow, affects coronary vascular resistance and contributes to endothelial dysfunction (Frohlich 1999).

Diabetes is an important cause of coronary heart disease morbidity and mortality worldwide. In fact, cardiovascular diseases account for two-thirds of deaths in patients with diabetes (Danaei et al. 2006). Diabetes type 2 has been associated with accelerated development of atherosclerosis which may be due to conditions such as insulin resistance, hyperinsulinemia and hyperglycemia (Hayden et al. 2000). In a meta-analysis, elevated fasting blood-glucose was considered to be one of the most important risk factors for CVD mortality (Danaei et al. 2006).

Metabolic syndrome has in a recent meta-analysis of accumulated evidence been linked to 2-fold increased risk of myocardial infarction (Mottillo et al. 2010). The metabolic syndrome is defined by having three or more of the following factors (Grundy et al. 2004):

- Abdominal obesity (waist circumference among women >88 cm and among men >102 cm)
- High triglyceride concentrations (>15 mg/l)
- Low HDL concentrations (women <50 mg/dl, men <40 mg/dl)
- Hypertension (>130/85 mm Hg)
- High fasting glucose (>11 mg/l)

Smoking is an established risk factor for coronary heart disease (Ambrose et al. 2004; Mosca et al. 2007). Not all toxic compounds of cigarette smoke are identified, however, cigarette smoke generates free radicals which may contribute to atherosclerosis progression (Ambrose et al. 2004).

There is strong evidence that **physical activity** is reducing the risk of coronary heart disease (Ahmed et al. 2012). Physical activity has been associated with favorable changes in markers associated with atherosclerosis such as triglyceride reduction, apolipoprotein B reduction, HDL increase, alteration in LDL particle size and decrease in coronary artery calcification (Ahmed et al. 2012).

Observational studies on dietary antioxidants and coronary heart disease

There is no previous study investigating the association between Total Antioxidant Capacity of diet and risk of coronary heart disease. However several prospective cohort studies have investigated how dietary intakes and blood concentrations of single antioxidants such as vitamin C, vitamin E and beta-carotene are associated with the risk

of coronary heart disease. These studies have reported mixed results (Asplund 2002). Many epidemiological studies focusing on carotenoids have observed that dietary intake and blood concentrations of carotenoids are inversely associated with the risk of coronary heart disease (Voutilainen et al. 2006). Observational studies focusing on polyphenols such as flavonols, flavones, catechins, and lignans suggest beneficial effects of both flavonoids and lignans on coronary heart disease (Arts et al. 2005).

Observational studies on dietary supplement use and coronary heart disease
Despite the widespread use of multivitamins there is limited data in relation to coronary heart disease incidence. Observational studies examining coronary heart disease incidence and mortality have reported mixed results. The majority of studies examining coronary heart disease incidence have reported statistically significant inverse associations (Rimm et al. 1998; Klipstein-Grobusch et al. 1999; Holmquist et al. 2003), however, one observed no association (Neuhouser et al. 2009). One study examining coronary heart disease mortality, observed an inverse association (Watkins et al. 2000) and another observed no association (Muntwyler et al. 2002).

Randomized-controlled trials of antioxidant supplements and coronary heart disease
Previous randomized controlled-trials testing antioxidant supplements containing one to three compounds have failed to observe any benefit on coronary heart disease (Rapola et al. 1997; Yusuf et al. 2000; Lee et al. 2005; Lonn et al. 2005; Cook et al. 2007; Sesso et al. 2008). Moreover, one randomized controlled trial studying the effect of a low-dose supplement of five antioxidants (including 120 mg ascorbic acid, 30 mg vitamin E, 6 mg beta carotene, 100 µg selenium, and 20 mg zinc) observed no significant association for ischemic CVD (Hercberg et al. 2004). Notably, in a meta-analysis of randomized controlled trials, high-doses and very high-doses of single supplements of vitamin A, β-carotene or vitamin E, was associated with higher mortality (Bjelakovic et al. 2008).

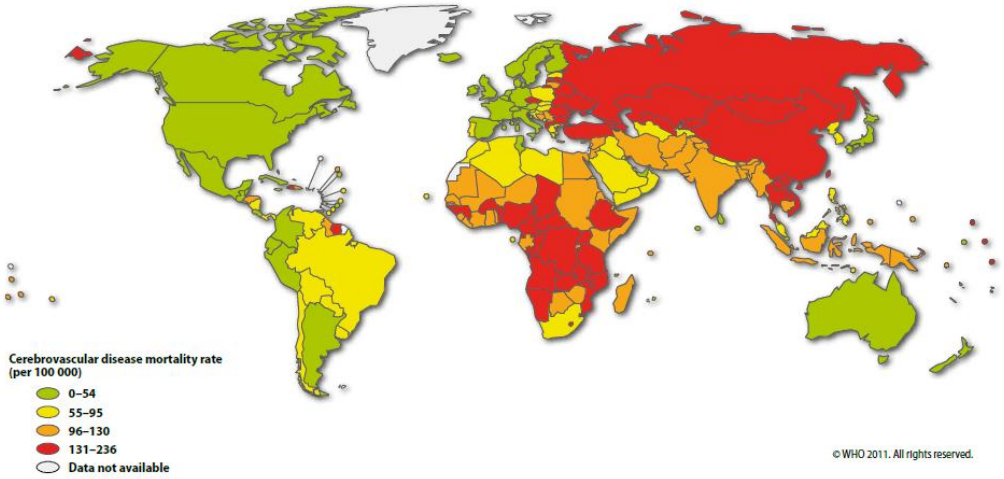
1.2.3 Stroke

The brain is a very complex organ consisting of tens of billion neurons and performs bewildering array of functions. Unlike many other organs such as the heart, the liver and the kidney, the brain is not replaceable if damaged. The brain requires a constant supply of oxygen and glucose to function. If there is a rupture in oxygen supply severe damage is caused quite immediately. Cerebral infarction and coronary heart disease share almost the same etiology characterized by the atherosclerotic process. Cerebral infarction is the most common stroke subtype and accounts for approximately 85% of all strokes (Socialstyrelsen 2009). Although hemorrhagic stroke is less common, it is causing the most damage referring to disability and mortality. The etiology is more well-studied cerebral infarction than for hemorrhagic stroke.

Descriptive Epidemiology

In **figure 1.4** the mortality rates per 100,000 world-wide are shown (WHO 2011). Stroke is the leading cause of death after heart disease in the world (Lopez et al. 2006) and a major cause of disability-adjusted life years (DALY) due to both increased mortality and living with disability. In Sweden, the incidence rate of stroke has been decreasing over time both among women and men (**figure 1.5**) (Socialstyrelsen 2012).

Men



Women

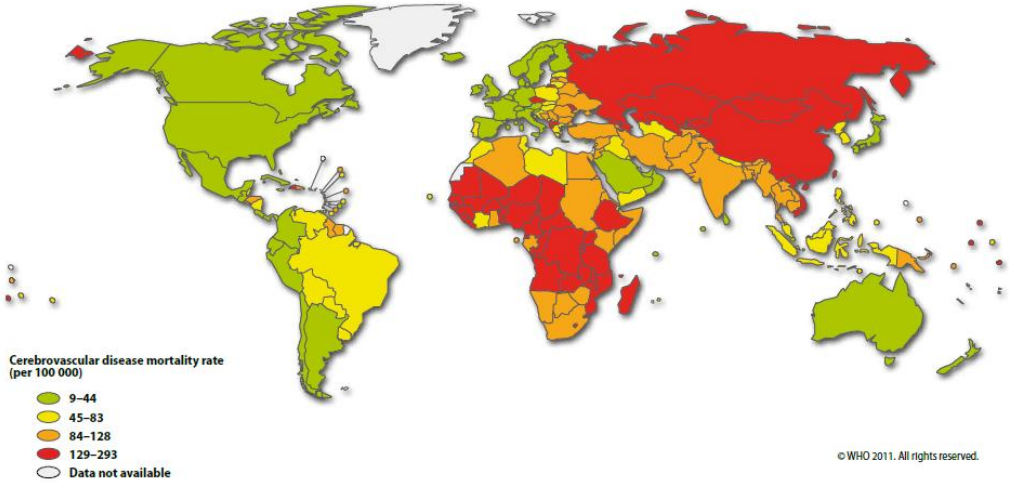


Figure 1.4. Age- and sex-adjusted mortality rates of stroke per 100,000 among men and women (WHO 2011).

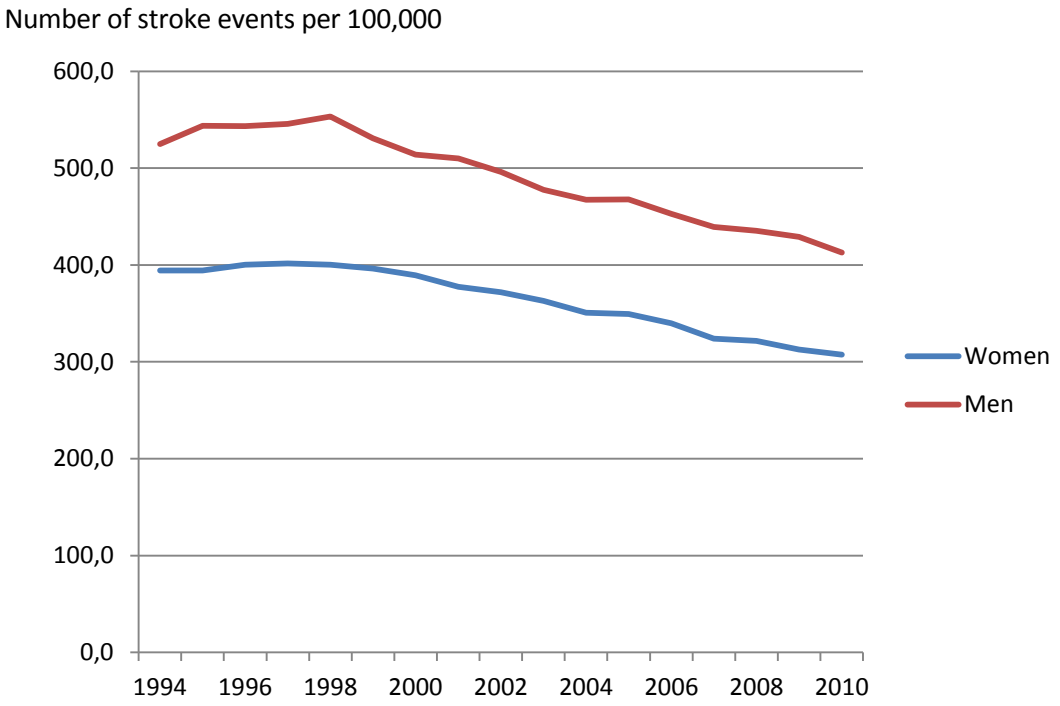


Figure 1.5. Trends in incidence rates of stroke per 100,000 in Sweden between 1994 and 2010. Rates are adjusted to the age-distribution in Sweden 2010 (Socialstyrelsen 2012).

Potential dietary risk factors

There is relatively limited evidence from observational studies on diet and the risk of stroke. Most of the studies have investigated total stroke and have not distinguished between cerebral infarction and hemorrhagic stroke.

Fruit and vegetable consumption has been inversely associated with stroke risk. In a meta-analysis of accumulated evidence, more than three servings of fruits and vegetables per day was inversely associated with both cerebral infarction and hemorrhagic stroke (He et al. 2006). Many constituents in fruits and vegetables have been hypothesized to have a protective effect e.g. antioxidants, dietary fiber, folate and potassium (Hankey 2012).

Coffee consumption has in moderate levels been weakly inversely associated with total stroke (Larsson et al. 2011). In the Swedish Mammography Cohort coffee consumption was investigated with different stroke subtypes and was associated with decreased risk of cerebral infarction and subarachnoid hemorrhage (Larsson et al. 2011). Coffee is high in polyphenols which are hypothesized to interfere with atherosclerotic process (Hankey 2012). On the other hand, caffeine in coffee have been suggested to increase risk of stroke by raising blood pressure (Hankey 2012). Indeed, an increase in cerebral infarction risk after coffee consumption has only been observed in one case-crossover study among infrequent consumers suggesting a potential role of caffeine as a trigger of stroke (Mostofsky et al. 2010).

Green and black tea consumption has been inversely associated with total stroke risk in a meta-analysis of prospective studies (Arab et al. 2009). Green and black tea contain polyphenols which are suggested to exert favorable effects on the atherosclerotic process (Hankey 2012).

Whole grain consumption was non-significantly inversely associated with the risk of total stroke in a meta-analysis of four prospective cohort studies (Mellen et al. 2008). Whole grains include dietary fiber, phytochemicals with antioxidant properties, B vitamins, minerals and various fatty acids. Whole grain consumption has been shown to have favorable effects on insulin sensitivity, endothelial function and inflammation (Hankey 2012).

Fish consumption (more than three servings per week) has been inversely associated with the risk of cerebral infarction and hemorrhagic stroke in observational studies (Larsson et al. 2011). Both fatty and lean fish consumption has been suggested to be beneficial. Fatty fish contains omega-3 fatty acids which have shown to have favorable effects on blood pressure, plasma triglycerides, and markers of thrombosis and inflammation (Hankey 2012).

Meat consumption, especially processed meat consumption, was in the Swedish Mammography Cohort associated with increased risk of cerebral infarction (Larsson et al. 2011). This finding was also observed among men (Larsson et al. 2011). In a recent study of two American prospective cohorts both unprocessed and processed red meat was associated with increased risk of total stroke (Bernstein et al. 2012). Processed meat is high in sodium which has been associated with increased blood pressure, a main risk factor for stroke (Hankey 2012).

Non-dietary risk factors

The role of hypercholesterolemia in stroke risk is unclear. Although atherosclerosis is detected in intracranial arteries, the severity of atherogenesis is much less than in extrachranial arteries and occurs much later in life (Napoli et al. 2006). It is not clear why this difference in atherosclerosis is observed however, intracranial arteries have much higher activity of endogenous antioxidant enzymes which dramatically decreases with age. Endothelial dysfunction has been suggested to be, independently from atherosclerosis, a risk factor for stroke (Napoli et al. 2006).

Hypertension is one of the most important risk factors for all types of stroke including cerebral infarction and hemorrhagic stroke (Dubow et al. 2011). Good control of hypertension has been linked to a dramatic reduction in stroke incidence and mortality. Hypertension has been shown to precede atherosclerosis progression, cause damage to the endothelium, and impair cerebral vasodilation (Dubow et al. 2011).

Diabetes is a well-established risk factor for cerebral infarction and is suggested to be an important risk factor also for hemorrhagic stroke (Luitse et al. 2012). In a meta-analysis of the accumulated evidence from prospective cohort studies diabetes was associated with increased risk of both cerebral infarction and hemorrhagic stroke (Sarwar et al. 2010).

Atrial fibrillation is characterized by the upper chambers of the heart malfunction in pumping all of the blood into the lower chambers which can cause a blood clot. When the clot breaks off it can block an artery in the brain, causing a stroke. Atrial fibrillation is associated with high risk of stroke and the risk varies by age and other medical conditions such as hypertension, diabetes, heart failure, and history of stroke (Ruff 2012).

Smoking is an established risk factor for all types of stroke. Plausible mechanisms by which smoking can increase the risk are numerous and include carboxyhemoglobinemia, increased platelet aggregability, elevated fibrinogen levels, lower levels of HDL-cholesterol, and direct effects of toxic compounds (Shah et al. 2010).

Physical activity has been inversely associated with risk of stroke. However, there is limited evidence on potential mechanisms of the favorable effects on stroke risk. On the other hand, several studies on physical activity and cardiovascular disease in general have contributed to understanding the complex physiologic effects. Physical activity has been shown to impair the atherosclerotic process and thereby reducing the risk of stroke (Alevizos et al. 2005).

