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OXIDATIVE STRESS AND AGE-RELATED CATARACT

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Oxidative Stress and Age-Related Cataract

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Age-related cataract is a clouding of the lens that leads to decreased vision. It increases with age and is one of the leading causes of blindness worldwide. The only treatment currently available is surgery. Therefore, it is important to identify modifiable risk factors for cataract prevention. The cause of cataract is not fully understood and may be multifactorial, involving oxidative stress, a condition of disrupted balance between oxidants and antioxidants. Oxidative damage to lens proteins and lipids is suggested to be involved in the development of cataract. Antioxidants may protect against oxidative damage.

The aim of this thesis was to examine factors related to oxidative stress, including biomarkers of exogenous/dietary and endogenous antioxidants, and systemic oxidative stress and inflammation, as well as vitamin supplement use and physical activity, with the risk of age-related cataract. The studies were based on women and men, born 1914-1952, in the population-based Swedish Mammography Cohort and the Cohort of Swedish Men. Information on diet and lifestyle factors was obtained from a self-administered questionnaire at baseline. Cases of age-related cataract were identified through linkage to registers.

The relationship between exogenous/dietary and endogenous antioxidants was examined in a cross-sectional study of women with and without a history of chronic diseases. High fruit and vegetable intake and high levels of plasma carotenoids were associated with lower plasma extracellular superoxide dismutase activity (an endogenous antioxidant enzyme) in healthy women but not in women with a history of chronic diseases. In a nested case-control study including women with and without incident cataract, higher levels of urinary 8-iso-prostaglandin $F_{2\alpha}$ (a biomarker for systemic oxidative stress) were associated with increased risk of cataract, but no association was observed for 15-keto-dihydro-prostaglandin $F_{2\alpha}$ (a biomarker for systemic inflammation). The association between dietary supplement use and risk of cataract was investigated prospectively in the cohorts. The use of single, high-dose supplements of vitamin C or E, as well as B vitamins, but not multivitamins (usually containing vitamin doses close to recommended daily intake), was associated with increased risk of cataract. The use of vitamin C supplements in combination with some oxidative stress-related factors, such as age and corticosteroid use, as well as in the long-term, may be associated with even higher risk. The association between physical activity and risk of cataract was also examined prospectively. Higher levels of total physical activity, especially long-term, and specific subtypes including walking/bicycling and work/occupational activity, were associated with lower risk of cataract in women and men. Conversely, high leisure time inactivity levels were associated with increased risk of cataract.

In conclusion, these results suggest that maintaining low systemic oxidative stress by having a healthier lifestyle, including eating a diet rich in antioxidants instead of taking high-dose supplements and being physically active may prevent cataract development in the general population.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Oxidativ stress är ett tillstånd som uppstår vid obalans mellan oxidanter och antioxidanter, där det bildas ett överskott av oxidanter eller finns otillräckligt med antioxidantaktivitet. Reaktiva syre- och kväveföreningar bildas vid olika cellulära processer, som t ex cellsignalering och cellmetabolism, men för stora mängder av dessa oxidanter kan leda till oxidativa skador på lipider, protein och DNA. Antioxidanter, både de som tillförs via kosten och kroppsegna, behövs för att skydda cellerna mot oxidativa skador. Oxidativ stress har associerats med åldrandet och olika sjukdomar som t ex grå starr. Åldersberoende grå starr är en vanlig ögonsjukdom som drabbar många äldre. Det innebär att ögats lins grumlas, vilket leder till nedsatt syn. Linsen utsätts dagligen för oxidation och det är därför viktigt att ha ett effektivt och välfungerande antioxidantsystem för att skydda ögat mot oxidativa skador. Orsaken till varför grå starr uppstår är idag inte fullständigt klarlagt, men en viktig del anses involvera oxidativ stress. Idag är den enda behandlingen att ersätta den grumliga linsen med en konstgjord lins genom operation. I Sverige är gråstarrsoperation den vanligast utförda operationen och enligt Nationella Kataraktregistrets senaste årsrapport utfördes ca 110,000 operationer under 2013. Det är därför viktigt att studera både skyddande faktorer och riskfaktorer för grå starr för att kunna förebygga uppkomsten av sjukdomen.

Syftet med denna avhandling var att studera sambanden mellan olika antioxidanter, samt hur faktorer relaterade till oxidativ stress, såsom biomarkörer för systemisk oxidativ stress och inflammation, samt vitamintillskott och fysisk aktivitet, påverkar risken för åldersberoende grå starr. Studierna i avhandlingen är baserade på kvinnor och män, 45-83 år, från de populationsbaserade kohorterna Svenska mammografikohorten (inklusive den kliniska subkohorten) som startades 1987 och Kohorten av svenska män som startades 1997. Deltagarna har vid start av studien besvarat ett frågeformulär med frågor relaterade till kost och livsstilsvanor. Kvinnorna i den kliniska subkohorten besvarade ytterligare ett frågeformulär samt deltog i en hälsoundersökning där de lämnade blod- och urinprover. Information om gråstarrsoperationer erhölls genom matchning mot olika register.

I det första delarbetet undersökte vi i en tvärsnittsstudie sambandet mellan antioxidanter från kosten (frukt- och grönsaksintag och karotenoider i blodet) och kroppsegna antioxidanter (plasma extracellulär superoxiddismutas-aktivitet, vilket är ett antioxidant enzym som produceras i kroppen och katalyserar oskadliggörande av superoxid som är en reaktiv syreförening) hos friska kvinnor och kvinnor med kroniska sjukdomar. Resultaten visade att friska kvinnor med ett högt intag av frukt och grönsaker samt höga nivåer av karotenoider i blodet hade lägre plasma superoxiddismutas-aktivitet. Det skulle kunna bero på en kompensatorisk nedreglering av kroppsegna antioxidanter vid högt antioxidantintag från kosten. Vi observerade däremot inget samband hos kvinnor med kroniska sjukdomar, såsom hjärt-kärlsjukdomar, diabetes eller cancer.

I det andra delarbetet studerade vi biomarkörer för systemisk oxidativ stress (8-iso-prostaglandin $F_{2\alpha}$) och inflammation (15-keto-dihydro-prostaglandin $F_{2\alpha}$) i urin hos kvinnor med och utan grå starr. För varje kvinna som hade utvecklat grå starr under uppföljningstiden 2003-2009 slumpades en frisk kontroll som matchats för ålder och datum för inlämning av urinprov. Kvinnor med grå starr hade högre 8-iso-prostaglandin $F_{2\alpha}$ nivåer (systemisk oxidativ stress) jämfört med friska kontroller. Däremot observerades ingen skillnad i nivåer av 15-keto-dihydro-prostaglandin $F_{2\alpha}$ (systemisk inflammation).

Resultaten från kohortstudierna i det tredje och fjärde delarbetet visade att män som använde enbart vitamin C eller vitamin E kosttillskott innehållande höga doser, men inte de som använde multivitaminer (vanligtvis innehållande flera vitaminer och/eller mineraler med doser nära det rekommenderade dagliga intaget), hade ökad risk för grå starr jämfört med de som inte använde kosttillskott. För vitamin C kosttillskott verkade sambandet vara starkare bland äldre män, kortikosteroid-användare och långtidsanvändare. Dessa resultat bekräftar en tidigare publicerad studie baserat på den Svenska Mammografikohorten som visade liknande resultat. Användning av kosttillskott innehållande höga doser av olika B vitaminer var också kopplat till ökad risk för grå starr hos kvinnor och män.

I det femte delarbetet undersökte vi i en kohortstudie sambandet mellan fysisk aktivitet och risk för grå starr hos kvinnor och män. Resultaten visade att de som hade hög total fysisk aktivitet, promenerade och/eller cyklade mer än 60 minuter per dag eller var mer fysiskt aktiva på arbetet hade lägre risk att utveckla grå starr, jämfört med de som var mindre fysiskt aktiva. Sambandet var ännu starkare för de som konsekvent hade en hög total fysisk aktivitet under en längre tidsperiod. Däremot var mycket stillasittande på fritiden associerat till ökad risk för grå starr. Jämfört med de två tidigare publicerade prospektiva studierna som undersökte fysisk aktivitet (främst träning) och risk för grå starr endast bland deltagare som redan tränade mycket, visar dessa resultat att fysisk aktivitet kan vara en skyddande faktor mot grå starr även i en generell population.

Resultaten i denna avhandling visar att samspelet mellan olika antioxidanter kan skilja sig beroende på hälsostatus och att systemisk oxidativ stress kan vara kopplat till grå starr. I en generell population med relativt god nutritionsstatus verkar användandet av kosttillskott i form av multivitaminer inte ha någon större påverkan på risken för grå starr, däremot kan användande av enskilda vitaminer i höga doser vara associerat med ökad risk. Fysisk aktivitet kan vara en skyddande faktor mot grå starr, medan mycket stillasittande kan vara kopplat till ökad risk. Sammanfattningsvis tyder resultaten på att minskad systemisk oxidativ stress, t ex genom att äta en allsidig kost rik på antioxidanter istället för att använda kosttillskott, och att vara fysiskt aktiv regelbundet kan minska risken för grå starr. För framtida studier kan det vara värdefullt att undersöka oxidativ stress och antioxidanter i olika populationer, t ex bland högrisk grupper med lågt antioxidantintag, samt med hänsyn till hälsostatus.

LIST OF SCIENTIFIC PAPERS

I. **Jinjin 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;14(1):9-14.

II. **Jinjin Zheng Selin**, Birgitta Ejdervik Lindblad, Susanne Rautiainen, Karl Michaëlsson, Ralf Morgenstern, Matteo Bottai, Samar Basu and Alicja Wolk. Are Increased Levels of Systemic Oxidative Stress and Inflammation Associated with Age-Related Cataract? *Antioxid Redox Signal*. 2014 Aug;21(5):700-4.

III. **Jinjin Zheng Selin**, Susanne Rautiainen, Birgitta Ejdervik Lindblad, Ralf Morgenstern and Alicja Wolk. High-Dose Supplements of Vitamins C and E, Low-Dose Multivitamins, and the Risk of Age-related Cataract: A Population-based Prospective Cohort Study of Men. *Am J Epidemiol*. 2013 Mar;177(6):548-55.

IV. **Jinjin Zheng Selin**, Birgitta Ejdervik Lindblad, Matteo Bottai, Ralf Morgenstern and Alicja Wolk. High-Dose B Vitamin Supplements and Risk of Age-Related Cataract: A Population-Based Prospective Study of Men and Women. *Submitted.*

V. **Jinjin Zheng Selin**, Nicola Orsini, Birgitta Ejdervik Lindblad and Alicja Wolk. Long-Term Physical Activity and Risk of Age-Related Cataract: A Population-Based Prospective Study of Male and Female Cohorts. *Ophthalmology*. 2015 Feb;122(2):274-80.

CONTENTS

LIST OF ABBREVIATIONS

BMI	Body Mass Index
CI	Confidence Interval
COSM	Cohort of Swedish Men
CVD	Cardiovascular Disease
EC-SOD	Extracellular Superoxide Dismutase
FFQ	Food Frequency Questionnaire
HR	Hazard Ratio
MET	Metabolic Equivalent
OR	Odds Ratio
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
RCT	Randomized Controlled Trial
RDI	Recommended Daily Intake
ROS	Reactive Oxygen Species
SMC	Swedish Mammography Cohort
SMC-C	Swedish Mammography Cohort–Clinical
SOD	Superoxide Dismutase
WHO	World Health Organization

1 INTRODUCTION

Oxidative stress is a condition of disrupted balance between oxidants and antioxidants, towards excessive amounts of oxidants or insufficient antioxidant activity.[1] It has been related to the aging process and a variety of diseases.[2-4] Age-related cataract is a clouding of the lens that leads to disturbed vision and is one of the major causes of visual impairment worldwide.[5] Oxidative stress is suggested to be involved in the etiology of cataract. Since the lens is under target of oxidation, there is a need of efficient antioxidant systems in the eyes to protect against oxidative damage. Some of the known risk factors for cataract include both non-modifiable (such as age and gender) and modifiable (lifestyle-related) factors.[5] Since the only treatment for cataract today is through surgery (cataract extraction), it is of public health importance to find modifiable risk factors for cataract prevention. The aim of this thesis was to examine the association between oxidative stress, including biomarkers of antioxidants and systemic oxidative stress and inflammation, as well as modifiable diet and lifestyle factors related to oxidative stress, such as vitamin supplement use and physical activity, and the risk of age-related cataract among women and men in two large, population-based cohorts in Sweden.