Alcohol consumption has been differently associated with the risks of cerebral infarction and hemorrhagic stroke (Hillbom et al. 2011). Some studies suggest that light to moderate alcohol consumption is associated with lower cerebral infarction risk whereas most studies have observed a linear association between alcohol consumption and hemorrhagic stroke. A recent meta-analysis reported a J-shaped association between alcohol consumption and cerebral infarction and suggested a linear association between alcohol consumption and hemorrhagic stroke. It was also reported that women had higher risks of stroke than men related to alcohol consumption (Patra et al. 2010).

Observational studies on dietary antioxidants and stroke

There is only one previous prospective study investigating the association between Total Antioxidant Capacity of diet and risk of stroke. This study investigated Total Antioxidant Capacity of diet with the FRAP method and included 112 cerebral infarctions and 48 hemorrhagic stroke cases among CVD-free men and women aged 50-61y. There was an inverse association observed for cerebral infarction (HR = 0.41; 95% CI = 0.23-0.74 for the highest vs lowest category) but non-significant increased

risk for hemorrhagic stroke (Del Rio et al. 2011). There is no study investigating the association between Total Antioxidant Capacity and risk of stroke among participants with CVD history at baseline.

Several epidemiologic studies have investigated the association between single antioxidants such as vitamin C, vitamin E and beta-carotene in relation to the risk of stroke and have reported inverse associations. Other carotenoids have also been inversely associated with the risk of stroke (Voutilainen et al. 2006). Limited research have been on plant polyphenols in relation to stroke (Arts et al. 2005).

Observational studies on multivitamin supplement use and stroke
Very few epidemiological studies have examined the association between a wide-spectrum low-dose multivitamin supplement use and the risk of stroke (Watkins et al. 2000; Neuhouser et al. 2009). One study examined the association between multivitamin use and stroke mortality and observed no significant association (Watkins et al. 2000). Another study reported no association with stroke risk (Neuhouser et al. 2009).

Randomized controlled trials on antioxidant supplements and stroke
There is only one randomized clinical trial investigating the association between a wide-spectrum low-dose multivitamin supplement on the risk of stroke mortality among Chinese men and women with esophageal dysplasia. In this study, multivitamin supplement was associated with lower risk of stroke mortality especially among men (Mark et al. 1996).

Several randomized clinical trials have tested the effect from high-dose single antioxidant supplements and have failed to show beneficial effects on stroke risk. A meta-analysis of clinical trials of vitamin E supplementation and stroke reported decreased risk of cerebral infarction but increased risk of hemorrhagic stroke (Schurks et al. 2010). Other clinical trials on high-dose antioxidants supplements of one to three compounds (Leppala et al. 2000; Sesso et al. 2008) and low-dose clinical trials testing low-dose antioxidant supplements of two to three compounds, have reported no effect on stroke risk (Qiao et al. 2009).

2 AIMS

The overall aim with this thesis was to examine whether Total Antioxidant Capacity of diet and multivitamin supplements were inversely associated with the risk of cardiovascular diseases.

The specific aims were:

- To investigate the validity and reproducibility of food-frequency questionnaire-based Total Antioxidant Capacity estimates.

- To investigate if Total Antioxidant Capacity of diet is of importance in primary and secondary prevention of myocardial infarction in women.

- To investigate if Total Antioxidant Capacity of diet is of importance in primary and secondary prevention of stroke and different stroke subtypes in women.

- To investigate if multivitamin supplement use is of importance in primary and secondary prevention of myocardial infarction in women.

- To evaluate the association between multivitamin supplement use and coronary heart disease by quantitatively summarizing accumulated evidence with a meta-analytic approach

3 PARTICIPANTS AND METHODS

3.1 STUDY POPULATION

The four studies of this thesis are based on prospective data from the population-based Swedish Mammography Cohort (SMC) (**paper II, III and V**) and the SMC-Clinical subcohort (SMC-C) (**paper I**).

3.1.1 The Swedish Mammography Cohort (SMC)

The SMC was established between 1987 and 1990 when all women born between 1914 and 1948 living in Uppsala and Västmanland counties were invited to a mammography screening program. They also, together with the invitation, received a questionnaire about diet and other lifestyle factors. Of 90 303 invited women, 66 651women (74%) completed the questionnaire. To update exposure data a second expanded questionnaire was sent in 1997 to all cohort members still alive and living in the study area. Of 56 030 women, 39 227 women (70%) returned their questionnaires. The 1997 questionnaire is used as the baseline exposure assessment in **paper II, III and V** because of more information on major known and potential confounders was available there.

A flow chart of the exclusions from the source population to obtain the study population used in **paper II, II and V** is shown in **Figure 3.1**. Women with an erroneous or missing national registration number, and with history of cancer except nonmelanoma skin cancer before baseline in September 15, 1997 were excluded **(paper II, III and V)**. For analyses on Total Antioxidant Capacity, intake women with implausible total energy intake were excluded (**paper II and V**).

For those analyses on myocardial infarction (**paper II and III**), women with diabetes before baseline were excluded because this diagnosis may lead to changes in dietary habits. The primary analyses of Total Antioxidant Capacity and myocardial infarction were performed among 32,561 CVD-free (free from stroke, myocardial infarction, angina pectoris, or congestive heart failure) women at baseline. Separate analyses were performed among 2566 women with CVD history at baseline **(new results)**. Women with CVD were identified by linkage to the Swedish Hospital Discharge Registry, *International Statistical Classification of Disease, 10th Revision (ICD-10),* code I11.0, I20-25, I50 and I60-69) (Socialstyrelsen).

In the analyses of multivitamin supplement use and myocardial infarction (**paper III**) women with missing information on dietary supplement use were excluded. The primary analyses of multivitamin supplement in relation to myocardial infarction were performed among 31,670 CVD-free (free from stroke, myocardial infarction or angina

pectoris) women. Separate analyses were performed among 2262 women with CVD history (code I11.0, I20-25, I50 and I60-69) (**paper III**).

In the analyses of stroke (**paper V**) the primary analyses were performed among 31,035 CVD-free (free from stroke, myocardial infarction, angina pectoris, atrial fibrillation or congestive heart failure) women at baseline as well as 5680 women with CVD history (code I11.0, I20-25, I48, I50 and I60-69).

3.1.2 The Swedish Mammography Cohort – Clinical (SMC-C)

Between 2003 and 2009 women who completed the 1987 and 1997 questionnaires and living in Uppsala county were invited to participate in a study called SMC-C by sending a third questionnaire about diet. Women completing the questionnaire were invited after an overnight fast for donation of blood, urine and fat tissue at Samariterhemmet in Uppsala. Women also completed an additional questionnaire regarding lifestyle factors e.g. smoking, use of some medications and prevalent medical conditions e.g. diabetes. Weight and height were measured by a nurse. Bone mineral density was measured with the dual energy X-ray absorptiometry (DXA). From 2007 and forward also women's office blood pressure was measured. All women provided a written consent. The Ethics Committee of Karolinska Institutet approved this investigation.

Of 8311 invited women, 5022 women (60%) participated. Between 2003 and 2004, 246 women were randomly chosen to participate in the validation study of Total Antioxidant Capacity intake (**paper I**). Women with missing information on dietary supplement use (n=8) and those reporting use of dietary supplements regularly (n=91) and occasionally (n=28) were excluded because the contribution from dietary supplements to Total Antioxidant Capacity intake was unknown. Women reporting extreme energy intake (±2.5 SD) were also excluded (n=10). Totally, 109 non-supplement using women were included in the validity study. For the reproducibility study (**paper I**), 360 women who completed questionnaires between September 2004 and February 2005 were chosen. Women were asked to complete the same questionnaires one year apart and 300 women agreed on participating in the reproducibility study.

3.2 METHODS

3.2.1 Exposure assessment

Dietary assessment at baseline 1997
All women in the Swedish Mammography Cohort completed a 96-item FFQ in 1997. The 1997 FFQ included questions on the average consumption of predefined food-items over the past year. For each question there were eight response categories ranging from "never or seldom" to "three or more times per day". Open questions were used to collect information on commonly consumed foods such as dairy products, coffee, tea, light beer, soft drinks, sugar/honey and bread.

Women recruited to the SMC-C between 2003 and 2004 (n=467) completed two FFQs, the 67-item FFQ used in 1987 and one 96-item FFQ used in 1997 to estimate diet in the Swedish Mammography Cohort. Those women recruited between 2004 and 2009 completed an expanded version of the 1997 FFQ including 123 items. That FFQ included more questions on dairy products, meat, fruits (4 questions) and vegetables (2 questions).

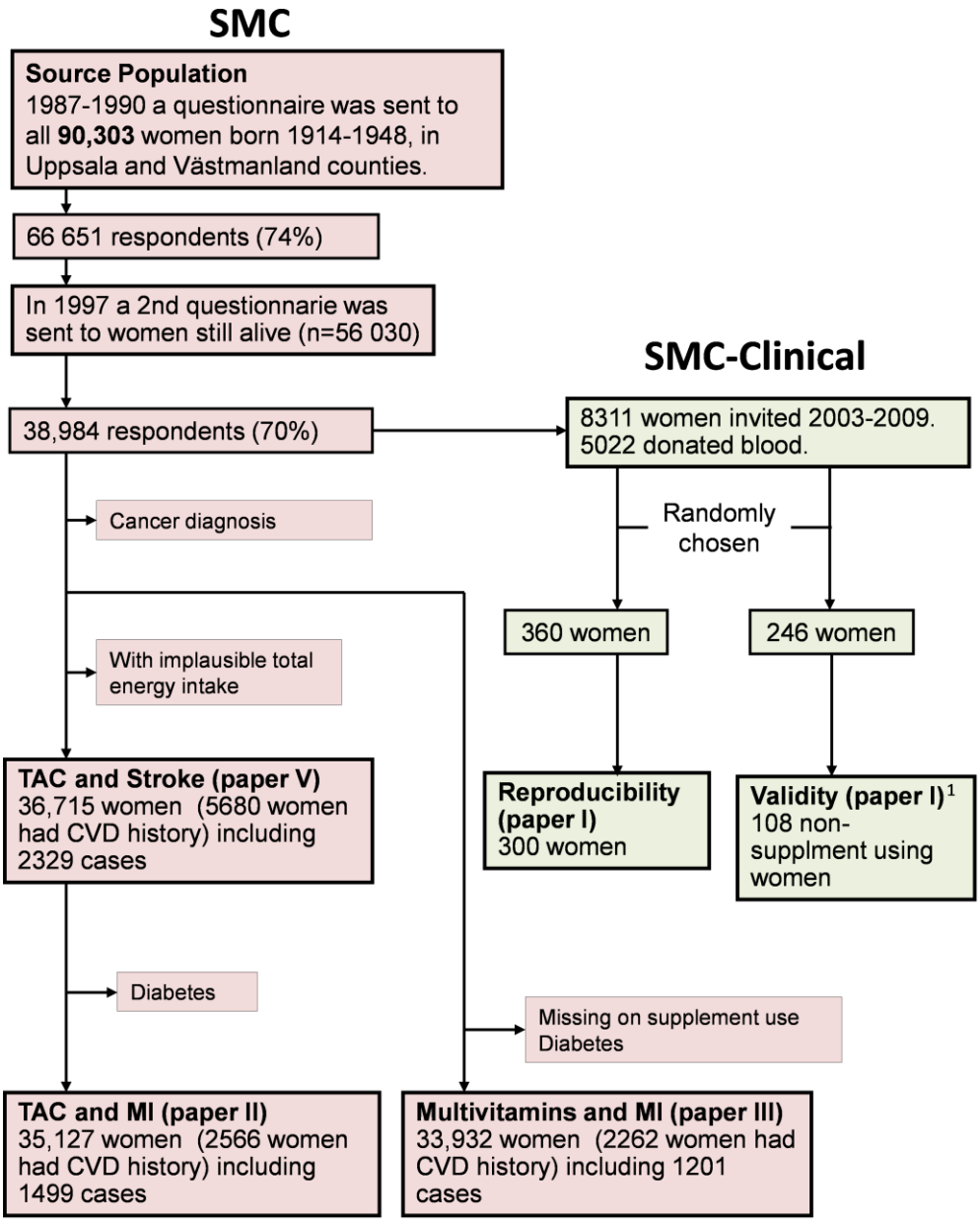


Figure 3.1. Source population, exclusions and study population for paper I-IV.
[1]138 women excluded: 127 dietary supplement users (*regularly users, occasionally users, and missing).* 10 with implausible total energy intake and one with extreme value on plasma Oxygen Radical Absorbance Capacity (ORAC). TAC: Total Antioxidant Capacity; CVD; Cardiovascular Disease; MI: Myocardial Infarction

Total Antioxidant Capacity of diet

Total Antioxidant Capacity was calculated by using databases over commonly consumed foods measured with the Oxygen Radical Absorbance Capacity (ORAC) (Prior et al. 2003; Sanchez-Moreno et al. 2003; Wu et al. 2004), the Total Radical-Trapping Antioxidant Parameters (TRAP) (Pellegrini et al. 2003; Pellegrini et al. 2006) and the Ferric Reducing Antioxidant Power (FRAP) assay (Halvorsen et al. 2006). The ORAC and the FRAP databases included foods from the United States whereas the TRAP database included foods from Italy.

The Total Antioxidant Capacity values were applied to the 1997 and 1997-expanded FFQs. If there were several foods analyzed for one item the mean value was calculated, e.g. the ORAC database included six types of apples and the mean value of these apples was applied to the FFQ. The FFQ-based ORAC, TRAP and FRAP estimates included values for fruits, vegetables, beverages (coffee, tea, wine, and fruit juices), grain products, chocolate and nuts. The FFQ-based TRAP estimate also included values for legumes and snacks whereas the FRAP estimate included values for legumes, snacks, salad dressing, and meat dishes.

Because of no available data on ORAC of coffee, this beverage was analyzed (Little Rock, Arkansas, the United States) in coffee brewed in a Swedish manner. A Total Antioxidant Capacity estimate, based on the ORAC assay, was calculated with coffee included. Because antioxidants in coffee and tea have been shown to be poorly absorbed in the intestines, the Total Antioxidant Capacity estimate was calculated by taking into account absorption (6% for coffee and 4 % for tea) (Natella et al. 2002).

Table 3.1 describes the amount of food-items covered by each Total Antioxidant Capacity estimate in the different FFQs. In **table 3.2** the different Total Antioxidant Capacity values are shown for a subsample of items included in the 1997 FFQ.

Table 3.1. Number of food items with available Total Antioxidant Capacity values in the food-frequency questionnaires (FFQ) used in the Swedish Mammography Cohort (SMC) and SMC-clinical.

	ORAC	TRAP	FRAP
1997 FFQ (96-items)			
Number of items covered	34	36	61
Number of fruit and vegetable items	17	16	16
1997 expanded FFQ (123-items)			
Number of items covered	47	49	76
Number of fruit and vegetable items	20	21	20

ORAC: Oxygen Radical Absorbance Capacity; TRAP: Total Radical-Trapping Antioxidant Parameters; FRAP: Ferric Reducing Antioxidant Power; FFQ: Food-Frequency Questionnaire,

Tabel 3.2. Total Antioxidant Capacity values for selected food items and the ranking order in parentheses.

	ORAC (µmol TE[1]/g)	TRAP (µmol TE/g)	FRAP (µmol[2]/g)
Chocolate, dark	1039.7 (1)	91.6 (1)	41.9 (1)
Coffee	57.0 (2)	56.0 (2)	12.5 (4)
Raspberry	49.3 (3)	10.5 (3)	23.3 (2)
Blueberry	32.7 (4)	9.3 (4)	21.5 (3)
Carrots	12.5 (5)	0.7 (7)	2.5 (6)
Tea	11.8 (6)	4.9 (5)	2.2 (7)
Apples	2.7 (7)	1.9 (6)	3.1 (5)

ORAC: Oxygen Radical Absorbance Capacity; TRAP: Total Radical-Trapping Antioxidant Parameters; FRAP: Ferric Reducing Antioxidant Power;

[1] Trolox Equivalents (TE)

[2] Amount of electron/hydrogen atoms donated in the redox reaction

Total Antioxidant Capacity in blood

To validate FFQ-based ORAC estimates, ORAC in whole plasma as well as ORAC in the lipophilic and hydrophilic part of plasma were measured among 246 women randomly chosen from SMC-clinical between 2003 and 2004. However, the contribution of dietary supplements to Total Antioxidant Capacity intake was unknown and therefore only those who reported they did not use any dietary supplements were included in the validation study (n=109).

To also validate FFQ-based TRAP and FRAP estimates, TRAP and FRAP were measured in whole plasma among 109 women not using dietary supplements.

Plasma Total Antioxidant Capacity values were analyzed in samples collected after a one night fast at one occasion. Blood was collected in evacuated tubes containing EDTA and thereafter centrifuged in a dark room immediately at 3000 X g in 10 minutes at 4°C. Plasma was separated and then stored approximately for three years in -80°C before plasma Total Antioxidant Capacity analyses.

The ORAC (Prior et al. 2003), TRAP (Serafini et al. 2002) and FRAP (Benzie et al. 1999) assays have previously been described elsewhere; here follows a brief summary. The experimental characteristics of the ORAC, TRAP and FRAP assay are described in **Table 3.3**. In the ORAC assay, flourescein was used as the fluorescent target and 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH) as the inducer of radical formation. Trolox, a water-soluble analogue of a-tocopherol, was used for the standard curve. Plasma extractions were performed to measure ORAC in the lipophilic and the hydrophilic fraction. To validate FFQ-based TRAP estimates TRAP was measured in whole plasma. To measure fluorescence R-phycoerythrin was used and AAPH as the radical. The results were standardized using Trolox. For validation of FFQ-FRAP

estimates FRAP in whole plasma was measured for comparision. Reduction of colourless ferric-tripyridyltriazine (TPTZ-Fe^{3+}) to ferrous coloured TPTZ-Fe^{2+} was measured with spectrophotometry.

Table 3.3. Experimental characteristics of methods measuring Total Antioxidant Capacity in plasma (**Paper I**).

Method	Radical inducer	Oxidant	Flourescent target	Measurement	Standard	Plasma
ORAC	AAPH	ROO*	Flourescein	Flourescence (575 nm)	Trolox (AUC)	Whole Lipophilic Hydrophilic
TRAP	AAPH	ROO*	R-phycoerythrin	Flourescence (575 nm)	Trolox (lag-time)	Whole
FRAP	TPTZ-Fe^{3+}	TPTZ-Fe^{3+}	-	Absorbance (595 nm)	Fe(II)	Whole

ORAC: Oxygen Radical Absorbance Capacity; TRAP: Total Radical-trapping Antioxidant Parameter; FRAP: Ferric Reducing Antioxidant Potential; AAPH: 2,2'-azobis(2-amidino-propane) dihydrochloride; ROO*: Peroxyl radical; TPTZ: 2,4,6-tri(2-pyridyl)-1,3,5-triazine.

Dietary Supplements

The questionnaire completed in 1997 collected information on dietary supplement use. There were predefined questions on use and duration of use for multivitamins (with or without minerals), vitamin C, vitamin E, vitamin B12, calcium and fish oil. The questionnaire also included questions on the use of beta-carotene, selenium, zinc, coenzyme-Q10, ginseng, B-vitamins and magnesium.

The average doses of vitamins and minerals of the above mentioned supplements have been estimated in two study populations (Holmquist et al. 2003; Messerer et al. 2008). Multivitamin supplements have been estimated to contain doses close to recommended daily allowances of vitamins and minerals (**Table 4**).

Table 3.4. Estimated doses of vitamins and minerals included in multivitamin supplements on the Swedish market (Holmquist et al. 2003; Messerer et al. 2008).

	Average dose (% of recommended daily allowances)
Vitamins	
Vitamin C	60 mg (100%)
Vitamin D	10 µg (100%)
Vitamin E	9 mg (113%)
Thiamine	1.2 mg (120%)
Riboflavin	1.4 mg (117%)
Vitamin B6	1.8 mg (150%)
Vitamin B12	3 µg (150%)
Folic acid	400 µg (133%)
Minerals	
Iron	10 mg (100%)
Zinc	15 mg (214%)
Cupper	2 mg (222%)
Calcium	120 mg (15%)
Magnesium	50 mg (18%)
Chromium	50 µg (100%)
Selenium	50 µg (125%)
Iodine	150 µg (100%)

The sensitivity and specificity of self-reported dietary supplement use have been estimated in a subgroup of men from the Cohort of Swedish Men. These men were participating in fourteen 24-h recall interviews on diet and dietary supplement use. The sensitivity and the specificity of multivitamin use were 69% and 98%, respectively (Messerer et al. 2004).

Other covariates and lifestyle factors

Body mass index (BMI) was calculated by dividing reported weight (kg) by reported height (m^2). Self-reported weight and height is highly correlated with measured values in Swedish women (r=0.9 and r=1.0, respectively) (Kuskowska-Wolk et al. 1992). Women were categorized into never, past, or current smokers, and number of cigarettes smoked per day was calculated for both current and past smokers. The daily alcohol intake was based on consumption frequencies of specific alcoholic beverages and self-reports of average sizes for those specific drinks. The validity of questionnaire-based alcohol intake as compared to diet records in the women was high (r=0 .9) (Wolk A, unpublished data). Physical activity levels were estimated by multiplying the reported duration of five predefined activities (occupation, housework, walking or cycling, leisure-time exercise and inactive leisure time) by the intensity of these activities and expressed as multiples of the metabolic equivalent per day (MET, kcal kg^{-1} x h^{-1}) of sitting quietly for 1 h. The validity of the reported total physical activity against activity

records in these women were satisfactory (r = 0.6) (Orsini et al. 2008). Education was assessed with six categories ranging from 6 years of basic education to university studies. Information on the use of hormone replacement therapy, use of aspirin, hypertension, hypercholesterolemia and family history of myocardial infarction before age 60 were collected through the questionnaire. Diabetes was assessed from the questionnaire and through linkage to the Swedish Hospital Discharge Registry.

3.2.2 Case ascertainment and follow-up

In the analyses of myocardial infarction (**paper II and III**) and stroke (**paper V**), all women were followed from September 15 1997 until the date of myocardial infarction/stroke, death or the end of follow-up (31 December 2007 (myocardial infarction) or 2009 (stroke). The cases of cerebral infarction (I63), intracerebral hemorrhage (I61), subarachnoid hemorrhage (I60) and unspecified stroke (I64) as well as fatal and nonfatal myocardial infarction (I21) were identified by linkage of the national registration number of the SMC participants to the Swedish Hospital Discharge Registry and the Cause of Death Registry. These registries are considered nearly complete. The registry for 1995 were thoroughly validated and revealed high sensitivity (94%) and positive predictive value (86 %) for MI (Hammar et al. 2001)**.** Date of deaths were ascertained through the Cause of Death Registry.

3.2.3 Statistical Analyses

Validity and reproducibility study (Paper I)
To examine whether the validity group of 108 women (One woman with unreliable plasma ORAC data was excluded) **(paper I)** is representative of the SMC 1997 in baseline characteristics (age, energy intake, BMI, FFQ-based TAC estimates, education and smoking) these women were compared to non-supplement using women in the SMC (n=7154) using the same age range (54-73 years) as in the validity study. Differences in background characteristics were examined with *t*-tests and χ^2 tests. The distribution of FFQ-based Total Antioxidant Capacity estimates and plasma Total Antioxidant Capacity concentrations were tested with residual and goodness-of-fit analyses and they showed no evidence of departure from normality, therefore untransformed variables were used in the analysis.

Major food contributors to the FFQ-based Total Antioxidant Capacity estimates were assessed by calculating the percentage of contribution of each food item to each Total Antioxidant Capacity estimate.

Validity was examined with the Pearson correlation coefficients. The Pearson's partial correlation coefficients were used to examine whether date of completion of the FFQ

had any impact on the results. Deattenuated Pearson correlations were obtained by using data on within-person variation (Willett 1998) in the FFQ-based Total Antioxidant Capacity estimates from 300 women included in the reproducibility study. Additionally, the FFQ-ORAC estimate was plotted against whole plasma-ORAC.

The reproducibility of the three FFQ-TAC estimates was assessed by calculating the intraclass correlation between two identical FFQs completed one year apart. Statistical analyses were performed with SAS (version 9.1; SAS Institute, Cary, NC, USA) and STATA software (version 9.2).

Cohort studies of myocardial infarction and stroke (paper II, III and V)
In analyses of Total Antioxidant Capacity of diet in relation to myocardial infarction and stroke, the Cox proportional hazards models with age as the time-scale were used to estimate hazard ratios (HR), with 95 percent confidence intervals (Cox 1972) using the PHREG command in SAS (version 9.2; SAS Institute, Inc., Cary, North Carolina). Risk estimates were adjusted for potential risk factors. Missing data on a covariate was treated as a separate category. To assess trends across quintiles of dietary Total Antioxidant Capacity the median value of each category to create a single continuous variable was used. Additional analyses of the linear trend between Total Antioxidant Capacity and myocardial infarction (**paper II**) was performed by calculating a p-value for the linearity assumption by testing whether the quadratic term was equal to zero.

Women were categorized into quintiles of Total Antioxidant Capacity of diet to the association with myocardial infarction **(paper II)** and stroke **(paper V)**. Women with CVD history were categorized into quartiles of Total Antioxidant Capacity of diet because of smaller number of cases within specific subtypes of the disease. Women who reported they used dietary supplements were categorized into users of multivitamins only, users of multivitamins in combination with other supplements, users of other supplements than multivitamins and non-supplement users **(paper III)**. Characteristics differences between the categories of dietary supplement use were investigated by using analysis of variance.

The proportional hazards assumption was tested by calculating scaled Schoenfeld's residuals (**paper II and V**) and no evidence of violation of this assumption was found. In the study of multivitamin use and myocardial infarction, the proportional hazard assumption was tested by entering the product of multivitamin use and the natural logarithm of time in the model; no evidence of violation of this assumption was found (**paper III**).

To further examine whether the associations differed by known risk factors for stroke and myocardial infarction, subgroup analyses were performed. Test of interactions were

performed with the likelihood ratio test and the Walds test. All *p* values shown are two-sided. P-values less than 0.05 were considered statistically significant.

The Total Antioxidant Capacity of diet was also examined as a continuous variable by investigating every standard deviation increment (4000 µmol/TE/day in **paper II** (taking into account coffee and tea absorption) and 5000 µmol/TE/day in **paper V**). In the sensitivity analyses, these estimates were further corrected for bias due to measurement error with the regression calibration method correcting for both random and systematic error (Spiegelman et al. 1997). The validity coefficient between Total Antioxidant Capacity from diet and plasma (r=0.3) was used to correct the risk estimates for myocardial infarction and stroke.

3.3 META-ANALYSIS

Search Strategy
The MEDLINE and EMBASE databases were used to search for relevant literature to be included in the meta-analysis. The literature search was performed until Mars 29, 2012 using the search terms: *multivitamin* combined with *coronary heart disease, ischemic heart disease, myocardial infarction or cardiovascular disease*. To search for additional relevant studies a review of reference lists of retrieved articles was done.

Study Selection
Studies excluding participants with coronary heart disease were included in the meta-analysis if they met the following criteria: (1) prospective cohort design; (2) the exposure of interest was multivitamin use alone or in combination with other dietary supplements; (3) the outcome was incident myocardial infarction or coronary heart disease; and (4) relevant studies reporting relative risk estimates with 95% confidence intervals (CIs). The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement was used for planning, conducting and reporting of this systematic review (Moher et al. 2009).

Data Extraction
From each study the following data were excluded: the first author's last name, year of publication, country where the study was performed, study period, participant sex and age, sample size (cohort size), measure and exposure range, covariates in the analysis, and relative risk estimates with corresponding 95% CIs. Those relative risk estimates that reflected the greatest degree of control for potential confounders were included in the main analysis. The study quality was examined by using the 9-star Newcastle-Ottawa Scale (Wells GA et al.). Data extraction and study quality evaluation was

conducted independently by two authors (SR. and AÅ), with discrepancies resolved by agreement.

Statistical Analysis (Paper V)

Relative risk estimates of studies of participants with no coronary heart disease at baseline were summarized by using a random-effects model which considers both within-study and between-study variation. One study reported relative risks separately for different categories of multivitamin use and these relative risks estimates were combined (Rimm et al. 1998) and then the summarized relative risk estimate was included in the meta-analysis.

The calculation of statistical heterogeneity among studies was done by using the Q and I^2 statistics (Higgins et al. 2002). Stratified analyses were done by type of outcome, geographic area and sex. Publication bias was assessed by using the Egger regression asymmetry test (Egger et al. 1997), Begg rank correlation test (Begg et al. 1994) and a nonparametric iterative trim-and-fill procedure by Duval and Tweedy which estimates the number and outcomes of potentially missing studies resulting from publication bias (Duval et al. 2000; Duval et al. 2000). All statistical analyses were performed with Stata software, version 10 (Stata Corp, College Station, Texas). P <0.05 was considered statistically significant.

4 RESULTS

4.1 TOTAL ANTIOXIDANT CAPACITY OF DIET (PAPER I + NEW RESULTS)

Validity and Reproducibility of Total Antioxidant Capacity (Paper I, coffee not included)

Women in the validation study had a slightly higher BMI, were better educated and had a lower energy intake compared to the SMC comparison group. Women included in the validation study were similar to excluded women (regular and occasional supplement users) with regard to age, energy intake, BMI, smoking and education. However, women in the validation study had statistically significantly lower FFQ-based ORAC (13%) and TRAP (4%) estimates as compared to SMC comparison group.

Women in the reproducibility study were older, less likely to be smokers, had a higher energy intake and were better educated as compared to the SMC comparison group. The reproducibility group also had higher TAC estimates due to more food items with TAC values in the expanded 1997 FFQ.

The validity of FFQ-based Total Antioxidant Capacity estimates compared with Total Antioxidant Capacity in plasma was assessed with Pearson correlation coefficients. The FFQ-based ORAC estimate was statistically significantly ($p<0.05$) correlated with ORAC in whole plasma ($r=0.23$), ORAC in the lipophilic part of plasma ($r=0.21$) and TRAP in whole plasma ($r=0.22$). The FFQ-based TRAP estimate was statistically significantly correlated with ORAC in whole plasma ($r=0.20$). The FFQ-based FRAP estimate was statistically significantly correlated with ORAC in whole plasma ($r=0.22$) and TRAP in whole plasma ($r=0.20$). The intraclass correlations in the reproducibility study for the FFQ-based TAC estimates ranged between 0.55 and 0.68.