2 BACKGROUND

2.1 OXIDATIVE STRESS

Oxygen is a molecule essential for the existence of life, but it has also the property of becoming toxic through the generation of reactive oxygen species (ROS).[1] Antioxidants protect cells and tissues from oxidative damage by scavenging free radicals. During normal physiological conditions there is a balance between levels of oxidants and antioxidants. However, if this balance is disrupted towards excessive amounts of oxidants or insufficient antioxidant activity, a condition known as oxidative stress arises (**Figure 1**).[1]

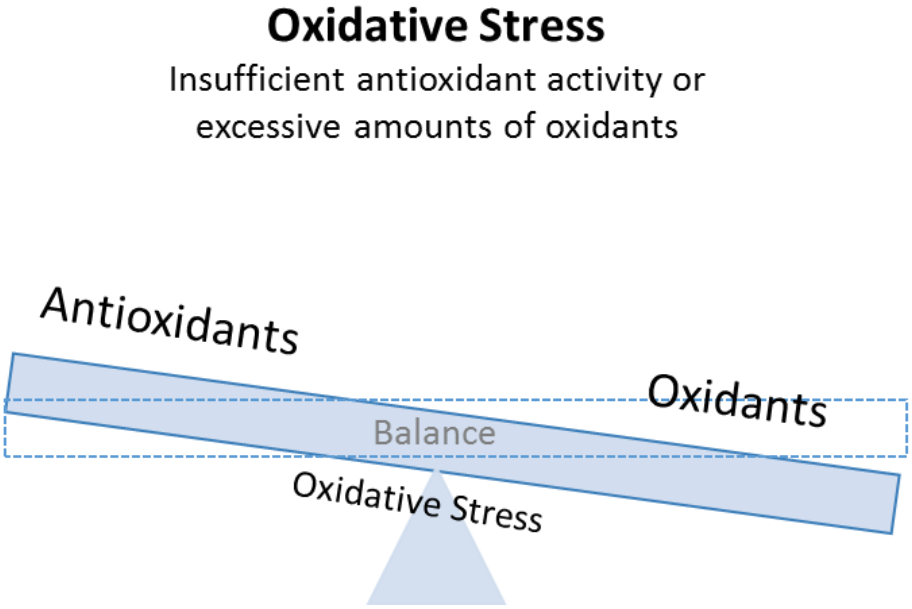

Figure 1. Oxidants and antioxidants under normal conditions (balance) and during oxidative stress (disrupted balance due to insufficient antioxidant activity or excessive oxidants levels).

Several biomarkers of oxidative stress have been used to assess oxidative stress *in vivo*, including biomarkers of lipid peroxidation such as thiobarbituric acid reactive substances and malondialdehyde, and different assays have been developed to measure the biomarkers.[6] However, it has been difficult to measure the "true" oxidative stress levels because of the reactivity of ROS. In the 1990's, the isoprostanes were discovered by Morrow et al.[7] and are today considered as one of the most reliable biomarkers for measuring oxidative stress *in vivo*. F2-isoprostanes, including the most abundant 8-iso-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), are formed during free radical-catalyzed peroxidation of arachidonic acid and can be measured in a variety of body fluids, such as blood and urine.[8] Several methods have been developed and used to measure levels of F2-isoprostanes, including mass spectrophotometry, enzyme- and radioimmunoassays.[6,8,9]

2.1.1 Oxidants

Reactive species, including reactive oxygen and nitrogen species (ROS and RNS), are free radicals (i.e. molecules with unpaired electrons), and non-radicals, that are partly generated through cellular processes such as cell metabolism and signaling.[1] Low levels of reactive species are vital for proper cell function and have an important role in cell signaling (e.g. regulating expression of antioxidant enzymes and other molecules), but excessive amounts can be harmful and lead to increased oxidative stress.[1] ROS and RNS can start rapid chain reactions with for example long-chain polyunsaturated fatty acids leading to lipid peroxidation and oxidative damage of cells and tissues. Proteins and nucleic acids are also target for oxidation.[10] Several endogenous and exogenous/environmental factors may influence or induce ROS production (**Figure 2**).

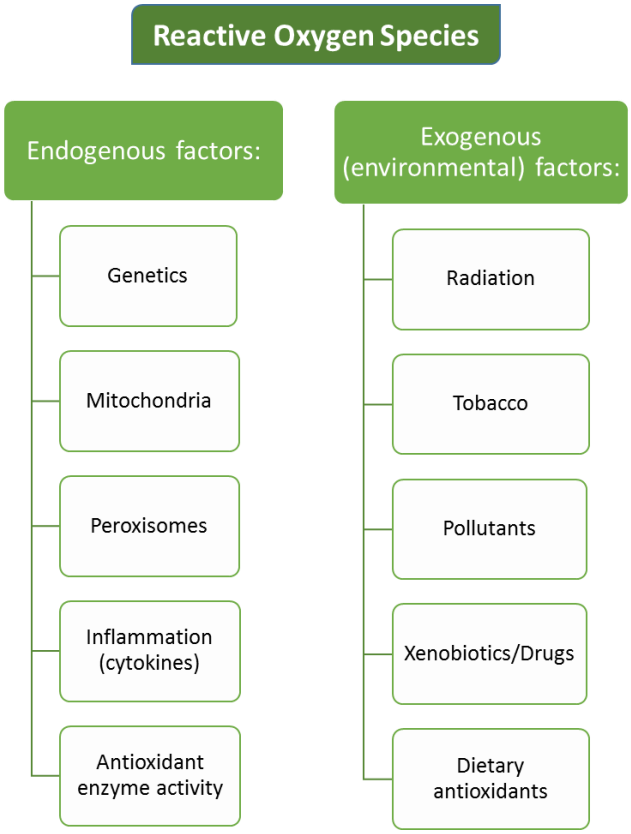

Figure 2. Examples of endogenous and exogenous (environmental) factors influencing or inducing the generation of reactive oxygen species.

The superoxide anion ($O_2^{\bullet-}$) is a common ROS and free radical produced by many cells during for example cell metabolism. Superoxide can react and form hydrogen peroxide (H_2O_2), a non-radical, which in turn may lead to generation of the very reactive hydroxyl radical ($OH^{\bullet}$) that can cause oxidative damage (**Figure 3**). Superoxide may also react with nitric oxide ($NO^{\bullet}$) to form peroxynitrite ($ONOO^-$, example of RNS). Nitric oxide has an important role in cell signaling and in the central nervous system by regulating blood pressure as a vasodilator.[11]

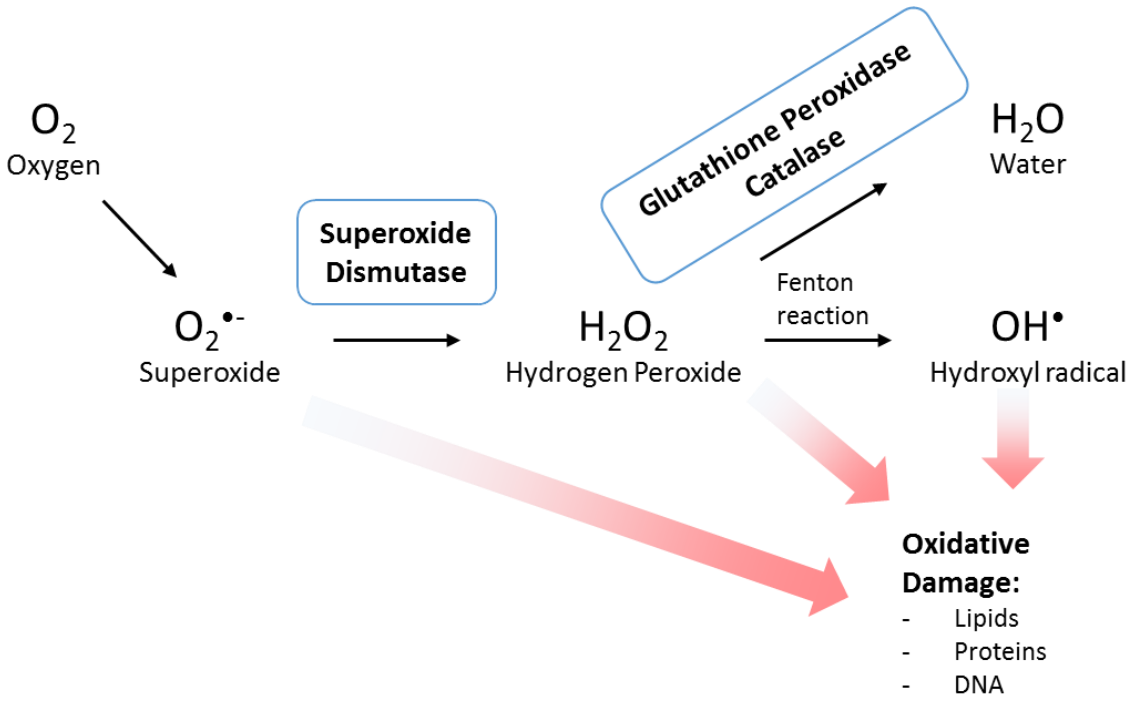

Figure 3. A schematic overview of common reactive oxygen species generated from oxygen (O_2), including superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), that may lead to oxidative damage of lipids, proteins and DNA. The defense by endogenous antioxidants (enzymes), including superoxide dismutase, glutathione peroxidase and catalase, is also shown.

2.1.2 Antioxidants

Antioxidants have been defined as "any substance that delays, prevents or removes oxidative damage to a target molecule".[1] Antioxidants participate in redox-reactions and reduce the formation of reactive species through donation of electron(s). The oxidized form of the antioxidant may in turn be reduced by other antioxidant enzymes/systems back into its reduced (active) state. Different types of antioxidants include exogenous (dietary) and endogenous antioxidants (produced in the body) and they may co-operate in a complex network and have synergistic effects against oxidative stress.[12] Antioxidants may also be divided into enzymatic and non-enzymatic ones. The levels and activity of antioxidants may be influenced by several factors, such as genetic factors, the redox-status of a cell, diet and health condition/diseases (e.g. inflammation).[1,13] An important mechanism of the cellular defense against oxidative stress is the regulation of antioxidant expression by different transcription factors related to oxidative stress and inflammation, such as nuclear factor κβ, retinoic acid (a metabolite of vitamin A) and nuclear factor E2-related factor 2 (Nrf2).[14,15] The transcription factors can activate different signaling pathways by binding to responsive elements on genes, for example Nrf2 binds to the antioxidant response element, which is an enhancer sequence found on genes encoding antioxidant/detoxifying enzymes (sensitive to changes in redox status).[16]

2.1.2.1 Exogenous antioxidants

Exogenous antioxidants are provided from dietary sources, mainly from fruit and vegetables, which are rich in vitamins and antioxidants.[10] Vitamins are a heterogenic group of nutrients that are essential for the body. Observational epidemiological studies have shown that high fruit and vegetable intake and blood concentrations of different vitamins may be protective against many diseases, including several cancers, cardiovascular disease (CVD) and cataract.[17-27] An overview of the common dietary antioxidants carotenoids, vitamin C and vitamin E and their dietary sources, physiological functions and antioxidant properties are presented in **Table 1**.

Table 1. Summary of dietary sources and antioxidant/physiological functions of antioxidants

Antioxidants	Dietary sources	Antioxidant properties	Other physiological functions
Carotenoids	Fruit and vegetables (especially those with yellow/red color and green leafy vegetables)	Lutein/zeaxanthin in the eye (absorbs blue light) Prevents lipid peroxidation	Pro-vitamin A[a]: Vision Growth and development Immune system
Vitamin C	Fruit and vegetables (especially citrus fruits, broccoli and pepper)	Scavenges reactive species Regenerates other antioxidants	Collagen formation Cofactor for enzymes Immune system
Vitamin E	Vegetable oils, cereals, nuts and legumes	Prevents lipid peroxidation Protects cell membranes against oxidative damage Regenerates other antioxidants	Cell signaling and gene expression Neurological function

[a] The carotenoids α-carotene, β-carotene and β-cryptoxanthin are pro-vitamin A.