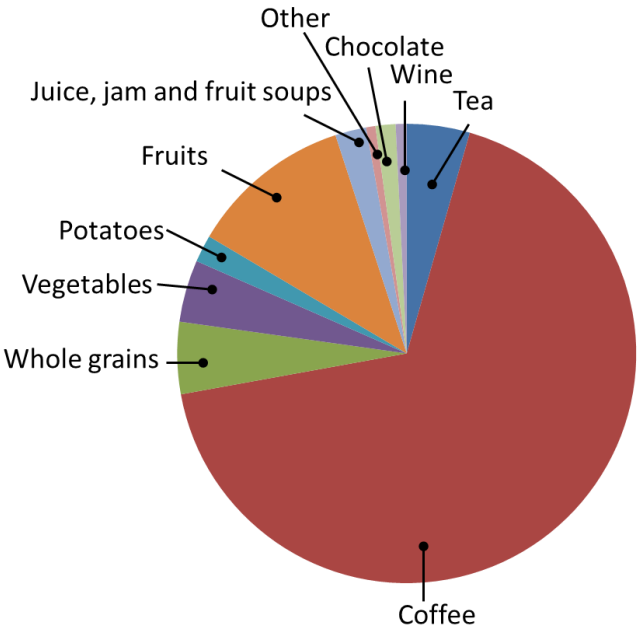


Figure 4.1.a. Contributors to crude food frequency questionnaire (FFQ)-based Total Antioxidant Capacity of diet estimate. The mean FFQ-based Total Antioxidant Capacity estimates was 40,977 Trolox Equivalents.

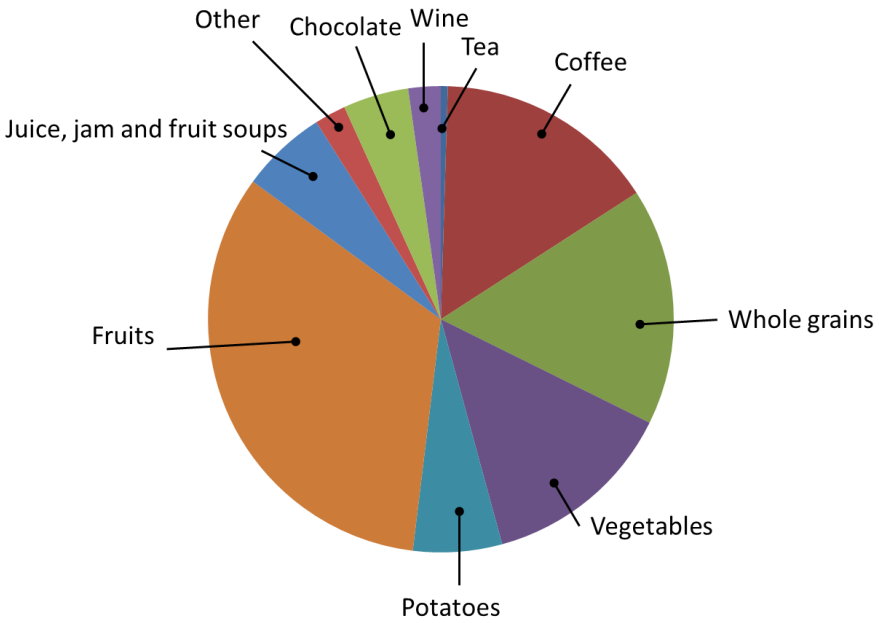


Figure 4.1.b. Contributors to the food frequency questionnaire (FFQ)-based Total Antioxidant Capacity of diet estimate taking into account coffee and tea absorption. The mean FFQ-based Total Antioxidant Capacity estimate was 12,426 Trolox Equivalents.

Validity of dietary Total Antioxidant Capacity estimates (coffee included)
Because antioxidants in coffee and tea have been shown to be poorly absorbed, we took into account absorption (6% for coffee and 4 % for tea) (Natella et al. 2002) when calculating Total Antioxidant Capacity of diet based on the ORAC assay. **Figure 4.1.a-b** shows major contributors to crude and adjusted (absorption of antioxidants in coffee and tea) FFQ-ORAC estimates. In the crude estimate coffee was the major contributor, however, when adjusting for coffee and tea absorption fruits, vegetables and whole grains were the major contributors to Total Antioxidant Capacity of diet. The FFQ-ORAC estimate taking into account coffee and tea absorption was statistically significantly correlated with ORAC in whole plasma (r=0.24) and ORAC in lipophilic part of plasma (r=0.28) (**Figure 2b**).

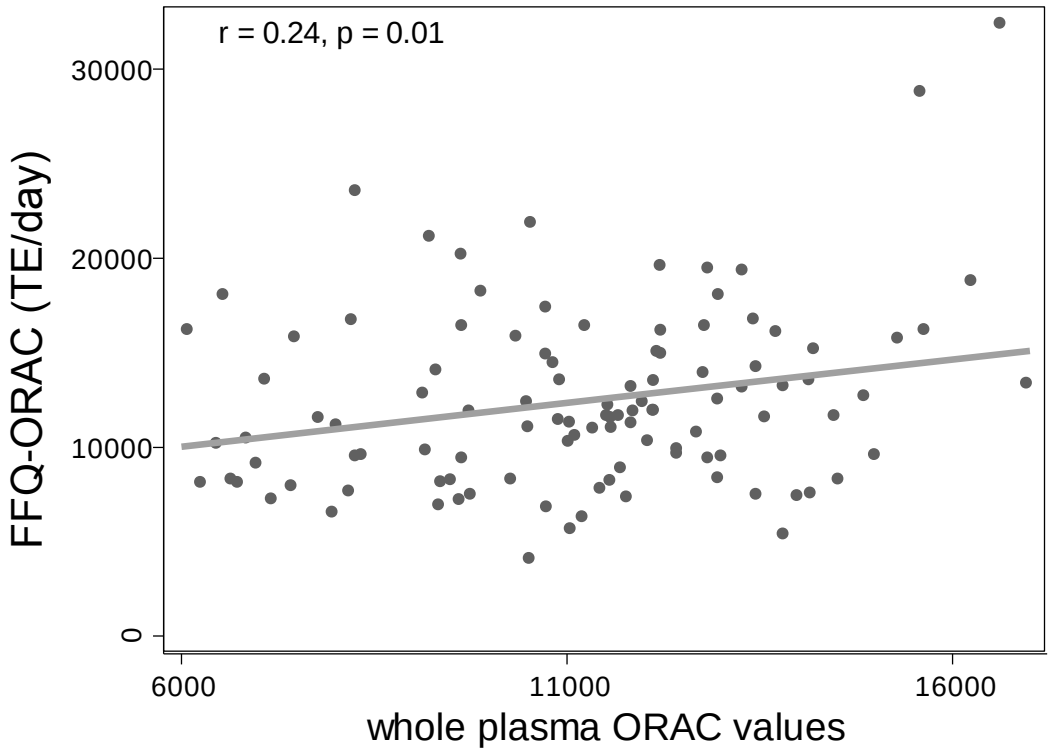


Figure 4.2. Scatterplot between FFQ-based Total Antioxidant Capacity estimate (taking into account coffee and tea absorption) and ORAC in whole plasma.

The reproducibility of the FFQ-ORAC estimate as measured with intra-class correlation was 0.61 (95% CI: 0.54 – 0.68). The deattenuated Pearson correlation coefficients between FFQ-ORAC estimate and ORAC in whole plasma and ORAC in lipophilic part of plasma were 0.28 and 0.32, respectively.

4.2 TOTAL ANTIOXIDANT CAPACITY OF DIET AND MYOCARDIAL INFARCTION (PAPER II + NEW RESULTS)

Cardiovascular disease (CVD)-free cohort

Women were followed until December 31, 2007, an average of 10.3 years (321,434 person-years), and 1114 cases of incident myocardial infarction were identified. Baseline characteristics are presented in **Table 1, paper II**. Women with higher Total Antioxidant Capacity of diet were more likely to be non-smokers, have ≥12y of education, and to have hypercholesterolemia, have higher consumption of fruit and vegetables (2-fold), whole grains (15%), coffee (34%) and chocolate (38%) as well as lower intake of saturated fatty acids (27%) and lower intake of monounsaturated fatty acids (19%).

The HRs of incident myocardial infarction by quintiles of Total Antioxidant Capacity of diet are presented in **Figure 4.3.** The HR among women in the highest quintile of Total Antioxidant Capacity of diet, compared to the lowest, was 0.80 (95% CI: 0.67–0.97 %, p for trend=0.02). To further investigate whether the apparent inverse association was explained by consumption of fruit and vegetables, sensitivity analyses were performed by adding the continuous fruits and vegetable variable (servings/day) to the model and results were similar (HR = 0.81 (95% CI: 0.64–1.02)). The HR in the highest quintile of Total Antioxidant Capacity of diet, compared to the lowest, for nonfatal myocardial infarction was 0.78 (95% CI: 0.65–0.95) and for fatal myocardial infarction 0.75 (95% CI: 0.48–1.20).

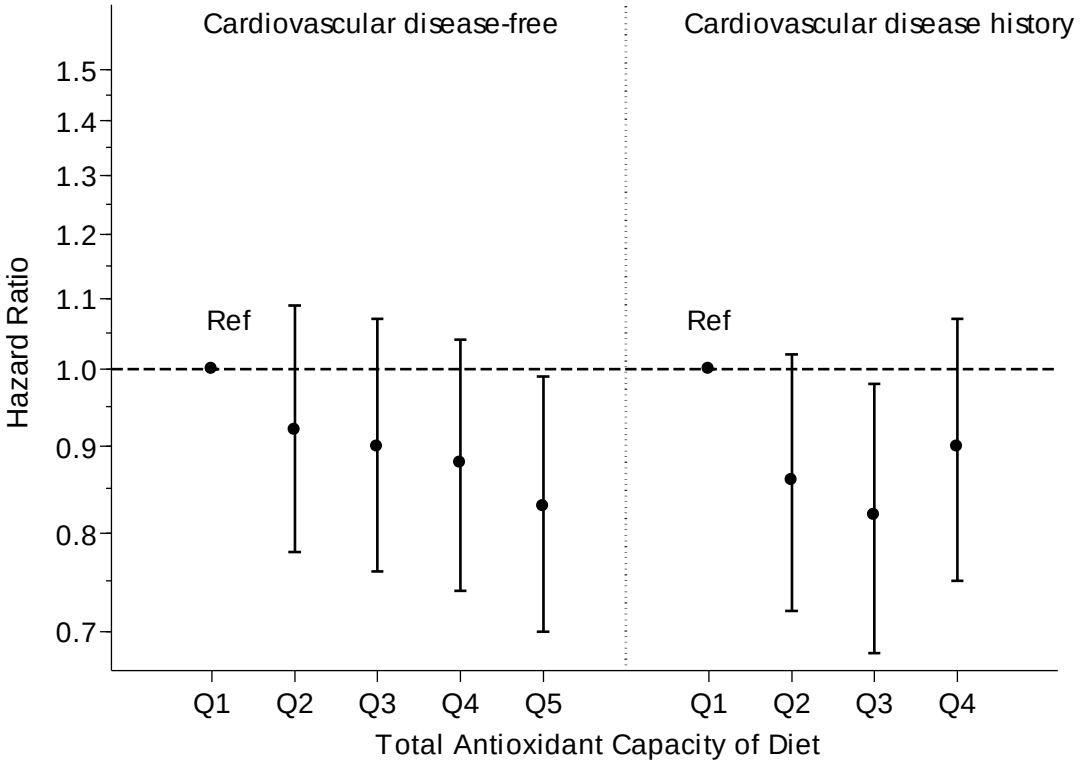


Figure 4.3. The association between Total Antioxidant Capacity of diet and myocardial infarction among cardiovascular disease-free women (n=32,561) and women with cardiovascular disease history at baseline (n=2566). Dots represent point estimates and bars represent 95% confidence intervals.

Linear and quadratic association between Total Antioxidant Capacity of diet and incident myocardial infarction are presented in **figure 4.4**. There was no significant evidence of departure from a linear change in the risk of myocardial infarction related with every unit increase in Total Antioxidant Capacity (p for non-linearity=0.25)**.** An increment of 4000 ORAC units/day (corresponding to approximately 1 standard deviation in the study population) was associated with HR=0.94 (95% CI: 0.88–1.00). When correcting for measurement error the HR was 0.79.

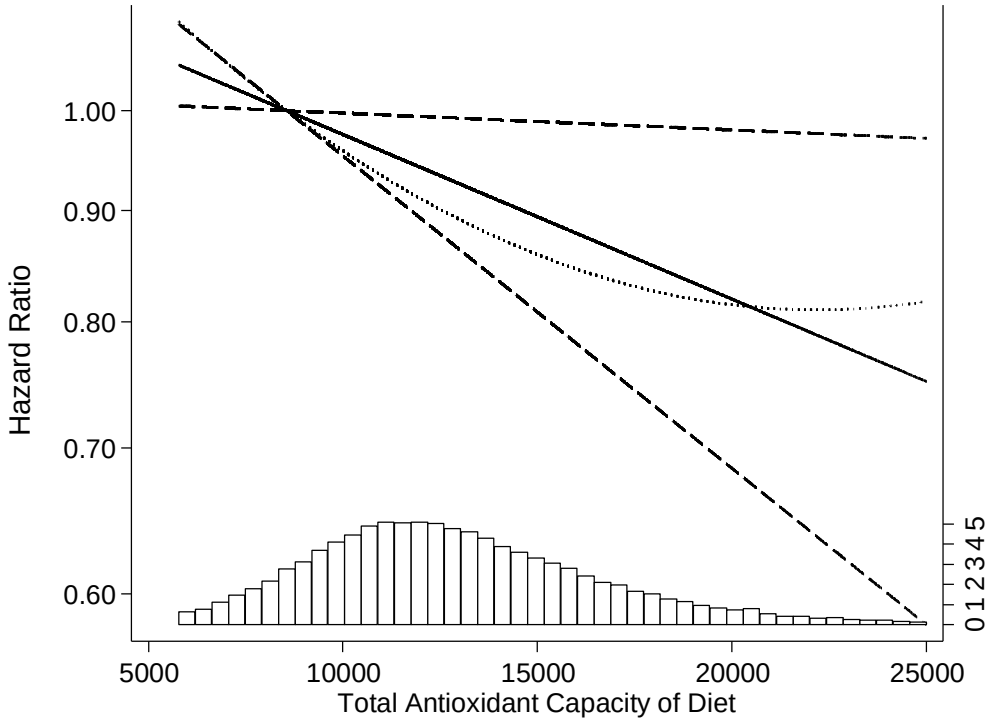


Figure 4.4. Multivariable-adjusted hazard ratios for incident myocardial infarction according to Total Antioxidant Capacity of diet (Trolox Equivalents per day). Dotted line represents point estimates of the quadratic trend. Dashed lines represent 95 % confidence intervals for the linear trend. The distribution of Total Antioxidant Capacity is presented at the bottom of the figure as a histogram.

Women having myocardial infarction during the first three years of follow-up were excluded to investigate whether the observed association could be due to reversed causality, as cardiovascular risk factors may be associated with alterations in dietary habits. There was no indication of influence of reversed causality (HR in the highest quintile=0.81 (95% CI: 0.68–0.98).

In **Figure 4.5** subgroup analyses were performed to investigate whether the inverse association between dietary Total Antioxidant Capacity and myocardial infarction varied by potential risk factors such as age, body mass index, smoking, saturated fatty acid intake and multivitamin supplement use. The association was somewhat stronger among women >65 years, women with body mass index >25 and current smokers, comparing the highest quintile with the lowest. However, the tests for interaction were not significant (all P for interaction>0.16).

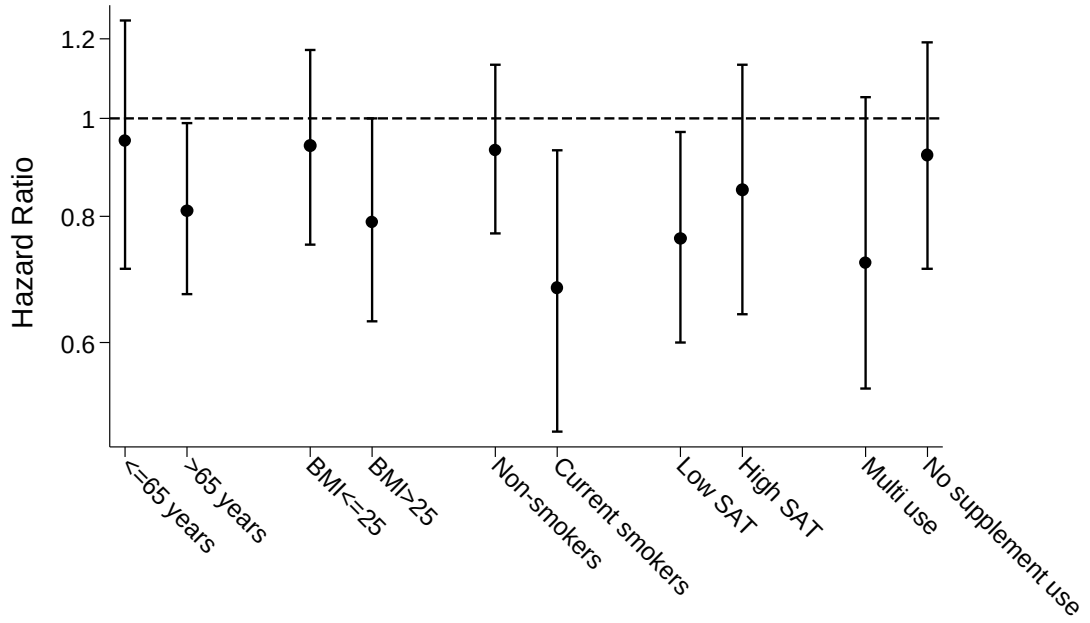


Figure 4.5. Subgroup analyses of the association between Total Antioxidant Capacity of diet and myocardial infarction by potential risk factors. Dots represent point estimates in the highest quintiles compared to the lowest and bars represent 95% confidence intervals. BMI: Body Mass Index; SAT: Saturated fatty acids

Cohort with Cardiovascular disease (CVD) history
Women with CVD history were followed during a median an average of 10.3 years (22,189 person-years) and 335 myocardial infarction cases (313 nonfatal and 70 fatal) were identified. There was no association observed between Total Antioxidant Capacity of diet and myocardial infarction (**Figure 4.3**).

4.3 MULTIVITAMIN USE AND MYOCARDIAL INFARCTION (PAPER III)

Cardiovascular disease (CVD)-free cohort
Women were followed until December 31, 2007, a median of 10.3 years (312,805 person-years), and 932 incident myocardial infarction cases (775 non-fatal and 157 fatal) were identified among CVD-free women at baseline. Baseline characteristics are presented in **Table 1, paper III** by categories of multivitamin use. Women using multivitamins only compared to non-users of any supplements were older, had higher education, less likely to be smoke, were leaner, more likely to consume alcohol, were more likely to use postmenopausal hormones, had slightly higher quality of diet, were less physically active and more likely to be non-hypercholesterolemic and have normal blood pressure.

In the multivariable analysis of CVD-free women, multivitamin supplements use only, as compared to non-use of supplements, was associated with lower risk of myocardial infarction (HR=0.73; 95 % CI: 0.57–0.93, **Figure 4.6**). Women using multivitamins together with other supplements also had lower risk of myocardial infarction (HR=0.70; 95 % CI: 0.57–0.87). The lower risk was similar for women reporting they used multivitamin regularly and occasionally. Using multivitamin ≥5y before baseline was associated with even lower risk of myocardial infarction (HR=0.59; 95% CI: 0.44–0.80). The HR for using multivitamins <5y was 0.82 (95% CI: 0.60–1.12). No significant association was observed for the use of other supplements than multivitamins.

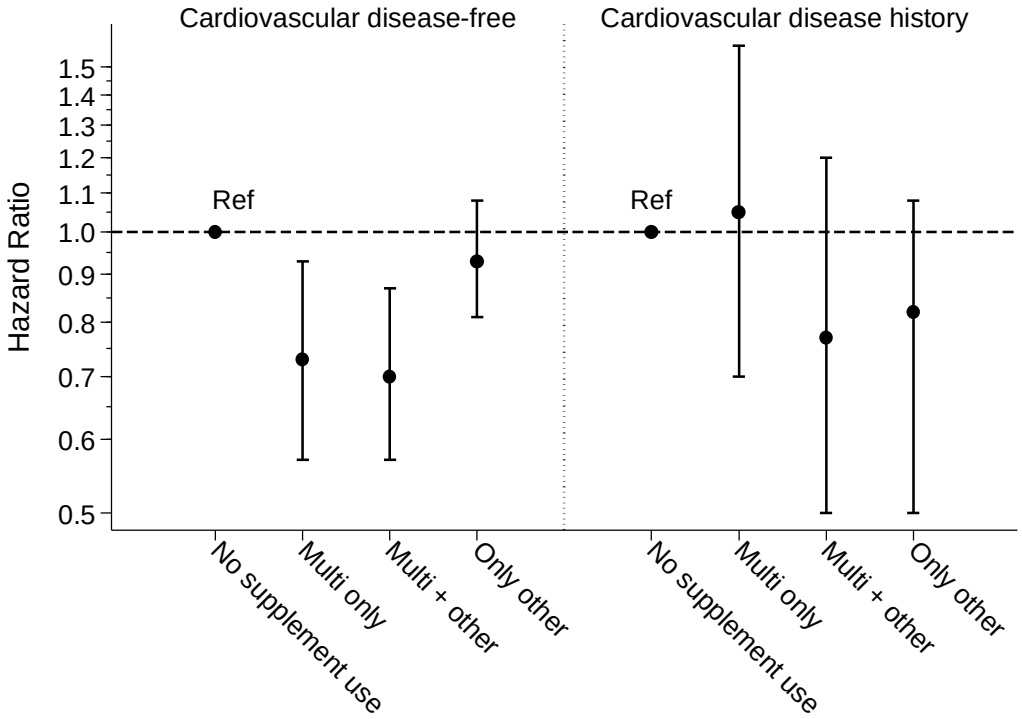


Figure 4.6. The association between multivitamin use and myocardial infarction among cardiovascular disease-free (n=31,670) women and women with cardiovascular disease history at baseline (n=2262). Dots represent point estimates and bars represent 95% confidence intervals.

Sensitivity analyses were performed to examine if reversed causality may have had any impact on the results by excluding cases during the first three years of follow-up. The results were similar after these exclusions among women using multivitamin only (HR=0.70, 95% CI: 0.52, 0.93) and women using multivitamins together with other supplements (HR=0.79, 95% CI: 0.62, 0.99).

Cohort with cardiovascular disease (CVD) history
Women were followed-up during a median of 10.3 years (19,813 person-years) and 269 cases (219 non-fatal and 50 fatal) were identified among women with history of CVD. Women using multivitamins only were younger, more educated, leaner, less physically active or less likely to be hypertensive. In multivariable-adjusted analysis there was no significant association observed between use of multivitamins and myocardial infarction (**Figure 4.6**).

Additional analyses were performed to investigate whether the association between multivitamin supplement use and myocardial infarction varied by CVD history at baseline. There was no statistical significant interaction observed between use of multivitamin and CVD history (P for interaction=0.11).

4.4 MULTIVITAMIN USE AND CORONARY HEART DISEASE (PAPER IV)

Literature Search
In the literature search 40 potentially relevant articles were identified that were likely to have investigated the association between multivitamin use and incident coronary heart disease (**Figure 4.7)**. From them 28 articles were excluded because they had not reported relative risks for the association between multivitamin use and incident coronary heart disease. Three articles were excluded because participants with prevalent coronary heart disease at baseline were not excluded (Losonczy et al. 1996; Iso et al. 2007; Neuhouser et al. 2009). One article was excluded because it was a duplicate report on the same study population (Stampfer et al. 1993). Two articles were excluded because they did not examine incident myocardial infarction or coronary heart disease (Watkins et al. 2000; Muntwyler et al. 2002). One article with case-control design was excluded (Holmquist et al. 2003). The remaining five articles were considered relevant examining the association between multivitamin use and coronary heart disease incidence and were included in the meta-analysis (Rimm et al. 1993; Rimm et al. 1998; Klipstein-Grobusch et al. 1999; Ishihara et al. 2008; Rautiainen et al. 2010).

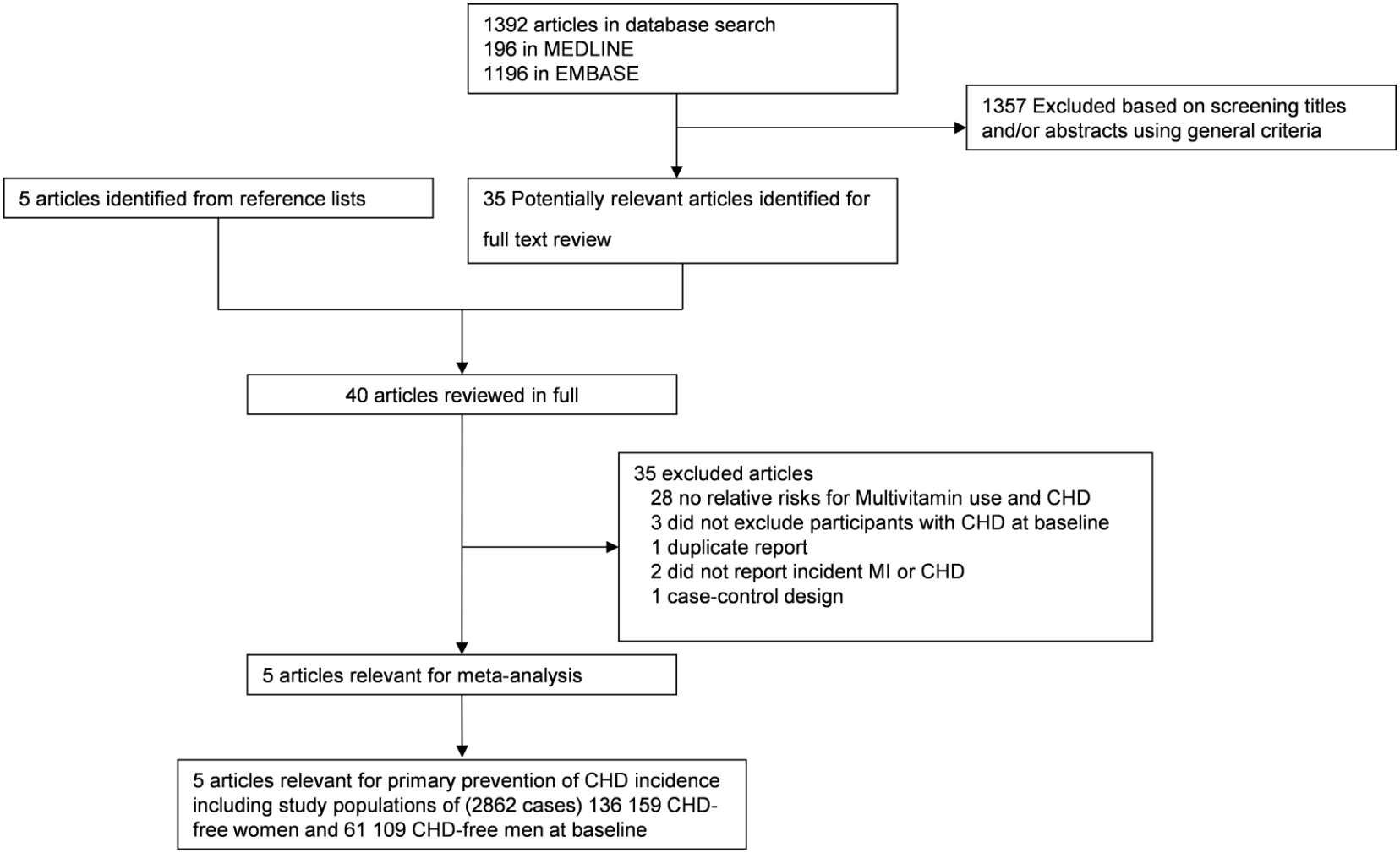


Figure 4.7. Selection of studies included in the meta-analysis. CHD: Coronary Heart Disease; MI: Myocardial Infarction

Study Characteristics

The five prospective cohort studies included in the meta-analyses (including 2862 incident cases of coronary heart disease and published between 1993 and 2010), were conducted in the United States (n=2), in Europe (n=2) and in Asia (n=1) (**Table 1, paper IV)**. The studies provided risk estimates adjusted for age (all five studies), body mass index (all five studies), smoking (all five studies), physical activity (four studies), alcohol (four studies) and covariates estimating healthy diet (all five studies).

Use vs non-use of multivitamins

The relative risks for each prospective cohort study and the summary relative risk from these studies for use vs non-use of multivitamins are shown in **Figure 4.8**. The summarized results indicated that multivitamin use was associated with lower risk of coronary heart disease (relative risk=0.78, 95% CI: 0.70 – 0.86). Two studies (Rimm et al. 1998; Rautiainen et al. 2010) reported both age-adjusted as well as multivariable-adjusted relative risks and when comparing the summary relative risk of the different adjustments, a slightly stronger association for the age-/age- and sex-adjusted model (relative risk =0.69, 95% CI: 0.61 – 0.79) was observed as compared to the multivariable-adjusted model (relative risk=0.78, 95% CI: 0.66 – 0.91). There was no evidence of publication bias when performing the Egger test (p=0.23) and the Begg test (p=0.46).

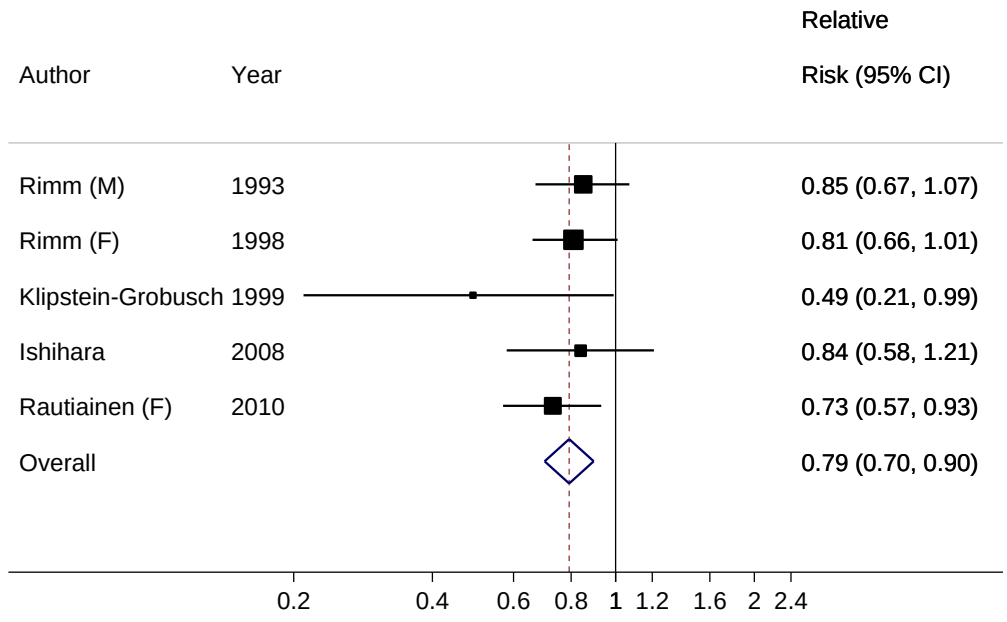


Figure 4.8. Relative risks of coronary heart disease (CHD) among CVD-free study populations by categories of multivitamin use (no vs. yes) in prospective cohort studies.

Stratified analyses
The summary results of the association between multivitamin use and coronary heart disease were slightly stronger among European studies (relative risk= 0.70; 95% CI: 0.56 – 0.89) than among American studies (relative risk= 0.83; 95% CI: 0.71 – 0.97). Results stratified by sex were similar among women (relative risk= 0.77; 95% CI: 0.66 – 0.91) and men (relative risk= 0.85; 95% CI: 0.67 – 1.07).