Carotenoids are lipophilic compounds found as natural pigments in plants, fungi and algae. The bioavailability of carotenoids may be influenced by cooking method, for example heating tomatoes in oil improves the bioavailability of lycopene.[28] The antioxidant potential of the carotenoids includes scavenging radicals generated from lipid peroxidation.[23] In humans, major carotenoids found in plasma are lycopene, lutein/zeaxanthin, α-carotene, β-carotene and β-cryptoxanthin.[20,23] Among the carotenoids, α-carotene, β-carotene and β-cryptoxanthin can be converted into vitamin A in the body and are called pro-vitamin A. Carotenoids are considered as reliable biomarkers for estimating fruit and vegetable intake.[29]

Vitamin C (ascorbic acid/ascorbate and its derivatives) is a hydrophilic nutrient found in most fruit and vegetables. The content of vitamin C may be reduced during long-term storage or cooking.[10] Vitamin C has antioxidant properties through protection against reactive species and the regeneration of other antioxidants, including vitamin E.[1] Dehydroascorbic acid is the oxidized form of vitamin C that can be reduced back to ascorbic acid (reduced form) by other antioxidants, such as glutathione. However, at high concentrations and especially in the presence of metal ions, it can also be a pro-oxidant (inducing radicals).[30,31]

Vitamin E consists of a group of 8 lipophilic compounds with similar structures, including tocopherols and tocotrienols. Dietary sources rich in vitamin E are vegetable oils, cereals and nuts. Vitamin E has antioxidant properties; it scavenges radicals and protects other oxidizable compounds, such as polyunsaturated fatty acids, vitamin A and D. As a result, a large proportion of the vitamin may be lost during long-term storage or cooking.[10] Vitamin E at high concentrations may also have pro-oxidative properties.[32,33]

In addition to the previously described antioxidant vitamins, B vitamins are a group of hydrophilic compounds found in a variety of foods, especially in cereals and meat, and have an important role in cell metabolism.[10] Some experimental studies indicate that B vitamins may have antioxidant properties[34-37] by for example inhibiting formation of advanced glycation end products[38] and protein oxidation in lens cells.[39] B vitamins at high concentrations have also shown toxic and pro-oxidative properties.[40,41]

2.1.2.2 Endogenous antioxidants

Antioxidants that are produced endogenously include different antioxidant enzymes and other non-enzymatic molecules such as glutathione.[1] One important group of enzymatic antioxidants is the superoxide dismutase (SOD) family, with the main function to scavenge superoxide anions (**Figure 3**). Three types of SODs have been identified in humans: cytosolic Cu/Zn SOD (SOD1), mitochondrial Mn-SOD (SOD2) and extracellular Cu/Zn SOD (SOD3; EC-SOD).[42] The EC-SOD was discovered in 1982 by Marklund et al.[43] and is a hydrophobic glycoprotein found in extracellular fluids. Different molecules may regulate EC-SOD gene expression, such as pro-inflammatory cytokines and other antioxidants.[44,45] It has also been suggested that a mild pro-oxidative state (through intake of natural pro-oxidants in foods) in the gastrointestinal tract may lead to up-regulation of endogenous antioxidants.[46] Studies have shown altered levels and activity of EC-SOD in association with chronic diseases, such as diabetes and CVD.[47-49] Carotenoids, being an important group of exogenous (dietary) antioxidants, are suggested to have antioxidant potential and may be involved in the regulation of gene expression of endogenous/enzymatic antioxidants.[23,50] Examples of other antioxidant enzymes are glutathione peroxidase and catalase, which catalyzes the reduction of hydrogen peroxide in different reactions (**Figure 3**).

Glutathione is an important non-enzymatic antioxidant produced endogenously from different amino acids that can prevent oxidative damage by reducing disulfide bonds. It exists in both a reduced (glutathione) and oxidized (glutathione disulfide) state. The enzyme glutathione reductase converts the oxidized form (glutathione disulfide) back into its reduced state.[11] Glutathione is also important for the function of other antioxidants, such as glutaredoxins/thioltransferases.[51]

2.2 AGE-RELATED CATARACT

Age-related cataract is defined as a clouding/opacification of the lens, which leads to impairment of the vision and is one of the leading causes of low vision and blindness in the world.[52] The lens is normally transparent and located in a collagen capsule behind the pupil in the eye. Incoming light passes through the lens and is refracted to the retina. Through accommodation, the shape of the lens is adjusted by contraction and relaxation of ciliary muscles to allow the eye to see sharply and focus at different distances (**Figure 4**).

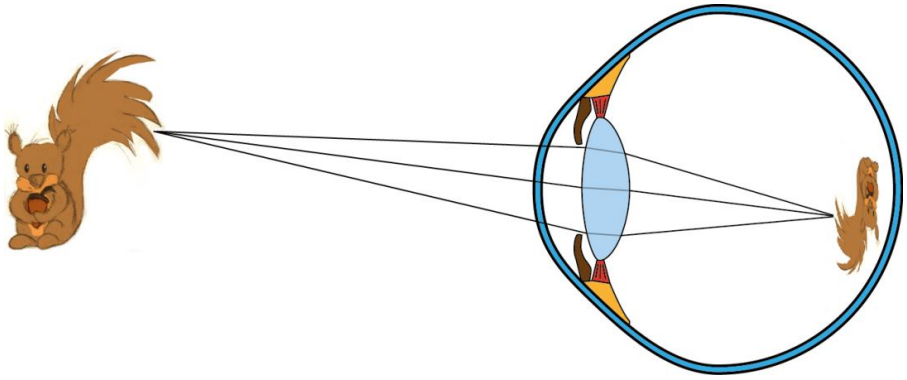

Figure 4. In the eye an image is formed at the retina from light refracted in the lens.

The cell types found in the lens are the epithelial cells (found on the anterior side of the lens) and the fiber cells. The lens is continuously growing as the epithelial cells divide into new fiber cells and pushing already existing fiber cells into the center of the lens, which becomes denser, thicker and less elastic with increasing age. In order to get optimal visual acuity it is important that the lens is clear. The transparency of the lens is due to densely packed lens fiber cells with a minimum of extracellular space and the absence of cell organelles in the fiber cells. Oxidative damage to lens proteins (mostly crystallins) contribute to degradation of the normal architecture and cloudiness of the lens. Some of the symptoms of cataract include cloudy/blurry vision, glare, decreased night vision, double vision and need for brighter light when reading. There are three subtypes of cataract depending on its location in the lens: nuclear, cortical and posterior subcapsular cataracts. The symptoms may vary between the subtypes. Nuclear cataract has been shown to be the predominant subtype in many populations and includes opacification of the nucleus of the lens, that is when lens proteins of the older fiber cells deteriorate/oxidize and the lens become denser and less elastic.[53,54] The development of nuclear cataract may progress slowly and leads to impairment of mostly distant vision. Cortical cataract refers to opacities in the lens cortex (the peripheral edge of the lens) and occurs when the younger lens fiber cells deteriorate and change in water content, leading to blurred vision and glare. In posterior subcapsular cataract, the opacification is located on the back surface of the lens, beneath the lens capsule, which can lead to difficulties when reading and glare. Risk groups for this subtype of cataract include

people who have diabetes or are using corticosteroids.[5] Posterior subcapsular cataract may develop relatively rapidly and give rise to notable symptoms within months.

The etiology of cataract is suggested to be multifactorial. Risk factors for cataract include both non-modifiable factors such as age, gender and genetics, as well as modifiable factors such as smoking, alcohol, ultraviolet radiation, overweight, hypertension, diabetes and use of corticosteroids.[5,55-62]

2.2.1 Prevalence and incidence

According to the World Health Organization's (WHO) latest assessment, approximately 51% of the world blindness may be due to cataract, representing about 20 million people (2010).[52] The access to cataract extraction (surgery) differs between countries and in many countries the availability of cataract extraction is limited.[63] As people live longer, the incidence of cataract is expected to increase worldwide, with an estimated doubling by the year 2020.[63]

2.2.2 Cataract extraction

Cataract extraction (surgery) is the only treatment available for cataract today. It is performed on an outpatient basis and is a common (some variation depending on country) and generally safe surgery. Briefly, the cataractous/cloudy lens is removed and replaced by, in most cases, an artificial lens. In Sweden, cataract extraction is the most commonly performed surgery with approximately 110,000 operations/year (**Figure 5**).[64]

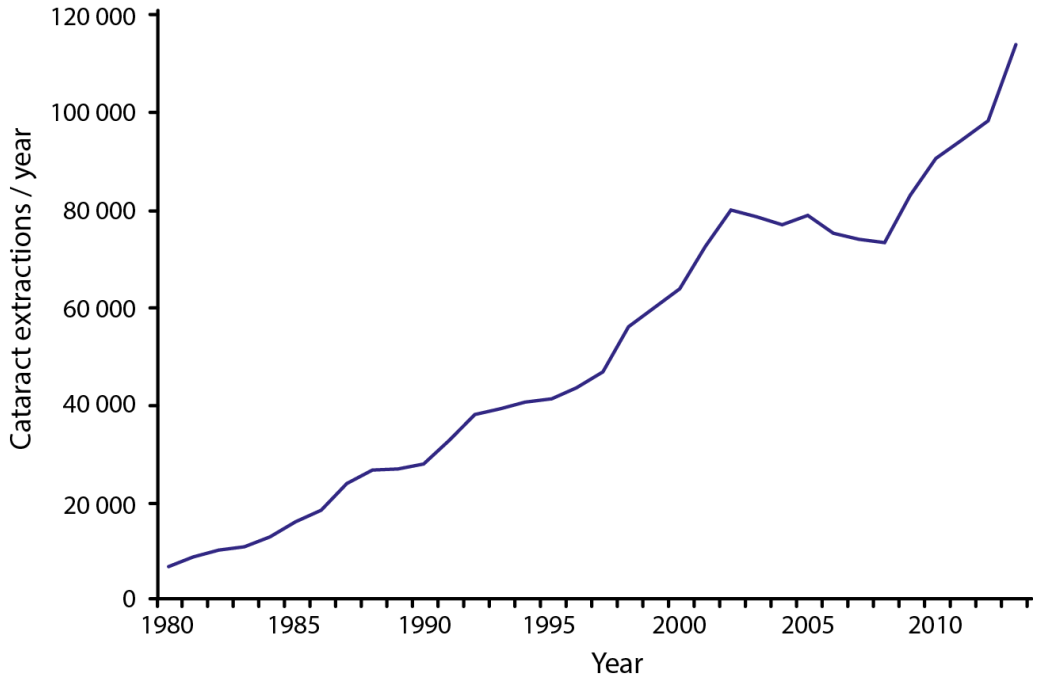

Figure 5. Numbers of cataract extractions in Sweden between 1980 and 2013 (data from the Swedish National Cataract Register).[64]

2.3 OXIDATIVE STRESS AND AGE-RELATED CATARACT

2.3.1 Oxidants and antioxidants in the lens

Oxidative damage of the lens is suggested to be involved in the etiology of age-related cataract, and lipid oxidation and glycation of proteins in the lens have been associated with cataract development.[30,51,65] Several biomarkers of oxidative stress have been shown to be higher in the cataract lens as compared to non-cataract lens (in both humans[65,66] and animals[30,67]).

Antioxidants in the eye protect the lens from oxidative stress.[51] Several exogenous/dietary antioxidants have been detected in different parts of the human lens, which include the hydrophilic ascorbate (vitamin C)[68] and the fat-soluble lutein/zeaxanthin (the only carotenoids), vitamin E and retinol.[69] The concentrations of the fat-soluble antioxidants in the lens have been shown to be approximately 2-3-fold higher in the younger and more metabolically active epithelial/cortical layers of the lens as compared to the older nuclear layers of the lens.[69] The lens contains relatively large amounts of ascorbate, which scavenges reactive oxygen species such as superoxide.[68] However, ascorbic acid may also have pro-oxidative properties and contribute to aging of the lens crystallins (protein oxidation by the Maillard reaction), especially in the presence of high metal concentrations.[30,31] Vitamin E (α-tocopherol) supplementation has been shown to protect, in a dose-response manner, against ultraviolet radiation induced cataract.[70] Moreover, high concentrations of lutein/zeaxanthin in the lens have been shown to decrease oxidative stress.[71]

The human lens also includes endogenous antioxidants, such as SOD and glutathione.[51] The levels of SOD in different ocular tissues varies and SOD activity was found to be highest in the retina and lowest in the lens (15-fold less compared to the average human tissue) and the majority was cytosolic CuZn-SOD.[72] The enzymes seem to have an important role in protecting the eye and the lens against oxidative stress. Knock-out mice lacking cytosolic CuZn-SOD have been shown to develop cataract faster than control mice and they also had higher levels of intracellular superoxide-derived oxidative stress.[73] Human cataract lens has been shown to have lower CuZn-SOD activity, as compared to non-cataract lens.[74]

Glutathione, another important antioxidant in the lens, may co-operate with vitamin C to scavenge ROS and is found at high levels in the lens.[51] There are also two repair systems in the lens, the glutathione-dependent glutaredoxin/thioltransferase system and the NADPH-dependent thioredoxin system that help to maintain redox homeostasis by reducing oxidized proteins and enzymes.[51,67] Experimental studies have shown for example that knock-out mice lacking glutaredoxin 2 developed age-related cataract faster than age-matched controls.[66]

2.3.2 Systemic oxidative stress and inflammation

Previous cross-sectional[75] and case-control[76-78] studies have observed higher concentrations of systemic oxidative stress biomarkers, such as malondialdehyde and hydroxyoctadeca-dienoic acid, in patients with cataract. However, few studies (one cross-sectional[75] and one case-control[79] study) have examined the association between 8-iso-$PGF_{2\alpha}$, which is considered as one of the most reliable biomarkers of oxidative stress *in vivo*,[7] and risk of cataract. Only the case-control study observed a statistically significant positive association between plasma 8-iso-$PGF_{2\alpha}$ concentrations and cataract risk.

High levels of chronic or acute inflammation may induce oxidative stress. Inflammation has also been suggested to increase the risk of cataract. However, few studies have investigated the association of systemic inflammation with risk of cataract and reported inconsistent results. Two studies have observed a positive association for the inflammatory biomarkers serum C-reactive protein[80] and interleukin-6[81] with risk of cataract, but others found no association between serum C-reactive protein and cataract risk.[81,82]

2.3.3 Antioxidants in diet and supplements

Observational epidemiological studies have shown an inverse association between diets rich in antioxidants such as high fruit and vegetable intake and high intakes of β-carotene, lutein/zeaxanthin, vitamin C and vitamin E, and risk of cataract, that has been attributed to the ability of antioxidants to prevent oxidation processes in the lens and systemically.[83-87] A meta-analysis of observational studies reported an inverse association between concentrations of some blood antioxidants and vitamins, including α-carotene, lutein/zeaxanthin and vitamin E, and risk of cataract, especially in populations with micronutrient deficiencies at baseline.[88] Moreover, high total antioxidant capacity (a measure that takes into account the levels and synergistic effects of many antioxidants in the diet) has also been associated with decreased risk of cataract extractions in a population-based prospective cohort study of women.[89]

Although several studies have observed an inverse association between diets rich in antioxidants and cataract, observational studies examining the use of dietary supplements containing an individual or a combination of antioxidants/vitamins have shown inconsistent results (previously reviewed by Mares[90,91] and Seddon[92]). A summary of the observational studies examining dietary supplement use and risk of cataract is shown in **Table 2.**[84,87,93-102] Randomized controlled trials (RCTs) investigating the effect of high-dose supplements, including vitamin C, vitamin E and β-carotene, either individually or in a combination, with risk of cataract, have mostly shown no effect, although a small deceleration in cataract progression was reported in one trial[103] (previously reviewed by Mares,[90,91] Mathew et al.,[104] and Chew[105]). The RCTs investigating the effect of dietary supplement use for cataract prevention are summarized in **Table 3.**[103,106-116]

In general, several large RCTs investigating the effect of single, high-dose vitamin/ antioxidant supplements for prevention of chronic diseases have failed to show any protective effect.[117-121] Indeed, some RCTs have even reported harmful effects of high-dose antioxidant supplements for risk of CVD,[122] some types of cancers[123-125] and mortality.[126]

For the use of multivitamins (containing doses close to recommended daily intake, RDI), on the other hand, three RCTs have reported a modestly decreased risk of total and nuclear cataracts.[114-116] One of the trials (the Linxian Cataract Studies) was based on a study population with micronutrient deficiencies at baseline,[114] while the other two trials were conducted among generally well-nourished study populations (the Italian-American Clinical Trial of Nutritional Supplements and Age-Related Cataract and Physicians' Health Study II).[115,116]

2.3.4 Physical activity

Physical activity is associated with several health benefits, such as reduced oxidative stress and inflammation levels, improved lipid profiles and insulin sensitivity.[127-129] It is a protective factor for many chronic diseases, including diabetes and coronary heart disease.[127,130] On the other hand, physical inactivity has been associated with higher levels of oxidative stress[129,131,132] and increased risk of many diseases.[130] Only two previous prospective cohort studies have observed, in physically active populations, that higher moderate (walking)[133] and vigorous (running)[133,134] physical activity levels were associated with decreased risk of cataract in men[133,134] and women.[133] One case-control study conducted at a hospital setting observed that lower physical activity levels were associated with increased risk of cataract.[135] However, little is known about the association between total and specific types of physical activity and risk of age-related cataract in a general population.

Table 2. Observational studies of dietary supplements, including multivitamins, vitamin C, E and/or B vitamins, and cataract risk

Study (Country) & Author	Study Population	Follow-up	Supplements	Outcome	Results
Cohort studies					
NHS (USA) Hankinson et al.[84]	n=50,825 women (45-67 y)	8 y	Multivitamins Vitamin C Vitamin E	Cataract extraction	No association ↓ Risk for vitamin C ≥10 y (RR=0.55) No association
PHS (USA) Seddon et al.[93]	n=17,744 men (40-84 y)	5 y	Multivitamins Vitamin C Vitamin E	Cataract incidence and extraction	↓ Risk (RR=0.73) Vitamin C and/or E ↑ Risk (RR=1.32; NS)
LSC (USA) Leske et al.[95]	n=764 (>40 y)	4.8 y	Multivitamins	Nuclear opacities	↓ Risk (RR=0.70)
NHS (USA) Chasan-Taber et al.[96]	n=73,956 women (>45 y)	12 y	Multivitamins Vitamin C Vitamin E	Cataract extraction	No associations
BDES (USA) Mares-Perlman et al.[97]	n=3089 (43-86 y)	5 y	Multivitamins Vitamin C Vitamin E	Incident cataract	No associations for ≤10 y Long-term (>10 y), all supplements: ↓ Risk for nuclear (RR=0.60) and cortical cataracts (RR=0.40)
NHS (USA) Taylor et al.[100]	n=492 women (53-73 y)	13-15 y	Multivitamins Vitamin C Vitamin E	Cortical and posterior subcapsular opacities	No association ↓ Risk of cortical opacities for ages <60 y and long-term use (≥10 y) (RR=0.40)
BDES (USA) Klein et al.[101]	n=2375-4926 (43-86 y)	15 y	Multivitamins Vitamin C Vitamin E B vitamins	Incident cataract	No associations
WHS (USA) Christen et al.[87]	n=35,551 women (≥45)	10 y	Vitamin C Vitamin E	Incident cataract	No association ↓ Risk (RR=0.86)
SMC (Sweden) Rautiainen et al.[102]	n=24,593 women (49-83 y)	8.2 y	Multivitamins Vitamin C	Cataract extraction	No association ↑ Risk (RR=1.25)

Table 2 (continued). Observational studies of dietary supplements, including multivitamins, vitamin C, E and/or B vitamins, and cataract risk

Study (Country) & Author	Study Population	Follow-up	Supplements	Outcome	Results
Cross-sectional studies					
BES (Barbados) Leske et al.[94]	n=4314 (41-84 y)	-	Multivitamins Vitamin C Vitamin E	Lens opacities	Any supplement use: ↓ prevalence (RR=0.78)
NHS (USA) Jacques et al.[98]	n=478 women (53-73 y)	-	Multivitamins Vitamin C Vitamin E	Prevalence of nuclear opacities	Long-term (≥10 y) multivitamins (RR=0.57) and vitamin C (RR=0.36) ↓ nuclear cataract
BMES (Australia) Kuzniarz et al.[99]	n=2873 (49-97 y)	-	Multivitamins Vitamin C Vitamin E B vitamins	Cataract prevalence (all sub-types)	↓ Nuclear and cortical cataracts (RR=0.60) No association No association ↓ Nuclear/cortical cataract (RR=0.6-0.8) ↑ posterior subcapsular cataract (RR=1.6)

Abbreviations: BES, Barbados Eye Study; BMES, Blue Mountain Eye Study; BDES, Beaver Dam Eye Study; LSC, Longitudinal Study of Cataract; NHS, Nurses' Health Study; PHS, Physicians' Health Study; SMC, Swedish Mammography Cohort; WHS, Women's Health Study.

Table 3. Randomized controlled trials investigating the effect of vitamin supplements on age-related cataract

Study (Country) & Author	Study population	Intervention (Dose, daily)	Duration	Endpoint	Results
ATBC (Finland) Teikari et al.[106]	n=28,934 (50-69 y)	β-carotene (20 mg) and/or vitamin E (50 mg) *vs.* placebo	5.7 y	Cataract extraction	No effect
AREDS (USA) AREDS research group.[107]	n=4629 (55-80 y)	β-carotene (15 mg), vitamin C (500 mg) and vitamin E (400 IU, ≈268 mg) *vs.* placebo	6.3 y	Cataract incidence and extraction	No effect
REACT (UK and USA) Chylack et al.[103]	n=158 (>40 y)	β-carotene (18 mg), vitamin C (750 mg) and vitamin E (600 mg) *vs.* placebo	3 y	Cataract progression	↓ Progression
PHS I (USA) Christen et al.[108]	n=22,071 (40-84 y)	β-carotene (50 mg on alternate days) *vs.* placebo	12 y	Cataract incidence and extraction	No effect
VECAT (Australia) McNeil et al.[109]	n=1193 (55-80 y)	Vitamin E (500 IU, ≈335 mg) *vs.* placebo	4 y	Cataract incidence, extraction and progression	No effect
APC (India) Gritz et al.[110]	n=798 (35-50 y)	Vitamin A (15 mg), vitamin C (500 mg) and vitamin E (400 IU, ≈268 mg) *vs.* placebo	5 y	Cataract progression	No effect
WHS (USA) Christen et al.[111]	n=39,876 (≥45 y)	Vitamin E (600 IU, ≈402 mg on alternate days) *vs.* placebo	9.7 y	Cataract incidence	No effect
PHS II (USA) Christen et al.[112]	n=11,545 (≥50 y)	Vitamin C (500 mg) and/or vitamin E (400 IU, ≈268 mg on alternate days) *vs.* placebo	8 y	Cataract incidence	No effect
SELECT Eye Endpoints (USA, Canada and Puerto Rico) Christen et al.[113]	n=11,267 (≥50 y)	Selenium (200 µg) and/or vitamin E (400 IU, ≈268 mg) *vs.* placebo	5.6 y	Cataract incidence	No effect
Linxian Cataract Studies (China) Sperduto et al.[114]	n=3249 (45-74 y)	A. Retinol (500 IU), zink (22 mg) B. Riboflavin (3 mg), niacin (40 mg) C. Vitamin C (120 mg), molybdenum (30 µg) D. Selenium (50 µg), vitamin E (30 mg), β-carotene (15 mg) *vs.* placebo	5-6 y	Cataract prevalence	Group B: ↓ Nuclear cataract ↑ Posterior subcapsular opacities (for ages 65-74 y)

Table 3 (continued). Randomized controlled trials investigating the effect of vitamin supplements on age-related cataract

Study (Country) & Author	Study population	Intervention (Dose, daily)	Duration	Endpoint	Results
Linxian Cataract Studies (China) Sperduto et al.[114]	n=2141 (45-74 y)	Multivitamins (≈ twice RDI) *vs*. placebo	5-6 y	Cataract prevalence	↓ Nuclear cataract (for ages 65-74 y)
CTNS (Italy) CTNS Study Group.[115]	n=1020 (55-75 y)	Multivitamins (≈RDI) *vs*. placebo	9 y	Cataract incidence, extraction and progression	↓Total and nuclear opacities ↑ Posterior subcapsular opacities
PHS II (USA) Christen et al.[116]	n=14,641(≥50 y)	Multivitamins (≈RDI) *vs*. placebo	11.2 y	Cataract incidence	↓Total and nuclear opacities

Abbreviations: APC, Antioxidants in Prevention of Cataracts; AREDS, Age-Related Eye Disease Study; ATBC, Alpha-Tocopherol Beta-Carotene; CTNS, Italian-American Clinical Trial of Nutritional Supplements and Age-Related Cataract; PHS, Physicians' Health Study; REACT, Roche European American Cataract Trial; SELECT, Selenium and Vitamin E Cancer Prevention Trial; VECAT, Vitamin E, Cataract and Age-related Maculopathy Trial; WHS, Women's Health Study.

3 AIMS

The overall aim of this thesis was to examine the role of oxidative stress biomarkers and oxidative stress-related diet and lifestyle factors with the risk of age-related cataract.

The specific aims were:

- To study the association between exogenous/dietary and endogenous antioxidants in women and whether this association differs in healthy women *vs*. those with a history of chronic diseases.

- To examine the association between systemic oxidative stress and inflammation, as measured by urinary biomarkers, and risk of cataract incidence in women.

- To prospectively assess the association between use of high-dose vitamin C and vitamin E supplements, as well as low-dose multivitamins, and risk of cataract incidence in men.

- To prospectively assess the association between high-dose B vitamins supplement use and risk of age-related cataract incidence in women and men.

- To prospectively assess the associations of total and specific types of physical activity, as well as leisure time inactivity, with risk of cataract incidence in women and men.

4 PARTICIPANTS AND METHODS

4.1 STUDY POPULATIONS

The papers in this thesis are based on two population-based studies:

1) The Swedish Mammography Cohort (SMC)
2) The Cohort of Swedish Men (COSM)

4.1.1 Swedish Mammography Cohort

The SMC was established in 1987-1990. All women born (n=90,303) between 1914 and 1948 and living in Uppsala and Västmanland Counties in central Sweden received an invitation to a mammography screening program and, together with the invitation, a mailed questionnaire including questions regarding diet (67-item food frequency questionnaire, FFQ) and lifestyle factors. Approximately 74% of the women responded. The aim of the SMC was to study the association between lifestyle factors and major chronic diseases, including age-related cataract. To update exposure information, a second extended follow-up questionnaire (including a 96-item FFQ) was sent in late fall 1997 to the women, aged 48-83 years, who were alive and still living in the study area. Approximately 70% of the women responded.

The study populations in **Papers IV and V** include women from the SMC. The baseline exposure assessment was based on data from the 1997 questionnaire, since it contained more information on known and potential confounders than the previous 1987 questionnaire. Briefly, for each study, participants were excluded if they had missing or erroneous personal identity number, turned in a blank questionnaire or died before January 1, 1998. To avoid influence of potential changes in diet and lifestyle factors, participants were also excluded if they had a previous cancer diagnosis other than nonmelanoma skin cancer, or a history of cardiovascular disease and/or diabetes. Moreover, those with cataract diagnosis or cataract extraction before the baseline 1998 and those with missing information on any of the exposure variables (e.g. dietary supplement use and physical activity levels) were also excluded. A more detailed description of the exclusions for each study is presented in the flow-chart (**Figure 6**) and the published papers.