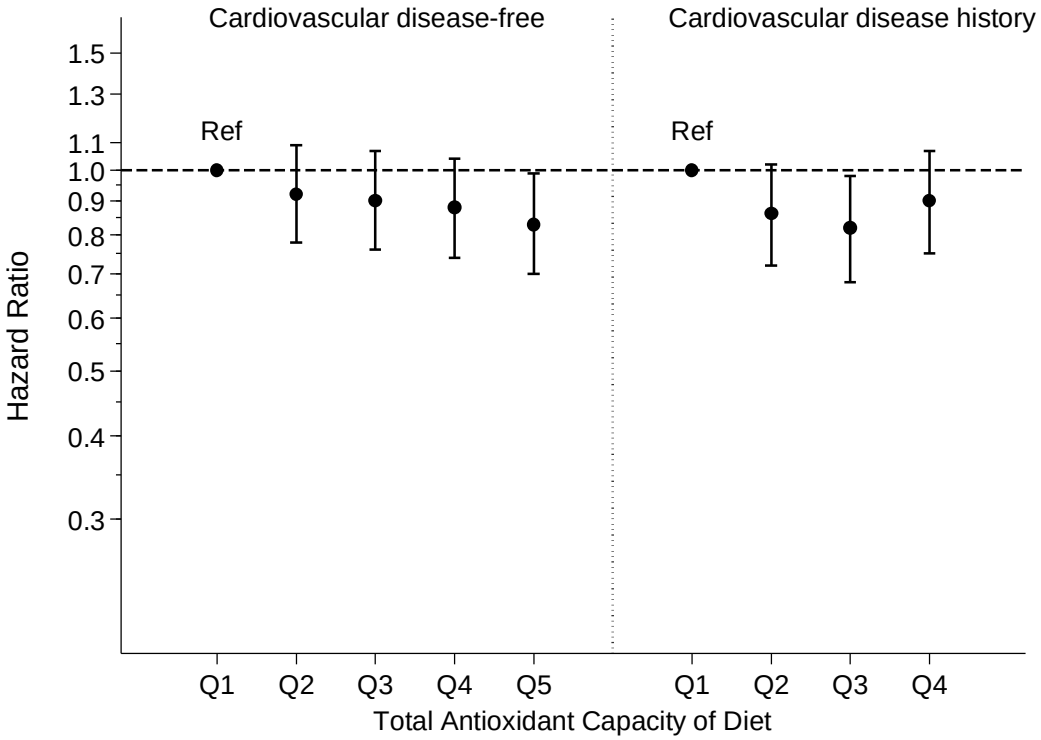
Duration of multivitamin use
Two studies examined duration of multivitamin use and incident coronary heart disease (Rimm et al. 1998; Rautiainen et al. 2010). When summarizing these two studies a risk reduction of 8 % (95 % CI: 8 – 13%, p for heterogeneity=0.21) for every three years of multivitamin use was observed.

4.5 TOTAL ANTIOXIDANT CAPACITY OF DIET AND STROKE (PAPER V)

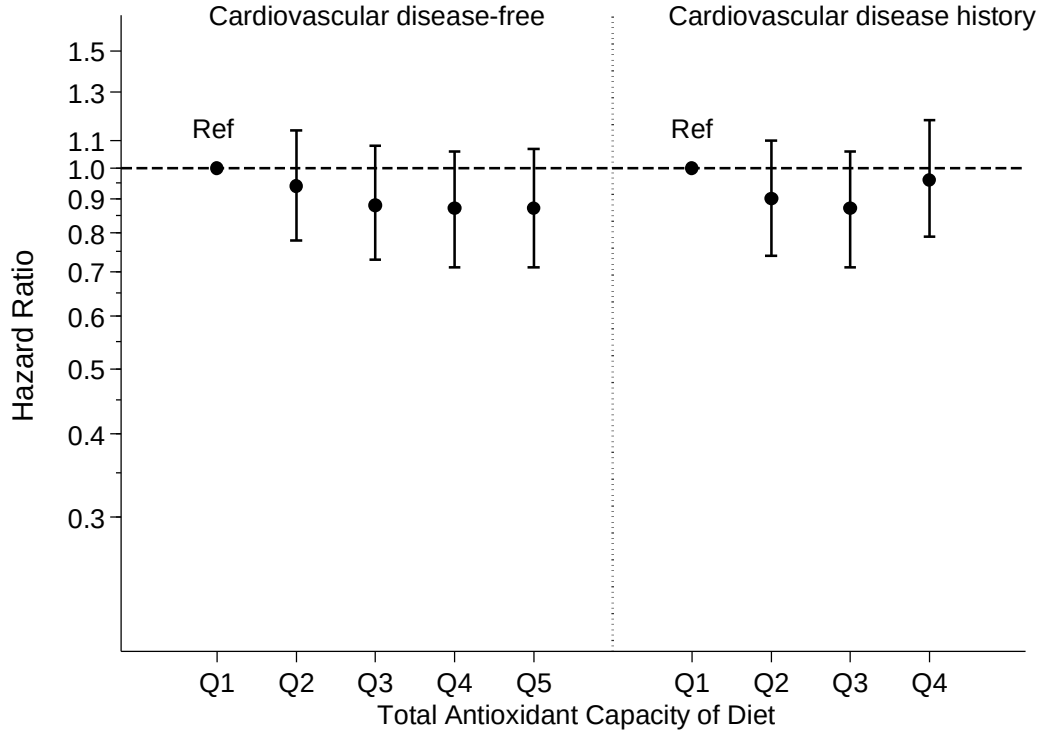
Cardiovascular disease (CVD)-free cohort
Women in the CVD-free cohort were followed until December 31, 2009, a median of 12.3 years (360,080 person-years), and a total of 1322 stroke cases (988 cerebral infarctions, 226 hemorrhagic strokes, 108 unspecified strokes) were identified. Women in the lowest quintile of Total Antioxidant Capacity of diet, as compared to those in the highest quintile were more likely to be non-smokers, have higher education, have hypercholesterolemia, and to use dietary supplements.

The HRs of total stroke, cerebral infarction and hemorrhagic stroke by quintiles of Total Antioxidant Capacity of diet are presented in **figure 4.8.** The multivariable-adjusted HR for total stroke among women in the highest quintile of Total Antioxidant Capacity of diet was 0.83 (95% CI: 0.70 – 0.99, p for trend=0.04). Adjustments for fruit and vegetable consumption (as a continuous variable), which is the major contributor to Total Antioxidant Capacity of diet, the association was no longer statistically significant (HR=0.89 (95% CI: 0.71–1.11). Total Antioxidant Capacity of diet was non-significantly inversely associated with both cerebral infarction and hemorrhagic stroke

Total Stroke



Cerebral Infarction



Hemorrhagic Stroke

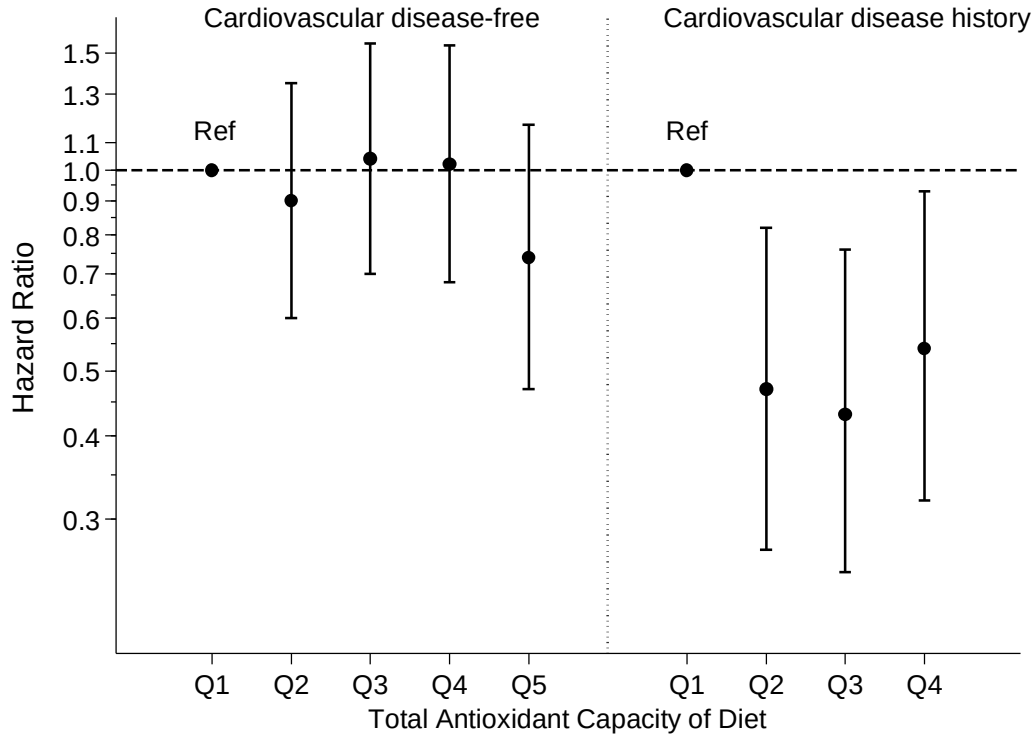


Figure 4.8. The association between multivitamin use and the risk of stroke among cardiovascular disease-free (n=31,035) women and women with cardiovascular disease history at baseline (n=5680). Dots represent point estimates and bars represent 95% confidence intervals.

The association between continuous Total Antioxidant Capacity of Diet and total stroke was further investigated in spline-analyses (**Figure 4.9**). An increment of 5000 ORAC TE units/day (corresponding to about 1 standard deviation in the study population) was associated with 6% (95% CI: 0 -11%) lower risk of total stroke. Correction for measurement error reviled a risk reduction of 9%.

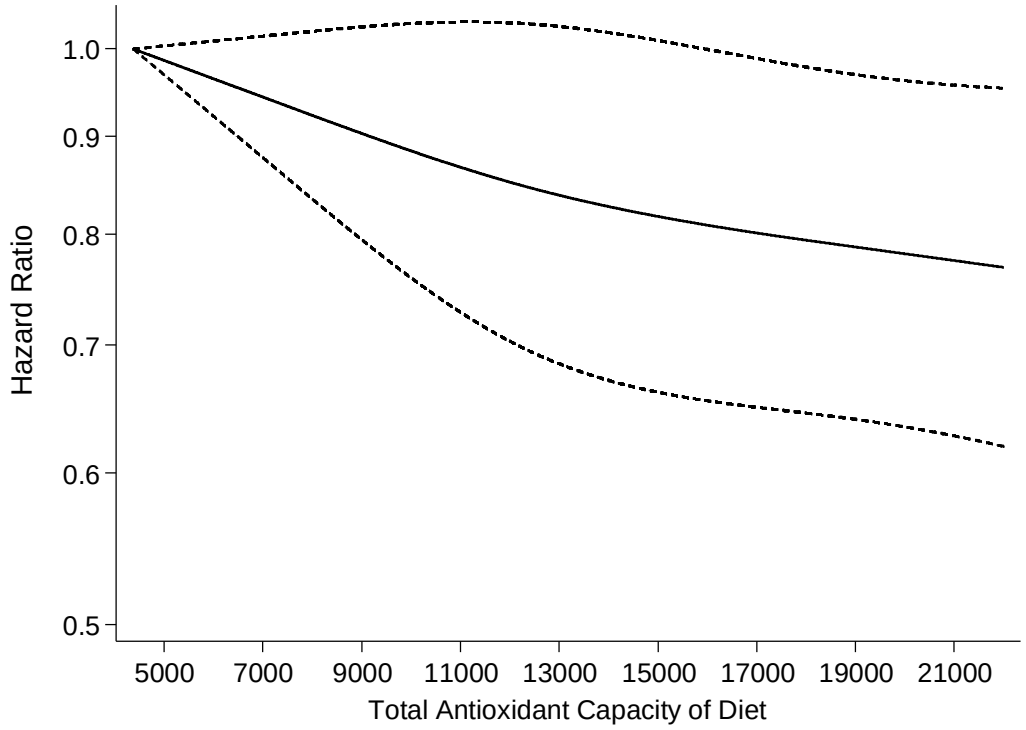


Figure 4.9. Multivariable-adjusted hazard ratios for incident total stroke among cardiovascular disease-free (n=31,035) women, according to dietary Total Antioxidant Capacity, based on the Oxygen Radical Absorbance Capacity assay (µmol trolox equivalents). Solid curve represents point estimates and dotted lines represent 95 % confidence intervals.

Stratified analyses by known risk factors for stroke are shown in **Figure 4.10**. The association between Total Antioxidant Capacity of diet and total stroke was stronger among younger women, overweight women, current alcohol drinkers and non-diabetic women, however, the interaction tests were not statistically significant (all p for interaction > 0.14).

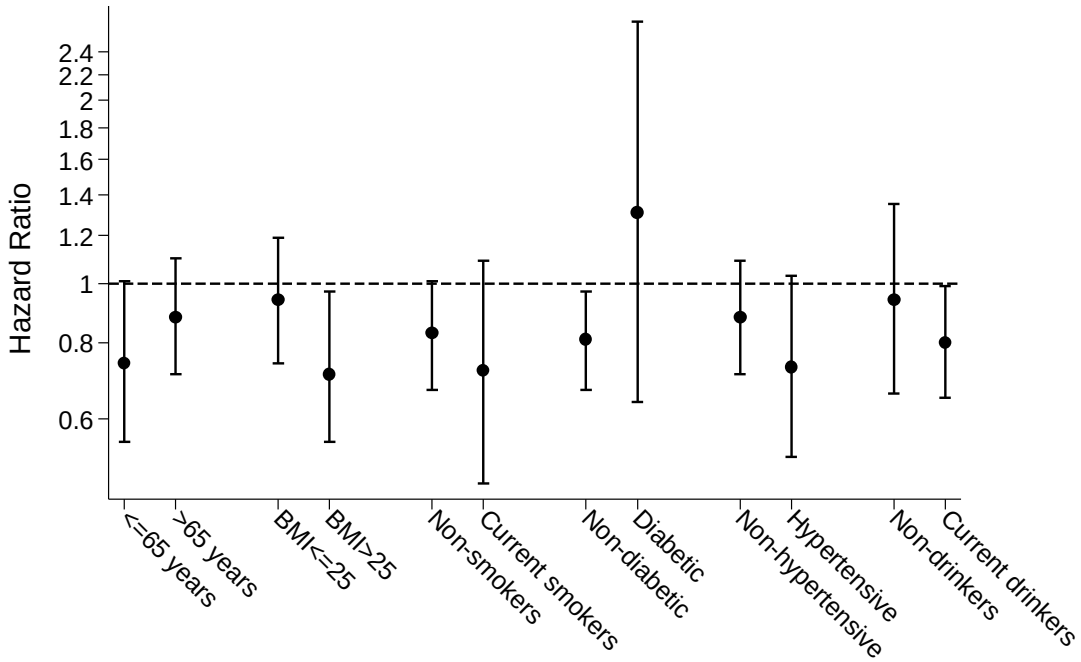


Figure 4.10. Subgroup analyses of the association between Total Antioxidant Capacity of diet and total stroke by potential risk factors. Dots represent point estimates in the highest quintiles compared to the lowest and bars represent 95% confidence intervals.

Cohort with Cardiovascular disease (CVD) history

Women with CVD history were followed during a median of 12.3 years (57,124 person-years) and 1007 stroke cases (796 cerebral infarctions and 100 hemorrhagic strokes, 111 unspecified strokes) were identified. Women in the highest quartile Total Antioxidant Capacity of diet were more likely to be non-smokers and to have higher education, hypercholesterolemia, hypertension, diabetes, and a family history of myocardial infarction compared with women in the lowest quartile of Total Antioxidant Capacity of Diet (Table 1, **paper V**). These women were also more likely to use aspirin and dietary supplements and had a higher consumption of fruits and vegetables (>2-fold) and tea (12-fold) but lower consumption of red meat (11%) and coffee (32%).