4.1.1.1 The Swedish Mammography Cohort – Clinical (SMC–C)

The clinical sub-cohort of the SMC, the SMC–C, was established between 2003 and 2009, a total of 8311 women (<85 years) living in Uppsala County were invited to complete a third questionnaire regarding diet and lifestyle factors (similar to the 1997 questionnaire) and to undergo a health examination (including measurements of weight, height, waist, hip and blood pressure, as well as a dual energy X-ray absorptiometry scan). Approximately 65% of the women completed the questionnaire and 61% participated in the health examination. Fasting blood and urine samples and other biologic materials (e.g. adipose tissue) were collected at the health examination.

Papers I and II (including biomarkers) are based on women from the SMC–C and recruited between 2003 and 2005. For **Paper I**, 157 women aged 55-74 years that had donated blood samples and answered the third questionnaire were randomly selected from the SMC–C and included in the study. Two women were excluded, because of fruit and vegetable intake outside the range of 133-1572 g/day (±2.5 standard deviation on a log scale). In **Paper II**, a total of 258 cases and 258 controls were included. Cases were defined as women with incident age-related cataract (diagnosis and/or cataract extraction after the date of urine sample collection and through December 2009). For each case, one control without cataract was randomly selected and matched for age (same year of birth) and date of urine sample collection (within 1-5 months). Excluded were women with a history of cataract diagnosis, cataract extraction, or cancer at the time of health examination and collection of urine samples.

4.1.2 Cohort of Swedish Men

The COSM was established in late fall 1997 with the aim to study lifestyle factors and major chronic diseases, including age-related cataract. All men (n=100,303), aged 45-79, living in central Sweden (Örebro and Västmanlands Counties) received a questionnaire regarding diet and lifestyle factors (same questionnaire as for the SMC, except for gender specific questions). Approximately 49% of the men completed the questionnaire.

The study populations in **Papers III-V** included participants from the COSM. **Paper III** was based on COSM participants only and **Papers IV and V** were based on participants from both the COSM and the SMC. The exposure assessment was based on data from the 1997 questionnaire (study baseline). The same exclusion criteria (as for the SMC) were applied in the studies and participants were excluded if they had missing or erroneous personal identity number, turned in a blank questionnaire, died before January 1, 1998, had a previous cancer diagnosis other than nonmelanoma skin cancer, or a history of cardiovascular disease and/or diabetes. Moreover, men with cataract diagnosis or cataract extraction before baseline and those with missing information on any of the exposure variables were also excluded. The flow-chart (**Figure 6**) shows a more detailed description of the exclusions made in each study and this information can also be found in the published papers.

4.2 METHODS

4.2.1 Study designs

Observational epidemiological studies examine the distribution, determinants (protective and risk factors) and/or occurrence of a disease, by making observations in a study population. Different types of observational studies include cross-sectional, case-control and cohort studies. The study design of each paper included in this thesis is presented in **Figure 6**.

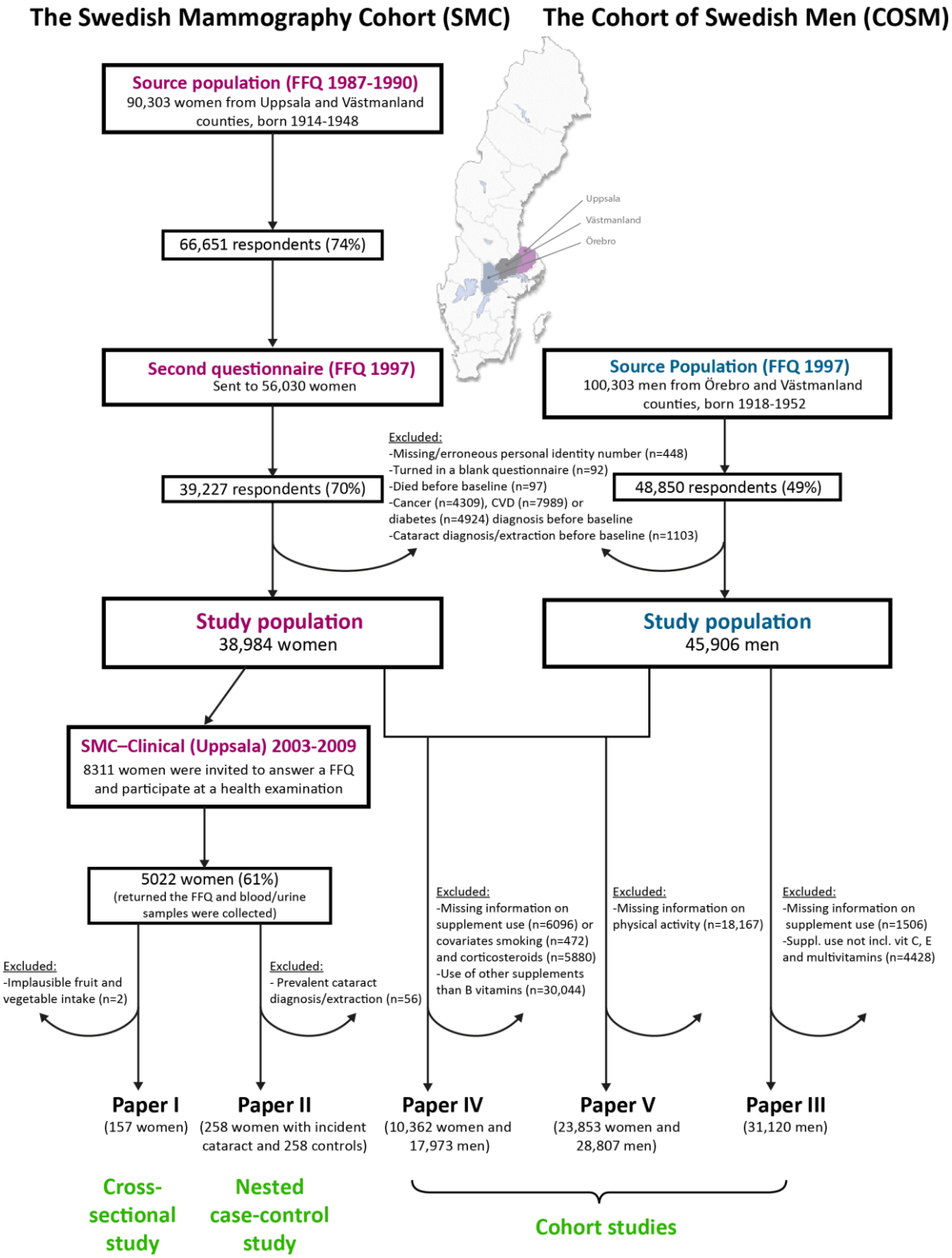

Figure 6. Flow-chart of the Swedish Mammography Cohort and the Cohort of Swedish Men, including source and study populations, exclusions and study designs for **Papers I-V**.

Paper I is a cross-sectional study, which is a type of study where the exposure and outcome of interest are collected at the same time, representing a "snapshot" of the population status at a single/specific point in time. **Paper II** is a nested case-control study, which is a case-control study conducted within ("nested") a cohort, the SMC–C, and includes incident cataract cases and controls without cataract matched on age and date of urine sample collection. **Papers III-V** are prospective cohort studies, which are based on participants from the COSM and/or SMC that are free from cataract at baseline (start of follow-up). The participants are followed over a period of time and cataract incidence is compared between exposed and unexposed individuals (i.e. dietary supplement users *vs*. non-users or high *vs*. low physical activity levels). Information on diet and lifestyle habits, as well as health status is collected during the study period through a self-administered questionnaire and linkage to registers.

4.2.2 Assessment of exposures and covariates

4.2.2.1 Biomarkers of antioxidants, oxidative stress and inflammation (Papers I and II)

Fasting blood and urine samples (one of each for all the study participants) were collected at the health examination. The blood samples were collected in evacuated tubes (containing EDTA) and immediately centrifuged for 10 min, at 4 °C, in a dark room. Plasma was separated from the blood samples and stored at -80 °C until analysis. The urine samples were also stored at -80 °C until analysis.

Plasma EC-SOD activity was analyzed with a commercial kit (Cayman Chemical Company, Ann Arbor, MI). In this assay, based on the xanthine oxidase system, superoxide was generated by adding diluted xanthine oxidase and the levels of superoxide (detected using a tetrazolium salt) were measured spectrophotometrically at 450 nm. The activity of EC-SOD was calculated from an equation based on the linear regression from the standard curve and defined as units (U). One unit was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. All the standards and plasma samples were analyzed in duplicates. The inter- and intra-assay coefficients of variations were 8.6% and 7.1%, respectively.

Plasma concentrations of lycopene, lutein/zeaxanthin, α-carotene, β-carotene and β-cryptoxanthin were measured using a previously described method.[136] Briefly, plasma carotenoids were separated and quantified using high-performance liquid chromatography and determined spectrophotometrically at 470 nm. The results obtained were compared with standard curves from lycopene, lutein/zeaxanthin, α-carotene, β-carotene and β-cryptoxanthin standards. Analysis of serum cholesterol was performed at Uppsala University Hospital. Concentrations of serum total cholesterol were measured with Konelab 20 analyzer (Thermo Electron Oy, Vantaa, Finland) and cholesterol reagent was purchased from Thermo Electron Corporation (ref. no 981813, Vantaa, Finland).

Analysis of urinary 8-iso-$PGF_{2\alpha}$, 15-keto-dihydro-$PGF_{2\alpha}$ and creatinine concentrations were performed at Uppsala University, using radioimmunoassays developed and previously described by Basu.[8,137] The intra-assay coefficient of variation was 14.5% at low and 12.2% at high concentrations for 8-iso-$PGF_{2\alpha}$. The detection limit of the assay was 23 pmol/L. For the 15-keto-dihydro-$PGF_{2\alpha}$ assay, the intra-assay coefficient of variation was 12.2-14.0% and the limit of detection was 45 pmol/L. Both urinary 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-PGF2α was corrected for urinary creatinine levels. Creatinine levels were measured by a colorimetric method described elsewhere.[138]

4.2.2.2 Diet and dietary supplements (Papers I, III and IV)

Fruit and vegetable intake was estimated from a 96-item FFQ completed by the participants at study baseline. They have answered questions on how often, on average, during the past year they had consumed different food items. There were eight different consumption frequency categories to choose from, ranging from never to three times or more daily, for each food item. The average consumption (g/day) of each food item was calculated by multiplying the frequency of consumption by age-specific portion sizes. The variable total fruit intake was created by adding the fruit items asked for in the FFQ, which included oranges/citrus fruits, orange/grapefruit juice, apples/pears, bananas, berries, fruit soups and other fruits. For total vegetable intake, the vegetable items asked for in the FFQ were added and included carrots, beetroots, lettuce/iceberg lettuce, cabbage, brussels sprouts, cauliflower, broccoli, tomatoes/tomato juice, pepper, spinach, green peas, onions, garlic, mixed vegetables, beans/lentils and soya products.

Intakes of single nutrients from the diet, such as vitamin C, vitamin E and B vitamins, were assessed based on the estimated average consumption of different food items from the FFQ. Nutrient intakes were estimated by multiplying the frequency of consumption of food items by age-specific portion sizes and the nutrient composition obtained from the Swedish National Food Agency.[139] The nutrient intakes were adjusted for energy intake using the residual method.[140] The 1997 FFQ has been validated among 248 men (aged 40-74 years) from the COSM, who received the same FFQ as the women in the SMC 1997. The FFQ had relatively good validity compared to 14 interviews (24-h recalls) for micronutrient intake, such as vitamin C, vitamin E and vitamin B6 (all correlation coefficients ≈0.6).[141]

Information on dietary supplement use was obtained from the FFQ completed by the participants at study baseline, which included a general question "Do you use vitamin, mineral, or other supplements?" and three pre-specified responses (no, regular use and occasional use). Followed by this question were more specific questions on dietary supplement use including pre-specified types of supplements (including multivitamins, vitamin C, vitamin E and B vitamins) and open answers about frequency and duration of use. Supplement users were defined as participants who reported regular or occasional supplement use. Duration (number of years) of dietary supplement use, including use of multivitamins

and single vitamins, such as vitamin C, vitamin E and vitamin B6, was inquired by open-ended questions. Long-term and short-term supplement users were defined as those who reported use ≥10 and <10 years before baseline, respectively. Supplement doses were estimated through information based on the commonly used supplements on the Swedish market during the study period.[142] The doses for single vitamins (vitamin C, vitamin E and B vitamins) were estimated to be approximately 10 times higher than the RDI and were defined as high-dose supplements. Multivitamins containing vitamin doses close to the RDI were defined as low-dose supplements. A validity study of the self-reported supplement use data for men in the COSM has shown relatively high validity (the sensitivity for the FFQ regarding use of any dietary supplements was 78% and the specificity was 93%).[143]

4.2.2.3 Assessment of physical activity (Paper V)

Levels of physical activity were assessed by using a self-administered questionnaire with 6 questions about physical activity and inactivity habits during the previous year (study baseline) and at age 30 years. The specific types of physical activities and the predefined alternatives for time spent on each activity (5-6 alternatives) included: walking/bicycling (hardly ever to more than 1.5 h daily), leisure time exercise (less than 1 h to more than 5 h weekly), work/occupational activity (mostly sitting to heavy manual labor), home/housework (less than 1 h to more than 8 h daily) and inactive leisure time (e.g. sitting/reading/watching TV; less than 1 h to more than 6 h daily). In addition, there was an open-ended question on duration of sleep and time spent sitting/laying down daily. Activity levels of the specific types of activities were calculated by multiplying the intensity (defined as metabolic equivalents, MET, kcal/kg/h) by the self-reported duration (in hours) of the activities.[144] Total physical activity (24 h) at baseline and at age 30 years were estimated by adding all specific activity types together. The self-reported physical activity data has been validated against 7-day activity records in women and in men from the SMC and the COSM and shown to estimate total physical activity satisfactory (Spearman's rank correlation: 0.6).[145,146]

4.2.2.4 Assessment of covariates

The self-administered questionnaire completed at baseline also included information on demographic, medical, lifestyle-related factors and other potential confounders, which were included as covariates in the multivariable-adjusted models in the papers. The following covariates were included (with some variation depending on the study, a more detailed description is presented in the statistical analysis section and in each paper): age, educational level, smoking status, alcohol intake, body mass index (BMI), abdominal obesity (defined as waist circumference ≥80 cm for women and ≥94 cm for men), self-reported health (information only available for men), history of CVD, diabetes or hypertension, and use of corticosteroids.