The association between Total Antioxidant Capacity of diet and stroke is presented in **Figure 4.8.** Women in the highest quartile of dietary TAC had 46 % (95 % CI: 7 – 68%) lower risk of hemorrhagic stroke; after adjustments for fruit and vegetable consumption the inverse association was not statistically significant (HR=0.59, 95% CI: 0.30–1.17). Total Antioxidant Capacity of diet was non-significantly associated with total stroke and cerebral infarction.

5 DISCUSSION

5.1 MAIN FINDINGS AND GENERAL DISCUSSION

5.1.1 Main Findings

The results in this thesis supports that Total Antioxidant Capacity of diet, an indicator of antioxidant content of foods, and multivitamin supplement use are associated with decreased risk of myocardial infarction among CVD-free women. No association was observed among women with CVD history. The Total Antioxidant Capacity of diet was also inversely associated with the risk of total stroke among CVD-free women but not among women with CVD history at baseline. These results suggest that Total Antioxidant Capacity of diet is of importance in primary but not secondary prevention of cardiovascular diseases, however, more studies are needed to confirm our results. The accumulated evidence indicates that multivitamin supplement use is of importance in primary prevention of coronary heart disease. Studies investigating a potential role of multivitamin supplement use in primary prevention of stroke and secondary prevention of myocardial infarction and stroke are needed.

Main findings on myocardial infarction in context of findings from other studies
This is the first study investigating dietary Total Antioxidant Capacity in relation to myocardial infarction. However, several foods which are major contributors to antioxidant intake have been linked to a decreased risk of coronary heart disease. In particular, high consumption of fruit and vegetables, which contributed to 44% of the dietary Total Antioxidant Capacity, have been inversely related to coronary heart disease in many studies (Dauchet et al. 2006). Whole grains (18% of Total Antioxidant Capacity) have also been inversely associated with coronary heart disease risk (Seal 2006; Kelly et al. 2007). Coffee consumption (14% of Total Antioxidant Capacity) has been inversely related to coronary heart disease in some, but not all studies (Cornelis et al. 2007). Chocolate consumption (4% of Total Antioxidant Capacity) has been associated with favourable effects on cardiovascular risk biomarkers such as flow-mediated dilation and diastolic blood pressure (Hooper et al. 2008).

Summary results from the meta-analysis of five prospective cohort studies indicated that the use of multivitamin supplements was consistently associated with lower risk of coronary heart disease incidence among CVD-free participants at baseline. Ingredients in multivitamin supplements (which sometimes also include minerals) that have been proposed to be protective against coronary heart disease are antioxidant vitamins (Fairfield et al. 2002; Thomson et al. 2007), B-vitamins (Fairfield et al. 2002; McNulty et al. 2008) and minerals e.g. magnesium and selenium (Brigelius-Flohe et al. 2003; Bo et al. 2008).

Main findings on stroke in context to findings from other studies
There is only one previous prospective study that investigated the association between Total Antioxidant Capacity of diet and stroke. That study used a CVD-free study population of women and men and included 112 cerebral infarctions and 48 hemorrhagic stroke cases. In this study with limited statistical power, Total Antioxidant Capacity of diet was inversely associated with the risk of cerebral infarction (HR = 0.41; 95% CI = 0.23-0.74 for highest vs lowest category) and a non-significant positive associated with the risk of hemorrhagic stroke (Del Rio et al. 2011). Previous studies have observed inverse associations between consumption of major contributors of Total Antioxidant Capacity such as fruit and vegetables (He et al. 2006), cereals (Ascherio et al. 1998; Oh et al. 2005; Larsson et al. 2009), tea (Arab et al. 2009), and chocolate (Mink et al. 2007; Buijsse et al. 2010) and risk of stroke. No previous study has examined the association between Total Antioxidant Capacity of diet and stroke risk in participants with CVD history at baseline.

5.1.2 General Discussion

Mechanisms involving antioxidants in CVD etiology
The initiating event in atherosclerosis is the impairment of the vascular endothelium and oxidative modification of LDL. This damage can be caused by infections, circulating toxins (e.g. cigarette smoke) or metabolites (e.g. glucose, LDL and homocysteine) (Madamanchi et al. 2005; Halliwell et al. 2007). Damage to the endothelium can also occur from mechanical forces e.g. turbulent blood flow caused by high blood pressure. Once the endothelium is injured more LDL are allowed into the vessel wall attracting monocytes which develop into macrophages that can secrete cytokines initiating local inflammation. The uptake of oxidized LDL by macrophages can lead to foam cell formation which will die by necrosis or apoptosis because of oxidation overload. This process leads to advanced atherosclerotic lesions (Madamanchi et al. 2005). The monocytes, macrophages and some enzymes (e.g. lipooxygenase enzymes) in the vessel wall will produce reactive oxygen species (e.g. O_2^{*-} and H_2O_2) (Poeckel et al. 2010). The presence of metals e.g. iron and/or cupper are suggested to accelerate the lipid peroxidation (Jomova et al. 2011).

Antioxidants such as alpha-tocopherol and beta-carotene are transported in LDL particles and have therefore been thought to inhibit oxidative modifications (Esterbauer et al. 1992), as described in the introduction. *In vitro* studies show that other antioxidants e.g. ubiquinol and vitamin C can recycle alpha-tocopherol. *In vitro* studies have also shown that polyphenols may protect LDL from oxidation by direct antioxidative effect as well as metal ion chelation and lipoxygenase inhibitaion (Gonzalez et al. 2011). Polyphenols have been shown to also improve endothelial function, reduced platelet aggregation, lower blood pressure, and exert anti-inflammatory effects (Grassi et al. 2009).

In summary, the development of atherosclerosis is a progression of many processes including hyperlipidemia, endothelial dysfunction, oxidation, inflammation, etc. If antioxidants play a role in this development, their role may not be equally important for all the different processes or across all the different stages of the disease (Moats et al. 2007). In already established atherosclerosis it may be too late for antioxidants to play any meaningful role. Moreover, patients with established CVD may already be well-managed medically – medicines such as statins have, besides the lipid-lowering effect, also antioxidative properties and other beneficial effects on endothelial function (Thomas et al. 2008) – hence further benefits of dietary antioxidants may not be seen. Indeed, when we previously examined the interplay between endogenous and exogenous antioxidants and assessed whether this interplay is different between CVD-free women and women with CVD history. Plasma extra cellular-SOD activity was statistically significantly inversely associated with plasma carotenoids and fruit and vegetable consumption among CVD-free women. However, no association was observed among women with CVD history. These results suggest that women with CVD history may be exposed to higher degree of oxidative stress and thus, dietary antioxidants are not sufficient to counterbalance the excessive amounts of reactive species whereby, the endogenous antioxidants defence system is activated (Zheng et al. 2011).

Total Antioxidant Capacity of diet as an antioxidant source

There are limited studies on Total Antioxidant Capacity of diet in relation to CVD. Total Antioxidant Capacity of diet provides a single estimate of antioxidant intake and is assumed to better measure antioxidant intake than the sum of individual antioxidants e.g. vitamins C, vitamin E, carotenoids and polyphenols, because it also reflects the synergistic and antagonistic effects between the compounds. One limitation with the databases used for calculation of Total Antioxidant Capacity of diet in this thesis is that they included American and Italian foods. It might have been more accurate to use Swedish foods because geographic location and growing conditions can affect the antioxidant content (Prior et al. 2005).

Previous observational studies focusing on antioxidants from diet in relation to CVD have mainly focused on individual antioxidants e.g. vitamin C, vitamin E and beta-carotene. Dietary intakes and blood concentrations of vitamin C and vitamin E have been inversely associated with cardiovascular diseases. Epidemiological studies focusing on carotenoids, a class of natural fat-soluble pigments found in plants, have reported inverse association between both dietary intake and blood concentrations of carotenoids and risk of coronary heart disease and stroke (Voutilainen et al. 2006).

More recent epidemiological studies have focused on plant polyphenols, a large group of natural antioxidants. However, these studies have only focused on subclasses of polyphenols such as flavonols, flavones, catechins and lignans and suggest beneficial

effects of both flavonoids and lignans on coronary heart disease (Arts et al. 2005). More research on other polyphenols and a potential role in stroke prevention is needed. However, there is limited evidence that polyphenol-rich foods modify antioxidative and oxidative biomarkers in humans (Hollman et al. 2011). Thus, a direct antioxidant effect of polyphenols in vivo is questionable, because of low blood concentrations as compared with other antioxidants and extensive metabolism following ingestion. Indeed, beneficial effects from polyphenol-rich foods (e.g. tea, coffee and chocolate) may be caused by other pathways than the chain-breaking reaction observed *in vitro*.

Dietary supplements as antioxidant sources

In contrast to dietary supplements, Total Antioxidant Capacity reflects all present antioxidants including thousands of compounds, all of them in doses present in our usual diet, and even takes into account their synergistic effects. Antioxidants derived from wide-spectrum low-dose multivitamin supplements may be important in primary prevention of CVD. This hypothesis has support from prospective cohort studies. However, results from these studies have been criticized of being confounded by a healthy behavior in general. An efficient way of dealing with such confounding is to perform randomized clinical trials. Today there is no published trial testing a low-dose wide-spectrum multivitamin supplement on the risk of incident myocardial infarction. The Suvimax trial tested the effect of a low-dose supplement mixture of five antioxidants (including 120 mg ascorbic acid, 30 mg vitamin E, 6 mg beta carotene, 100 µg selenium, and 20 mg zinc) and did not observe any association with incident ischemic cardiovascular disease risk (Hercberg et al. 2004). However, one study of Chinese women and men reported lower stroke mortality among those assigned to multivitamins (Mark et al. 1996). The Physicians Health Study II is the first study giving a low-dose wide-spectrum (including all essential vitamins and minerals) multivitamin to CVD-free men. This trial has just recently ended and no results are yet published.

It may seem contradictory that the results from observational studies on coronary heart disease risk (Rimm et al. 1993; Rimm et al. 1998; Klipstein-Grobusch et al. 1999; Holmquist et al. 2003; Ishihara et al. 2008) as well as from one randomized clinical trial on stroke risk (Mark et al. 1996) on multivitamins supplements are not in agreement with randomized clinical trials on high-dose antioxidant supplements containing one up to three compounds (Rapola et al. 1997; Yusuf et al. 2000; Lee et al. 2005; Lonn et al. 2005; Cook et al. 2007; Sesso et al. 2008). These trials have failed to see any benefit on coronary heart disease and stroke. Moreover, in a Cochrane review of randomized clinical trials, high-dose and very high-dose single supplements of vitamin A, β-carotene or vitamin E were associated with higher mortality (Bjelakovic et al. 2008). The reason for this apparent contradiction between observational studies on multivitamins and randomized clinical trials may be that the dose and the composition of antioxidants studied vary in different studies. *In vitro* studies have shown that

antioxidants in high-doses may exert pro-oxidative effects (Gutteridge et al. 2010). Another reason to the contradiction is that the majority of observational studies (Rimm et al. 1993; Rimm et al. 1998; Klipstein-Grobusch et al. 1999; Holmquist et al. 2003; Ishihara et al. 2008) and the Chinese trial (Mark et al. 1996) mainly studied primary prevention of CVD whereas, the majority of randomized clinical trials have investigated secondary prevention of CVD. We therefore performed separate analyses in CVD-free women and women with CVD history and our findings support the role of antioxidants in primary but not in secondary prevention of CVD.

5.2 METHODOLOGICAL CONSIDERATIONS

5.2.1 Information bias

Measurement error
Measurement error can cause bias in the estimated effect and is called information bias. The direction and magnitude of the bias varies depending on factors such as the distribution of errors, the actual value of the variable, actual values of other variables or measurement error in other variable (Rothman et al. 2008).

The measurement error in the FFQ-based Total Antioxidant Capacity estimates was investigated by comparing these estimates with Total Antioxidant Capacity in blood. A correlation of 0.3 was observed, indicating reasonable validity in the FFQ-based estimate used in **paper II** and **paper V**. When correcting the hazard ratios for measurement error by using information from the validation study an even stronger association was observed.

When including coffee and tea in the Total Antioxidant Capacity of diet, without taking into account coffee and tea absorption, no correlations with plasma Total Antioxidant Capacity values were observed. This may be explained by several factors. First, the main antioxidant in coffee is chlorogenic acid which can by the gut microflora be converted into caffeic acid. Chlorogenic acid has been hard to detect in blood after ingestion and blood concentrations of caffeic acid are rapidly decreasing suggesting that they might not be completely absorbed from the intestines or they might be rapidly metabolized or eliminated from the blood stream (Williamson et al. 2011). Moreover, a study measuring changes in Total Antioxidant Capacity of plasma after coffee and tea consumption observed only a weak increase (Natella et al. 2002; Moura-Nunes et al. 2009). Second, plasma samples in the validation study were taken after an overnight fasting state (>12h) which may also contribute to the lack of correlation with the Total Antioxidant Capacity of diet estimate not taking into account absorption. Third, coffee has a high content of Maillard products and these are contributing to the Total Antioxidant Capacity of coffee (Lara Manzocco 2001). The evidence, however, on

absorption rate and metabolism regarding Maillard products in humans is lacking (Somoza 2005).

Total Antioxidant Capacity in plasma

When examining validity of dietary exposures, biomarkers are suggested to be good reference methods because they are objective measures and not influenced by those biases associated with self-report of dietary intake which may be a more subjective measure (Jenab et al. 2009). It may therefore, be obvious to think that plasma Total Antioxidant Capacity measures should be used as the golden standard when validating Total Antioxidant Capacity intake. However, plasma Total Antioxidant Capacity is influenced by many factors, such as endogenous antioxidants; homeostatic control mechanisms of plasma antioxidants, stress, environment, pollution, inflammation and absorption; as well as the extent of the metabolism of dietary antioxidants. We found reasonable good correlations between FFQ-based Total Antioxidant Capacity estimates and ORAC in whole plasma and lipophilic part of plasma, however, stronger correlations would have been preferable. This can partly be explained by other factors besides dietary intake that can influence antioxidant biomarker values. It is also important to point out that Total Antioxidant Capacity in blood not necessarily reflect changes in various tissues. The antioxidant content varies between different organs according to their requirements (Elsayed 2001; Evelson et al. 2001).

Some assays are very sensitive to specific compounds in blood e.g. uric acid which is produced endogenously (McNaughton et al. 2005). Uric acid can provide as much as 60% of oxygen and free-radical scavenging in human serum (Ames et al. 1981) and has shown to contribute 7% of ORAC in whole serum, 20–60% of TRAP in whole serum, and 60% FRAP in whole serum (Lotito et al. 2006). Uric acid was analyzed for the women in the validation study and correlated significantly with ORAC in hydrophilic part of plasma and TRAP and FRAP. Uric acid has antioxidant properties, however, studies suggest that increased uric acid concentrations and gout are associated with subclinical atherosclerosis and increased risk of CVD (Gagliardi et al. 2009).

It may seem contradicting that the FFQ-based Total Antioxidant Capacity estimate, assessed with ORAC, only correlated with ORAC of the lipophilic and not the hydrophilic part of plasma. This may be explained by that the fact that hydrophilic antioxidants are bound to proteins and are lost during the extraction procedure (Prior et al. 2007). On the other hand, lipophilic antioxidants such as carotenoids and tocopherols will be extracted into the lipid fraction and these antioxidants have also previously have been shown to correlate with dietary antioxidant intake (McNaughton et al. 2005).