History of CVD was obtained from the National Inpatient and Outpatient Registers at the National Board of Health and Welfare. Diabetes history was obtained through the National Outpatient Register, National Diabetes Register and self-reports. History of hypertension was identified through linkage to the National Inpatient and Outpatient Registers and through self-reported data from the questionnaire. Information on cancer diagnosis was obtained through linkage to the Swedish National Cancer Register.

4.2.3 Case ascertainment and follow-up

Incident age-related cataract cases, defined as participants with cataract diagnosis and/or cataract extraction after baseline during follow-up **(Papers II-V)**, were identified through linkage to the National Outpatient and Inpatient Registers at the National Board of Health and Welfare (International Classification of Diseases (ICD)-10, code H25 and operation codes CJC, CJD, CJE, CJG). To complement the preceding data and for increased completeness, the cohorts were also matched against local cataract extraction registers from both public and private clinics (**Papers II-V**) and with the Swedish National Cataract Register (**Papers IV and V**), which has a coverage of approximately 97% of all cataract extractions in Sweden.[64,147] Cataracts that were considered to be congenital or secondary to ocular trauma, intraocular inflammation, or previous intraocular surgery were excluded (ICD-10, code H26). According to data from the Swedish National Cataract Register, the median pre-operative best corrected visual acuity in the operated eye with cataract was 20/50 Snellen Equivalents (corresponding to reading difficulties) and the median pre-operative visual acuity in the non-operated eye was 20/30 Snellen Equivalents for men and women during the study period.[64,147]

In **Paper II**, incident cataract cases (n=258) were defined as women with cataract diagnosis and/or cataract extraction after the date of urine sample collection and through December 2009. A control (woman without cataract diagnosis and/or cataract extraction during the follow-up time) was randomly selected for each case and matched for age (same year of birth) and date of urine sample collection (91.1% within 1 month and 8.9% within 2-5 months). In the prospective cohort studies (**Papers III-V**), the participants were followed from baseline (January 1, 1998), until the date of cataract diagnosis, cataract extraction, death or the end of follow-up, whichever occurred first. In **Paper III**, a total of 2963 cataract cases were identified during the follow-up period between January 1, 1998, and December 31, 2006. In **Papers IV and V**, a total of 5761 and 11,580 cataract cases were identified, respectively, during follow-up from January 1, 1998 to December 31, 2011. Date of death was identified through linkage to the Swedish Death Register.

The studies included in this thesis were approved by the Regional Ethical Board at Karolinska Institutet (Stockholm, Sweden) and informed consent was obtained from the participants.

4.2.4 Statistical analysis

The statistical analyses were performed with SAS software versions 9.2 and 9.3 (SAS Inc., Cary, NC) for analysis of linear regression, logistic regression and Cox proportional hazards models. Stata software versions 11 and 12 (StataCorp, College Station, TX) were used for Laplace regression, restricted cubic spline models and correction of risk estimates taking into account sensitivity and specificity of self-reported use of dietary supplements. All *P* values shown are 2-sided and *P* values <0.05 were considered as statistically significant.

4.2.4.1 Cross-sectional study (Paper I)

In **Paper I**, characteristic differences among the women were analyzed with analysis of variance. The distribution of plasma carotenoids, fruit and vegetable intake and plasma EC-SOD activity was tested using residual and goodness-of-fit analyses. Pearson correlation coefficients were calculated to assess the association between plasma carotenoids/fruit and vegetable intake and EC-SOD activity. Partial Pearson correlation coefficients were also calculated to control for the potential effects of age, BMI, smoking, education, supplement use and serum total cholesterol concentrations on the observed associations. The mean EC-SOD activity for each quartile of plasma carotenoids and fruit and vegetable intake in healthy and non-healthy women, respectively, was determined using a multivariable regression model adjusted for the same covariates as in the partial Pearson correlation model. The differences in mean EC-SOD activity across the quartiles and between healthy and non-healthy women were tested using Student's t test.

4.2.4.2 Nested case-control study (Paper II)

In **Paper II**, the differences in characteristics between cases and controls were tested using analysis of variance. Spearman correlation coefficients were calculated to assess the correlation between 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ levels and were adjusted for BMI, smoking, education, use of corticosteroids, history of diabetes and history of CVD. Conditional logistic regression was performed given the matched group to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for age-related cataract by 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ levels (median and quartiles). The multivariable model was adjusted for the same covariates as described above and additionally for potential dietary confounders, including alcohol intake, fruit and vegetable consumption and antioxidant supplement use (vitamin C, E, beta-carotene, zinc and multivitamins). To test for trends across the quartiles of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$, a single numeric variable was created using the median value of each quartile. In a sensitivity analysis, the conditional logistic regression was restricted to only include women without a history of diabetes or CVD and not using corticosteroids at baseline, because these factors have been associated with both increased oxidative stress and risk of cataract.[5] The dose-response relationship between 8-iso-$PGF_{2\alpha}$ as a continuous variable and risk of cataract was flexibly modeled using restricted cubic splines with three knots of the distribution (at 0.27, 0.42, and 0.75 nmol/mmol creatinine).

4.2.4.3 Cohort studies (Papers III-V)

In the prospective cohort studies, person-years of follow-up for each participant were calculated from baseline (January 1, 1998), until the date of cataract diagnosis, cataract extraction, death or the end of follow-up (**Paper III**: December 31, 2006; **Papers IV and V**: December 31, 2011), whichever came first. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% CIs[148] for cataract associated with dietary supplement use (including duration of use) and physical activity (at baseline and long-term). The estimates were adjusted for potential confounders, which in **Paper III** included age, smoking, abdominal obesity, educational level, history of hypertension, corticosteroid use, alcohol intake, fruit and vegetable intake, physical activity and self-reported health. In **Paper IV**, the model was adjusted for age, smoking status and corticosteroid use. Additional adjustments for other potential confounders only changed the risk estimates marginally and were not included in the final model. In **Paper V**, the model was adjusted for age, sex, smoking, abdominal obesity, history of hypertension, corticosteroid use, educational level, fruit and vegetable intake, antioxidant supplement use, and alcohol intake. Missing values for any of the potential confounders were handled in two different ways: either as a separate category in the model (missing indicator method; **Papers III and V**) or excluded from the analysis (complete subject analysis; **Papers IV and V**). The proportional hazard assumption was tested by including the product of the exposures of interest and the natural logarithm of time in the models and there was no departure from this assumption.

In **Paper III**, the men were categorized into non-supplement users and users of vitamin C, vitamin E or multivitamins only (including both regular and occasional users) or multiple supplements in addition to vitamin C or E. In **Paper IV**, women and men were categorized into non-supplement users and users of B vitamins (including vitamin B complex, folic acid and vitamin B6) or vitamin B6 only.

Sensitivity analysis was performed to evaluate the potential risk of reversed causality by excluding the first 4-7 years of follow-up. In **Paper III**, correction analyses[149] were applied to examine the effect of misclassification of self-reported supplement use on the risk estimates. Assumptions of sensitivity and specificity for the vitamin supplements were based on results from a previous validation study in men living in central Sweden.[143]

Stratified analyses were also performed to examine whether the risk between dietary supplement use and cataract differed by factors associated with oxidative stress, as well as vitamin intake from diet. The likelihood ratio test was used to test for interaction (on the multiplicative scale) between these factors and dietary supplement use. In addition, the attributable proportion of risk due to interaction (in excess of the additive effects between the two exposures of interest), was also calculated in **Paper III**.[150,151] Furthermore, in **Paper IV**, the cataract-free survival probabilities over time of follow-up among B vitamin supplement users and non-users were derived from Kaplan-Meier curves.

The difference in time to event (cataract diagnosis and/or extraction) between B vitamin users and non-users was estimated by applying Laplace regression.[152-154] The 10th percentile of survival (corresponds to the point in time when 10% of the group has had an event) was modeled in both groups and adjusted for the same covariates as in the proportional hazard multivariable model.

In **Paper V**, the dose-response relationship between total physical activity as a continuous variable and cataract incidence was examined using restricted cubic splines with three knots located at 10%, 50% and 90% of the distribution of total physical activity (MET*h/day).[155]

5 RESULTS

5.1 RELATIONSHIP BETWEEN EXOGENOUS AND ENDOGENOUS ANTIOXIDANTS (PAPER I)

The relationship between plasma carotenoids, fruit and vegetable intake (exogenous antioxidants) and plasma EC-SOD activity (endogenous antioxidant enzyme) was examined in a cross-sectional study including 95 healthy women and 62 non-healthy women (with a history of CVD, diabetes or cancer), randomly selected from the SMC–Clinical. Characteristics of the women in the highest and lowest quartiles of the sum of plasma carotenoids (including lycopene, lutein/zeaxanthin, α-carotene, β-carotene, and β-cryptoxanthin) are presented in **Table 1, Paper I**. Healthy women in the highest quartile of plasma carotenoids were statistically significantly younger, had higher education, higher fruit and vegetable intake and were more likely to be dietary supplement users, as compared to the lowest quartile. Non-healthy women in the highest quartile of carotenoids were younger, had lower BMI, higher education, higher serum total cholesterol concentrations and higher fruit and vegetable intake than in the lowest quartile.

In healthy women, plasma EC-SOD activity was statistically significantly inversely associated with plasma concentrations of α-carotene, β-carotene, β-cryptoxanthin and the sum of plasma carotenoids, as well as fruit and vegetable intake (**Table 4**). Pearson partial correlation coefficients ranged from -0.22 to -0.38. No statistically significant correlations were observed in non-healthy women.

Table 4. Pearson correlation coefficients of plasma carotenoids, fruit and vegetable intake and plasma extracellular superoxide dismutase activity in healthy and non-healthy women

	Extracellular superoxide dismutase activity (Endogenous antioxidant)			
	Healthy women (n=95)		**Non-healthy women (n=62)[a]**	
Exogenous antioxidants	**Crude**	**Partial[b]**	**Crude**	**Partial[b]**
Sum of carotenoids	-0.28 (P=0.006)	-0.31 (P=0.003)	0.008 (P=0.95)	0.05 (P=0.74)
Specific carotenoids				
Lycopene	-0.16 (P=0.12)	-0.20 (P=0.06)	0.01 (P=0.94)	0.04 (P=0.77)
Lutein/Zeaxanthin	-0.15 (P=0.15)	-0.18 (P=0.09)	-0.08 (P=0.56)	-0.06 (P=0.67)
α-carotene	-0.24 (P=0.02)	-0.22 (P=0.04)	0.11 (P=0.39)	0.14 (P=0.29)
β-carotene	-0.25 (P=0.01)	-0.25 (P=0.02)	0.05 (P=0.69)	0.08 (P=0.58)
β-cryptoxanthin	-0.22 (P=0.04)	-0.23 (P=0.03)	-0.04 (P=0.73)	-0.01 (P=0.94)
Fruit and vegetable intake	-0.39 (P=0.0001)	-0.38 (P=0.0002)	0.06 (P=0.66)	0.08 (P=0.56)

[a] Women with a history of cardiovascular disease, diabetes or cancer.

[b] Adjusted for age, BMI, smoking, education, supplement use and serum total cholesterol concentrations.

Multivariable regression models were used to calculate adjusted mean plasma EC-SOD activity in quartiles of the sum of plasma carotenoids (**Figure 7A**) and fruit and vegetable intake (**Figure 7B**) separately in healthy and non-healthy women. Healthy women in the highest quartile of plasma carotenoids and fruit and vegetable intake had 1.2 U/ml (P=0.01)

and 1.8 U/ml (P<0.0001) lower plasma EC-SOD activity, respectively, as compared to women in the lowest quartile. In non-healthy women, mean plasma EC-SOD activity was not significantly different across levels of plasma carotenoids or fruit and vegetable intake. Furthermore, the mean plasma EC-SOD activity was lower in healthy women in the highest quartile of plasma carotenoids (1.9 U/ml; P<0.001) and fruit and vegetable intake (2.0 U/ml; P<0.001), as compared to non-healthy women in the highest quartile.

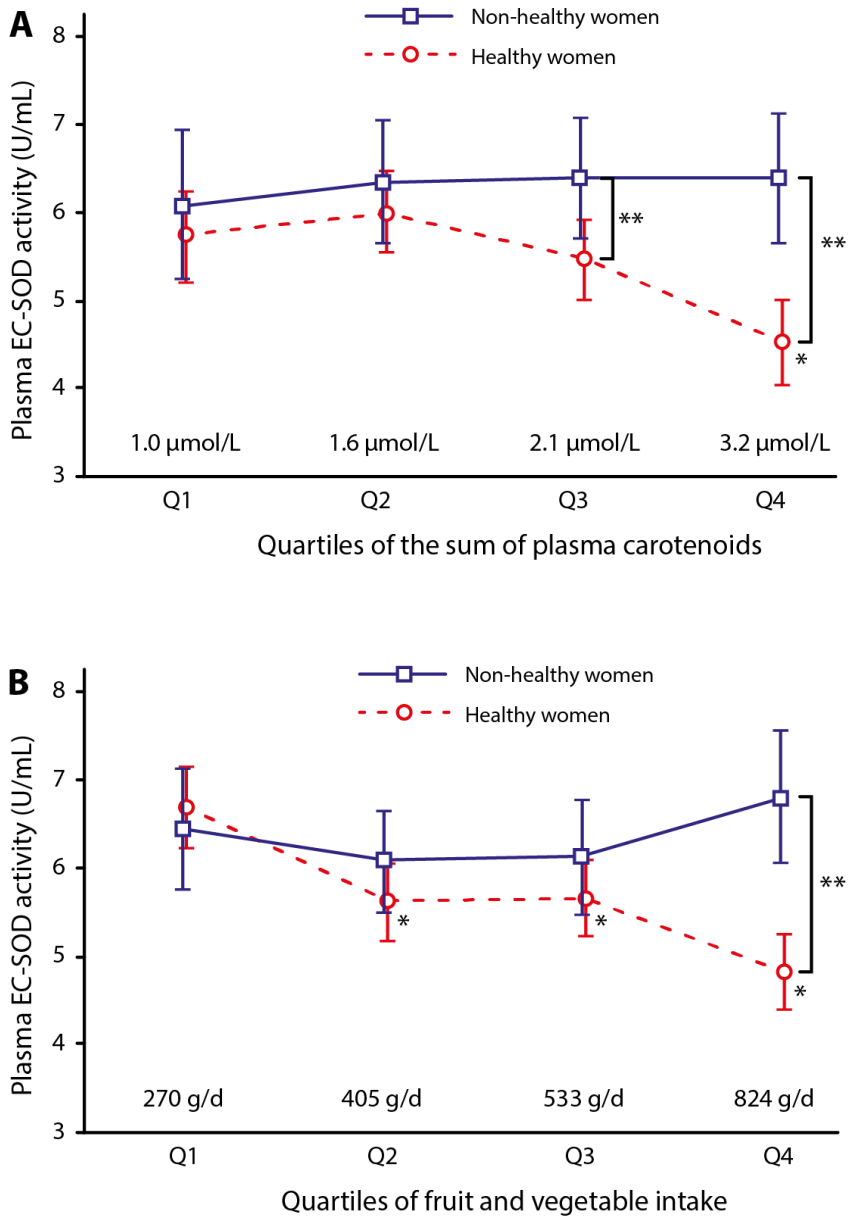

Figure 7. Mean (±SEM) plasma extracellular superoxide dismutase (EC-SOD) activity in quartiles (Q) of A) sum of plasma carotenoids and B) fruit and vegetable intake, in healthy women, n=95 (dashed line), and non-healthy women, n=62 (filled line). Estimated from a multivariable regression model. The numbers in the graphs represent the mean values in each quartile. *P<0.02 *vs*. Q1 in healthy women (Student's *t*-test). **Q3: P<0.05; Q4: P<0.001 difference between healthy and non-healthy women (Student's *t*-test).

5.2 SYSTEMIC OXIDATIVE STRESS, INFLAMMATION AND CATARACT (PAPER II)

This case-control study nested within the SMC included 258 women (cases) with incident age-related cataract diagnosis and/or cataract extraction and 258 women (controls) without cataract randomly selected and matched on age and date of urine sample collection. The cases were more likely to be corticosteroid users and to have a history of diabetes than the controls (**Table 1, Paper II**). The mean urinary 8-iso-$PGF_{2\alpha}$ (biomarker for systemic oxidative stress) level was higher in cases than controls (0.49±0.20 *vs*. 0.46±0.19 nmol/mmol creatinine; *P*=0.13). Urinary 15-keto-dihydro-$PGF_{2\alpha}$ (biomarker for systemic inflammation) levels did not differ between cases and controls. The ORs for cataract according to levels of urinary 8-iso-$PGF_{2\alpha}$ were derived from a restricted cubic spline model (**Figure 8**). The risk of cataract seemed to increase with higher levels of 8-iso-$PGF_{2\alpha}$.

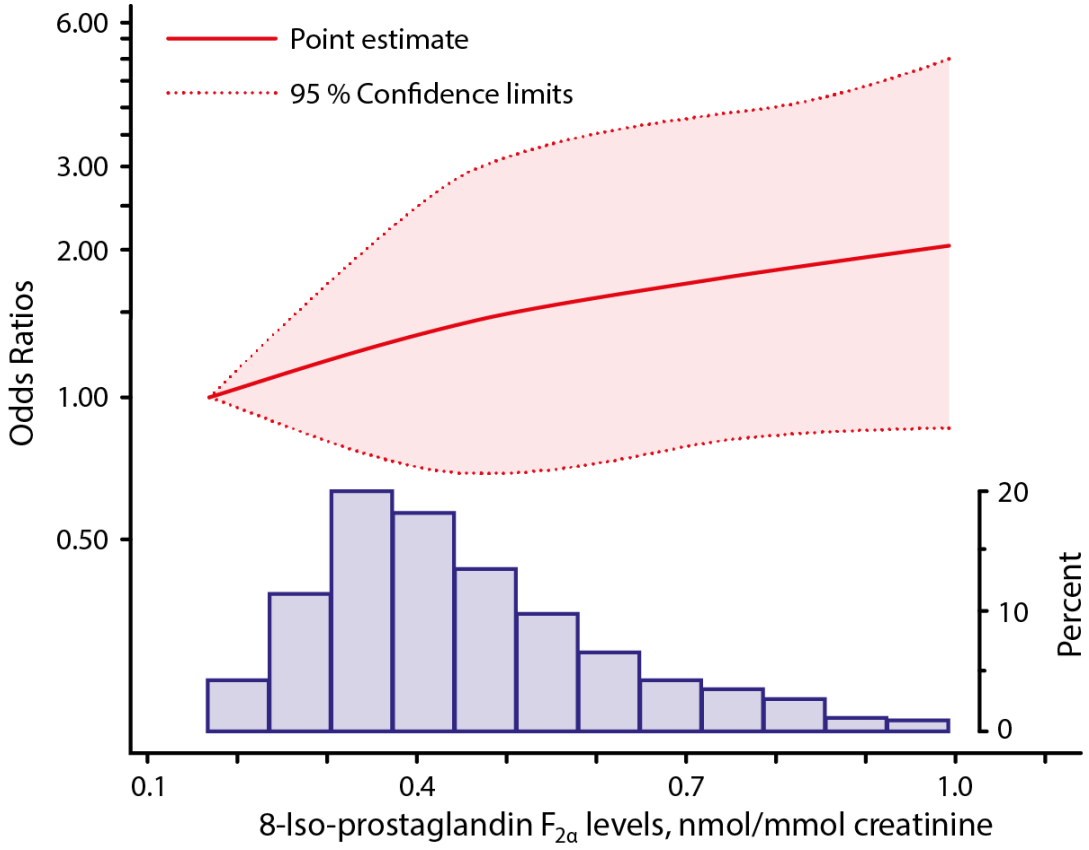

Figure 8. Multivariable-adjusted odds ratios and 95% confidence intervals for age-related cataract according to levels of urinary 8-iso-prostaglandin $F_{2\alpha}$. Data were fitted using a conditional logistic regression model. 8-Iso-prostaglandin $F_{2\alpha}$ was modeled using restricted cubic splines. The histogram shows the distribution of 8-iso-prostaglandin $F_{2\alpha}$ levels in the study population.

The ORs for cataract by median and quartiles of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ levels are presented in **Table 5**. Women with urinary 8-iso-$PGF_{2\alpha}$ levels above median (>0.42 nmol/mmol creatinine) had a statistically significantly 51% increased odds of cataract as compared to those below median and after adjusting for potential confounders (OR=1.51; 95% CI: 1.03-2.20). In a sensitivity analysis (**Table 3, Paper II**) including only women without a history of diabetes or CVD and not using corticosteroids, similar results were observed (the OR for women with 8-iso-$PGF_{2\alpha}$ levels above median compared to below median was 1.64; 95% CI: 1.01-2.68). No associations were observed for urinary 15-keto-dihydro-$PGF_{2\alpha}$ and cataract.

Table 5. Odds ratios[a] for levels of urinary 8-iso-prostaglandin $F_{2\alpha}$ and 15-keto-dihydro-prostaglandin $F_{2\alpha}$ and risk of cataract (n=516)

	No. of cases/ controls	Crude OR (95% CI)	Multivariable[b] OR (95% CI)
8-Iso-$PGF_{2\alpha}$ (nmol/mmol creatinine)			
≤Median (≤0.42)	116/142	1.00 (ref.)	1.00 (ref.)
>Median (>0.42)	142/116	1.57 (1.08-2.27)	1.51 (1.03-2.20)
Quartile 1 (<0.34)	58/71	1.00 (ref.)	1.00 (ref.)
Quartile 2 (0.34-0.42)	58/71	1.04 (0.62-1.75)	1.00 (0.58-1.73)
Quartile 3 (0.42-0.55)	72/57	1.60 (0.95-2.68)	1.52 (0.89-2.59)
Quartile 4 (>0.55)	70/59	1.60 (0.92-2.78)	1.48 (0.83-2.66)
P-trend		*P=0.047*	*P=0.09*
15-Keto-dihydro-$PGF_{2\alpha}$ (nmol/mmol creatinine)			
≤median (≤0.16)	127/131	1.00 (ref.)	1.00 (ref.)
>median (>0.16)	131/127	1.07 (0.75-1.51)	1.12 (0.78-1.62)
Quartile 1 (<0.11)	66/63	1.00 (ref.)	1.00 (ref.)
Quartile 2 (0.11-0.16)	61/68	0.86 (0.54-1.38)	0.79 (0.48-1.30)
Quartile 3 (0.16-0.22)	64/65	0.94 (0.57-1.55)	0.99 (0.59-1.66)
Quartile 4 (>0.22)	67/62	1.04 (0.64-1.69)	1.00 (0.60-1.66)
P-trend		*P=0.76*	*P=0.80*

Abbreviations: CI, confidence interval; OR, odds ratio; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

[a] Calculated using conditional logistic regression model.

[b] Adjusted for body mass index, smoking, education, use of corticosteroids, history of diabetes and history of cardiovascular disease.

5.3 DIETARY SUPPLEMENT USE AND CATARACT

5.3.1 Vitamin C, vitamin E and multivitamin supplements (Paper III)

This population-based prospective cohort study included a total of 31,120 men, aged 45-79 years at baseline. During the follow-up period between 1 January 1998 and 31 December 2006, 2963cases of incident age-related cataract were identified. In this study population, 5.3% of the men reported use of vitamin C supplements only, 0.5% use of vitamin E supplements only and 11.3% use of multivitamins only. Baseline characteristics by dietary supplement use are presented in **Table 1, Paper III**.

After adjusting for potential confounders, men who used high-dose vitamin C supplements only had a 21% increased risk of cataract as compared to non-supplement users (HR=1.21; 95% CI: 1.04-1.41) (**Figure 9)**. Users of high-dose vitamin E supplements only had a 59% increased risk of cataract (HR=1.59; 95% CI: 1.12-2.26). The use of multivitamins only (doses close to RDI) was not significantly associated with cataract risk (HR=0.96; 95% CI: 0.86-1.08). To check for potential reversed causality, the first 4 years of follow-up were excluded and the results remained similar for users of vitamin C only (HR=1.28; 95% CI: 1.07-1.53), vitamin E only (HR=1.62; 95% CI: 1.06-2.48) and multivitamins only (HR=0.90; 95% CI: 0.78-1.04). In a sensitivity analysis, the HRs were corrected for measurement error in self-reported use of supplements and were for vitamin C 3.0, vitamin E 3.2 and multivitamins 1.1.