There is only one previous study validating FFQ-based Total Antioxidant Capacity intake using FRAP and TEAC in whole plasma as references. This study observed weak correlations with plasma FRAP (r=0.17) and non-significant positive correlations plasma TEAC (r=0.11) (Pellegrini et al. 2007).

Several assays also fail in capturing antioxidant capacity from certain compounds. The ORAC and TRAP assay are due to their chemistry not capturing antioxidant capacity from carotenoids whereas, the FRAP assay is not capturing capacity from compounds with sulfate groups (Prior et al. 2005).

Although endogenous antioxidants are tightly regulated in vivo, there is a need to validate Total Antioxidant Capacity of diet with biomarkers of antioxidant efficiency to understand how diet can affect the redox defense homeostasis in humans.
The correlations between Total Antioxidant Capacity intake estimated with FFQs completed one year apart indicated good reproducibility, similar to reproducibility of other nutrients (Messerer et al. 2004; Levitan et al. 2007).

Misclassification
Measurement error for discrete variables are called misclassification (Rothman et al. 2008). Misclassification can either be non-differential or differential and can occur in both exposure and disease measurements. Non-differential misclassification is when the proportion of participants being misclassified is not dependent on other variables. Non-differential misclassification is most likely to give an underestimation of the true association. Differential misclassification occurs when the misclassification is dependent of other variables. Such bias is common in retrospective study deigns such as case-control studies and is called recall bias (Rothman et al. 2008).

Misclassification of exposure in **paper III** may have occurred if those reporting multivitamin use were not using multivitamin and vice versa. Misclassification of multivitamin use was investigated with sensitivity and specificity and used for correcting the hazard ratios. After this correction an even stronger association was observed. Misclassification of exposure may also have occurred from the consumption of fortified foods, which might have influenced the effect of multivitamin supplements. In Sweden, vitamin D is added to milk, butter and margarine and vitamin A is added to butter and margarine (Livsmedelsverket 1983; Livsmedelsverket 2006). Other vitamins and minerals may have been added to some foods (e.g, cereals and fruit juices), however, there is no mandatory fortification policy for other micronutrients. A stronger association with multivitamins might be expected when the food supply was not already fortified. Misclassification of the outcomes is very unlikely to have occurred due to almost complete and accurate information in the registries used for identification of cases.

5.2.2 Selection bias

Selection bias is defined as "both exposure and disease are affecting selection specifically, because they affect selection" (Rothman et al. 2008). Selection bias arises when participants selected are different from those not participating and may occur especially in a case-control design. Selection bias can also occur when the exposure is associated with a third factor causing the disease and if this bias occurs before exposure and disease it is classified as a confounder. One major reason to selection bias in cohort studies is loss of follow-up (Rothman et al. 2008).

Selection bias due to differences between participating and non-participating women is very unlikely to have any major impact on our results because the Swedish Mammography Cohort is a population-based cohort with high response rate. Selection bias due to loss of follow-up is unlikely to have appeared because women were almost completely followed-up.

5.2.3 Confounding

When looking at exposure-disease associations one must be very careful with the interpretation of the observed finding. In observational epidemiology it is important to think whether the observed association may be explained by a third factor which if it is associated with the exposure and the disease is called confounder (Rothman et al. 2008). There are three criteria for a confounding factor; 1) it must be an extraneous risk factor for the disease, 2) it must be associated with the exposure under study in the source population, and 3) it must not be in the causal chain from exposure to disease (Rothman et al. 2008). A confounder can lead to over- or underestimation of the true association. There are different ways of handling of confounding (Rothman et al. 2008). One way is to include the confounder as a covariate in the statistical model. A second way is to use restriction e.g. you exclude participants with that special characteristic at baseline. Randomization of the exposure as done in randomized clinical trials is an efficient way of removing the effect from known and unknown confounders. However, a successful randomization is dependent of a large number of participants (Rothman et al. 2008).

In observational epidemiology it is important to identify potential confounders and adjust for these factors. There is no single technique which identifies "real" confounders. It is very common to classify known risk factors for a disease as confounders, however, these are not confounders if they are not associated with the exposure. When the exposure-disease association is not adjusted for all potential confounders and/or identified potential confounders are not measured correctly, the association is influenced by residual confounding (Rothman et al. 2008).

In **paper II, III** and **V**, confounding was controlled for by restricting the analyses to women without cancer history at baseline. Moreover, we also excluded in **paper II** and **III** women with diabetes because these diagnoses may lead to changes in dietary habits and at the same time these women are at higher risk of myocardial infarction. We also handled confounding by including these factors as covariates in the statistical model. Those factors influencing the observed association most were age and smoking. Women with an antioxidant-rich diet or who use dietary supplements may be more health conscious and have other healthy behaviors. This might explain the observed inverse associations for myocardial infarction and stroke. However, when adjusted for several potential confounders associated with a healthy lifestyle the results remained unchanged.

In **paper V** we observed among women with CVD history at baseline, a reduced risk of hemorrhagic stroke in the highest three quartiles of Total Antioxidant Capacity of diet. However, these results should be interpreted with caution. These results may be confounded by that women in the lowest quartile were more likely to have a history of stroke and therefore be more likely to get hemorrhagic stroke (Fan et al. 2003). However, the inverse association remained when we adjusted for stroke history. Moreover, these results may also be confounded by the fact that women with CVD history may control their blood pressure or change their lifestyles because of knowledge of their disease. This might have produced a spurious inverse association between TAC of the diet and risk of hemorrhagic stroke.

Residual confounding may have affected observed findings, however, information was collected for a wide range of potential confounders and eventual measurement error in the potential confounders was investigated.

5.2.4 Effect modification

When studying exposure-disease associations it is important to investigate whether the association varies by a third factor, an effect-modifier (Rothman et al. 2008). One way to identify effect-modifiers is to perform stratified analysis. If the risk estimates differs across stratas, a potential effect modifier is present. A statistical test for interaction may be performed to give further evidence for a true effect modifier. Statistical tests can be performed on the additive scale and the multiplicative scale. Additive interaction is usually referred as biological interaction and the statistical tests provide the magnitude of the interaction. Multiplicative interaction is explored by creating an interaction term between exposure and the potential effect modifier in the statistical model (Rothman et al. 2008).

In paper **II, III** and **V,** identification of potential effect modifiers were investigated by stratified analysis of known risk factors. Interaction was investigated on the multiplicative scale. The association between Total Antioxidant Capacity of diet and

multivitamin supplement use and myocardial infarction was somewhat different across stratas for CVD at baseline. However, the interaction test on the multiplicative scale was not statistically significant.

5.2.5 Publication bias

When performing meta-analyses it is important to identify all studies investigating the exposure-disease association of interest. It is common to use computerized databases e.g. PubMed and EMBASE for the literature search. However, some studies may not be identified through such a literature search, especially not unpublished papers. Statistically significant results are more likely to be published than non-significant results (Rothman et al. 2008).

In **paper IV** only five studies were identified, all indicating an inverse association between multivitamin use and incident coronary heart disease among participants who were coronary heart disease-free at baseline. Publication bias was investigated by three different statistical tests such as the Egger regression asymmetry test (Egger et al. 1997), Begg rank correlation test (Begg et al. 1994) and a nonparametric iterative trim-and-fill procedure (Duval et al. 2000; Duval et al. 2000). None of these tests did find any evidence of publication bias. However, these tests have low power, especially when the number of studies is small. Hence, we cannot exclude potential publication bias.

5.2.6 Generalizability

When identifying an exposure-disease association it is important to think how the results should be generalized to the general public. It is common that study populations are restricted with regard to certain factors to avoid confounding, to gain cooperative behavior and attain accurate information. This may be more important than to have a representative sample of the natural population. However, having a homogenous population may sometimes affect the generalizability of the study findings (Rothman et al. 2008).

In the Swedish Mammography Cohort of middle-aged and elderly women, Total Antioxidant Capacity of diet was inversely associated with the risk of myocardial infarction among CVD-free women. These results may therefore, be generalized to women who are CVD-free. It is however, likely that also CVD-free men are benefiting from diet high in Total Antioxidant Capacity because atherosclerosis is also occurring among men. Multivitamin use has been inversely associated to coronary heart disease both among women and men and therefore, such an association may be generalized to both genders.

6 CONCLUSION

- FFQ-based estimates of Total Antioxidant Capacity of diet have reasonable validity and reproducibility and may be used in nutritional epidemiology to assess antioxidant intake.

- Total Antioxidant Capacity of diet may be of importance in primary prevention but not secondary prevention of myocardial infarction.

- Total Antioxidant Capacity of diet may be of importance in primary but not secondary prevention of total stroke.

- The use of multivitamin supplements might be of importance in primary but not secondary prevention of myocardial infarction.

- The summarized risk estimate of accumulated evidence from prospective cohort studies indicates that multivitamins may have a role in primary prevention of coronary heart disease.

7 FUTURE RESEARCH

The results in the thesis support that Total Antioxidant Capacity (TAC) of diet, an indicator of antioxidant content of foods, may play a role in primary prevention of myocardial infarction and stroke. Our study is the first addressing a potential role of TAC of diet in primary prevention of myocardial infarction. Only one previous study has investigated a role of TAC of diet in primary prevention of stroke. Future prospective cohort studies may consider using FFQ-based TAC estimates instead of sum of single antioxidants when assessing antioxidant intake. However, it is important to investigate whether all foods with a TAC should be considered as efficient antioxidant sources. When absorption of antioxidants from coffee and tea was taken into account in the FFQ-based TAC estimates, the validity was improved. Thus, absorption efficiency of antioxidants from foods should be taken into account when constructing databases for calculation of TAC estimates. Clinical studies investigating absorption efficiency of specific antioxidative compounds from foods would provide further knowledge in this area.

Our results suggest that TAC of diet does not play a role in secondary prevention of myocardial infarction and stroke. It is very important from both a clinical and public health point of view that future prospective cohort studies performs analyses separately among participants with or without prevalent CVD to evaluate whether a potential protective role varies in different stages of CVD progression.

It would be desirable, although difficult, to perform randomized clinical trials to investigate changes from TAC of diet on cardiovascular biomarkers among both CVD-free populations and populations with CVD history. This would give important insights in the specific mechanisms through which TAC of diet may induce changes in relevant biomarkers impacting the subsequent CVD risk.

The results of the thesis support a protective role of multivitamin supplements in primary prevention of coronary heart disease. Today there is limited evidence from observational studies on the role of multivitamins in stroke prevention and therefore, prospective cohort studies should investigate multivitamins in primary and secondary prevention of stroke and different stroke subtypes.

Randomized clinical trials are needed because they can provide the most convincing evidence of a potential role of multivitamins in primary and secondary prevention of CVD. The Suvimax trial is the only study testing a low-dose supplement of five antioxidants in ischemic CVD prevention. The recently ended Physicians Health Study II (men) is the first trial testing the role of a low-dose wide-spectrum (including all essential vitamins and minerals) supplement in primary prevention of CVD. Results from this trial will help to direct future research to answer the many questions that remain about multivitamin use.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Oxidativ stress innebär att det finns ett överskott av cellförstörande så kallade fria radikaler och andra reaktiva föreningar. Det kan i förlängningen leda till åderförkalkning som i sin tur kan orsaka hjärtkärlsjukdom. Antioxidanter, så som vitamin C, vitamin E, karotenoider och flavonoider, kan förhindra oxidativ stress genom att de oskadliggör de fria radikalerna. Intag av frukt och grönsaker, som är höga i antioxidantinnehåll, har i många epidemiologiska studier kopplats till lägre risk för hjärtkärlsjukdom. Tidigare studier har fokuserat på enstaka antioxidanter och rapporterat inkonsekventa resultat. I själva verket bidrar kosten till ett brett spektrum av antioxidanter och alla verkar i synergi med varandra i ett mycket komplext nätverk. Total Antioxidant Capacity (TAC) är ett koncept som syftar till att ge ett mått på ett livsmedels kapacitet i att bekämpa fria radikaler.

Multivitamintillskott är en annan källa till antioxidanter och ett vanligt antagande är att de är ett bra substitut till välbalanserad kost och kan förebygga uppkomsten av hjärtkärlsjukdom. Användandet av kosttillskott har stigit markant de senaste åren och det har rapporterats från både Sverige och USA att ca 50% av befolkningen använder någon typ av kosttillskott. Därför är det av stor betydelse ur ett folkhälsoperspektiv att studera användandet av kosttillskott. Många epidemiologiska studier har undersökt sambandet mellan användning av multivitamintillskott och risken för hjärtkärlsjukdom och flera av dessa studier har rapporterat lägre risk för hjärtinfarkt hos de som använder multivitamintillskott.

Tre av artiklarna som ligger till grund för denna avhandling bygger på en stor populationsbaserad studie av ca 40,000 kvinnor som har fyllt i ett frågeformulär om kostvanor, kosttillskottsanvändning och andra livsstilsfaktorer år 1997. Genom att tillämpa databaser med olika livsmedels TAC-värden kunde vi beräkna kvinnornas antioxidantintag. Vi kunde även via frågeformuläret identifiera vilka kvinnor som använde multivitamintillskott. De kvinnor som utvecklat hjärtinfarkt eller stroke identifierades med hjälp av det nationella patientregistret. Kvinnorna delades upp i två grupper, de utan hjärtkärlsjukdom vid studiens start och de som redan hade hjärtkärlsjukdom vi studiens start. Dessa två grupper följdes upp separat under studiens gång.

I det första delarbetet utvärderade vi hur bra frågeformuläret var att mäta TAC-intag genom att jämföra med TAC i blod hos 108 kvinnor. Vi fann att intaget överensstämde med TAC i blod. Till det totala TAC-intaget bidrog frukt och grönsaker med 44%, fullkornsprodukter med 16% och kaffe med 14%. Vidare undersökte vi om det fanns ett samband mellan TAC-intag och risken för att utveckla hjärtinfarkt och stroke. Bland kvinnor utan hjärtkärlsjukdom vid studiens start fann vi en statistiskt säkerställd lägre risk för hjärtinfarkt och stroke. Kvinnor med det högsta antioxidantintaget hade 20% lägre risk för hjärtinfarkt och 17% lägre risk för stroke

jämfört med kvinnor med det lägsta intaget. Bland kvinnor med hjärtkärlsjukdom vid studiens start fann vi inget samband med hjärtinfarkt eller stroke.

Eftersom vitamintillskott också bidrar till antioxidantintag undersökte vi även sambandet mellan användandet multivitamintillskott och risken för hjärtinfarkt. Vi fann att användet av multivitamintillkott var kopplat till 27% lägre risk för hjärtinfarkt hos kvinnor utan hjärtkärlsjukdom vid studiens start. Inget samband mellan multivitamintillskott och hjärtinfarkt observerades inom gruppen med hjärtkärlsjukdom vid studiens start. Vi utförde en systematisk granskning av litteraturen med avseende på multivitaminanvändning och risken för ischemisk hjärtsjukdom. De sammanfattade resultaten visade att multivitamintillskott var kopplat till 21% lägre risk för ischemisk hjärtsjukdom hos personer utan hjärtkärlsjukdom vid studiens start.

Sammanfattningsvis indikerar resultaten i denna avhandling att antioxidanter från kosten kan skydda mot insjuknandet i hjärtinfarkt och stroke hos kvinnor utan hjärtkärlsjukdom. Fler studier behövs för att undersöka om TAC är ett bra riktmärke för att skydda sig mot hjärtkärlsjukdom. Våra resultat indikerar också att användandet och multivitamintillskott skyddar mot hjärtinfarkt hos kvinnor utan hjärtkärlsjukdom vid studiens start. Det går inte helt och hållet att utesluta att resultaten från våra studier förklaras de som använder multivitaminer i allmänhet lever ett mer hälsosamt liv. Därför finns det ett stort behov and randomiserade kliniska studier eftersom de anses ge den mest övertygande bevisningen om ett eventuellt samband.

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