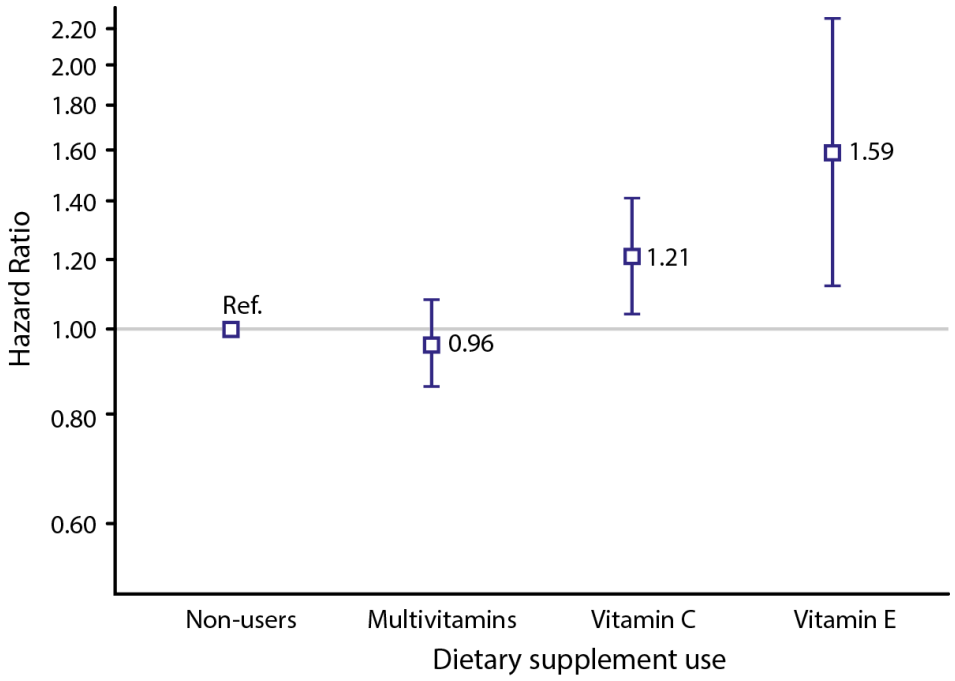

Figure 9. Multivariable-adjusted hazard ratios and 95% confidence intervals for dietary supplement use and risk of cataract in men.

Among men who took other supplements (one or more) in addition to vitamin C (276 cases) or vitamin E (169 cases), the HR was 1.06 (95% CI: 0.93-1.20) and 1.02 (95% CI: 0.87-1.20), respectively.

Long-term use of vitamin C supplements only (≥10 years before baseline) was associated with a 36% increased risk of cataract, as compared to no supplement use (HR=1.36; 95% CI: 1.02-1.81) (**Figure 10**). The difference between the risk estimates for duration of vitamin C supplement use was not statistically significant (*P*=0.71). Analysis of duration of vitamin E supplement use only was not available due to few cases. The use of multivitamins only ≥10 or <10 years before baseline was not associated with cataract risk.

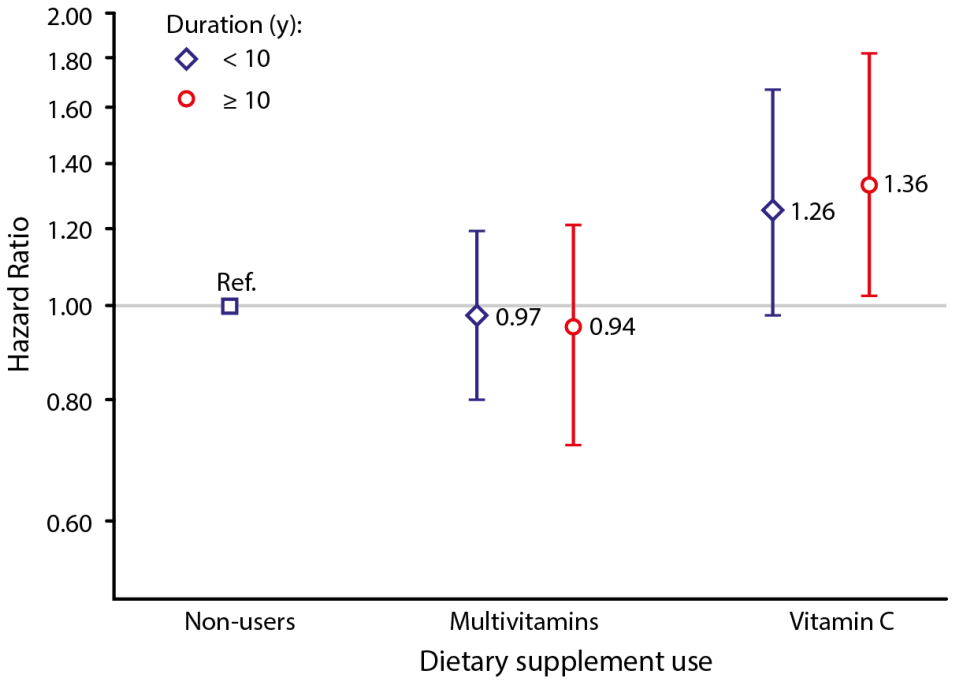

Figure 10. Multivariable-adjusted hazard ratios and 95% confidence intervals for duration of dietary supplement use (<10 and ≥10 years before baseline) and risk of cataract in men.

A stronger positive association between use of vitamin C supplements and risk of cataract was observed among older men (>65 years) and users of corticosteroids (**Table 6**). The attributable proportion due to interaction was borderline statistically significant for age and statistically significant for corticosteroid use on the additive scale. However, no statistically significant interactions were observed on the multiplicative scale. No statistically significant interaction (neither on the additive; all 95% CIs for interaction including 0, nor the multiplicative scale; all *P* for interaction >0.8) was observed between other oxidative stress-related factors, including smoking, abdominal obesity, history of hypertension and vitamin C from diet, and vitamin C supplement use. Since there were too few cases, potential interaction between use of vitamin E supplements and oxidative stress-related factors was not examined. Vitamins C or E intake from diet only among non-users was not associated with cataract risk.

Table 6. Multivariable-adjusted hazard ratios and 95% confidence intervals for vitamin C supplement use and risk of cataract by subgroups of age and corticosteroids

Subgroups	No supplement use (n=22,015)		Vitamin C Only (n=1652)		AP due to interaction[a]
	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	
Age					
≤65 y (n=18,311)	804	1.00 [Reference]	63	1.07 (0.83-1.38)	
>65 y (n=5356)	1133	1.48 (1.14-1.91)	125	1.92 (1.41-2.60)	0.19 (-0.01-0.40)
Corticosteroid use					
No (n=16,270)	1283	1.00 [Reference]	123	1.18 (0.98-1.42)	
Yes (n=1942)	239	1.33 (1.16-1.53)	31	2.11 (1.48-3.02)	0.29 (0.01-0.56)

Abbreviations: AP, attributable proportion; CI, confidence interval; HR, hazard ratio.

[a] The attributable proportion of risk due to interaction (in excess of the additive effects between the 2 exposures) and the corresponding 95% CI.

[b] Adjusted for age, smoking, abdominal obesity, educational level, history of hypertension, corticosteroid use, alcohol intake, fruit and vegetable intake, physical activity and self-reported health.

5.3.2 B vitamin supplements (Paper IV)

In this prospective cohort study of 10,362 women and 17,973 men, aged 45-83 years at baseline, a total of 5761 cases of incident age-related cataract (2677 cases in women and 3084 cases in men) were identified during follow-up (January 1998-December 2011). Among the women, 4.1% reported use of B vitamins only (including vitamin B complex, folic acid and vitamin B6) and 2.7% reported use of vitamin B6 only. The corresponding percentages among men were 1.4% and 0.8%, respectively. Baseline characteristics by B vitamin supplement use are presented in **Table 1, Paper IV**.

The use of B vitamins only was associated with a 20% increased risk of cataract in women (HR=1.20; 95% CI: 1.01-1.42) and a 54% increased risk in men (HR=1.54; 95% CI: 1.22-1.95), as compared to non-users. **Figure 11** shows the HRs and 95% CIs for B vitamin supplement use stratified by different age groups (<60, 60-69 and ≥70 years) in women and men separately. The use of B vitamins only was statistically significant positively associated with risk of cataract in younger age groups (<60 years for women and <70 years for men), but not in those ≥70 years (irrespective of sex).

In a sensitivity analysis, the first 4 years of follow-up were excluded to check for potential reversed causality and the results remained fairly unchanged; e.g. compared to no supplement use, the HR was 1.53 (95% CI: 1.08-2.17) for B vitamin use in women <60 years, and the corresponding HR for men was 2.02 (95% CI: 1.25-3.27).

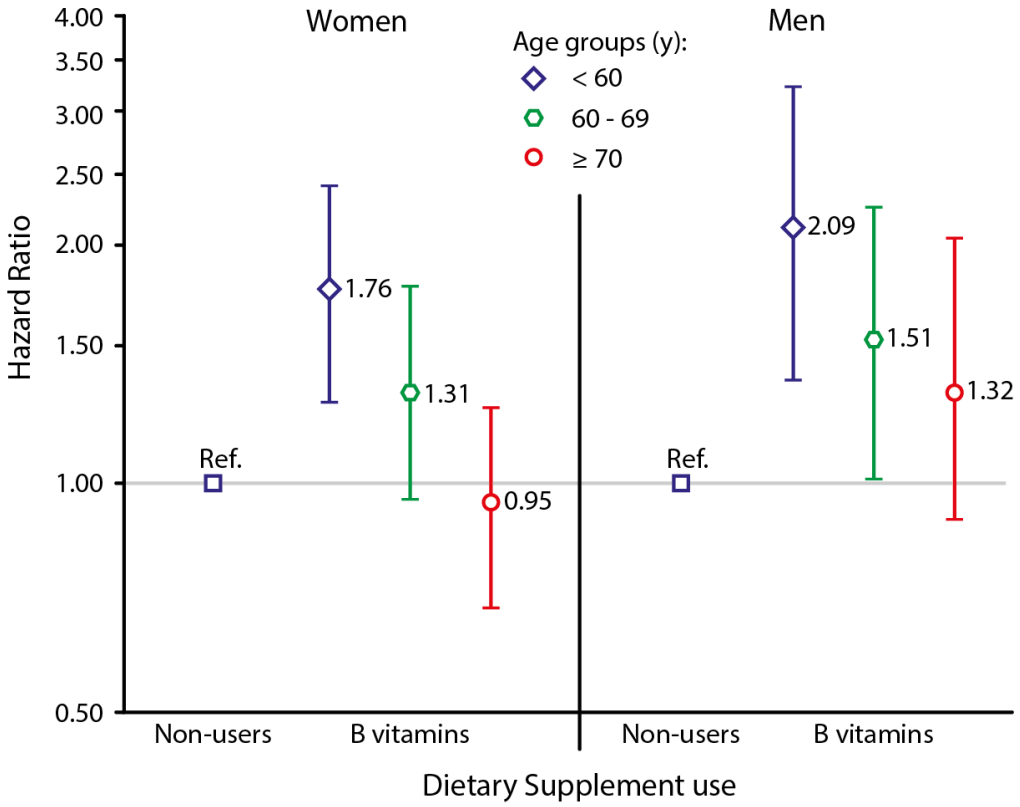

Figure 11. Multivariable-adjusted hazard ratios and 95% confidence intervals for B vitamin supplement use and risk of cataract by age groups (<60, 60-69 and ≥70 years) in women and men.

Laplace regression was applied to estimate the difference in time-to-event of cataract between B vitamin users and non-users, adjusted for the same covariates as in the proportional hazard multivariable model. The results showed that 10% of the non-users had the event about 10 years of study entry, while the same proportion (10%) among users of B vitamins had the event 1.4 years (95% CI: 0.42-2.41) earlier in women and 2.2 years (95% CI: 0.48-4.00) earlier in men.

Further analysis of potential effect modification by factors related to increased oxidative stress, such as smoking, corticosteroid use, abdominal obesity, history of hypertension, alcohol intake, and B vitamins from diet, showed no statistically significant interaction between B vitamin use and any of these factors (all *P* for interactions >0.08), except for smoking in women (*P* for interaction=0.01) (**Table 7**). B vitamins from diet were not associated with cataract.

Table 7. B vitamin supplements and risk of cataract by smoking status

Subgroups	B vitamin supplement use			
	No supplement use (n=9939)		B vitamins[a] (n=423)	
	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)
Women (n=10,362):				
Smoking				
Never (n=5373/6445)	1498	1.00 [Reference]	68	0.98 (0.77-1.25)
Ever (n=4989/11,528)	1042	1.12 (1.03-2.14)	69	1.68 (1.32-2.14)
P-value for interaction				0.01
Men (n=17,973):				
Smoking				
Never (n=5373/6445)	1057	1.00 [Reference]	22	1.75 (1.15-2.66)
Ever (n=4989/11,528)	1955	1.17 (1.09-1.27)	50	1.72 (1.30-2.29)
P-value for interaction				0.50

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] B vitamins include vitamin B complex, folic acid and vitamin B6 supplements.

[b] Adjusted for age and corticosteroid use.

Long-term use (≥10 years before baseline) of vitamin B6 supplements, based on an analysis of a small number of cases (27 cases in men and 47 cases in women), was associated with a 3-fold increased risk of cataract in men (HR=3.28; 95% CI: 1.82-5.93), but not in women (**Figure 12**). Data for duration of use of other B vitamin supplements was not available.

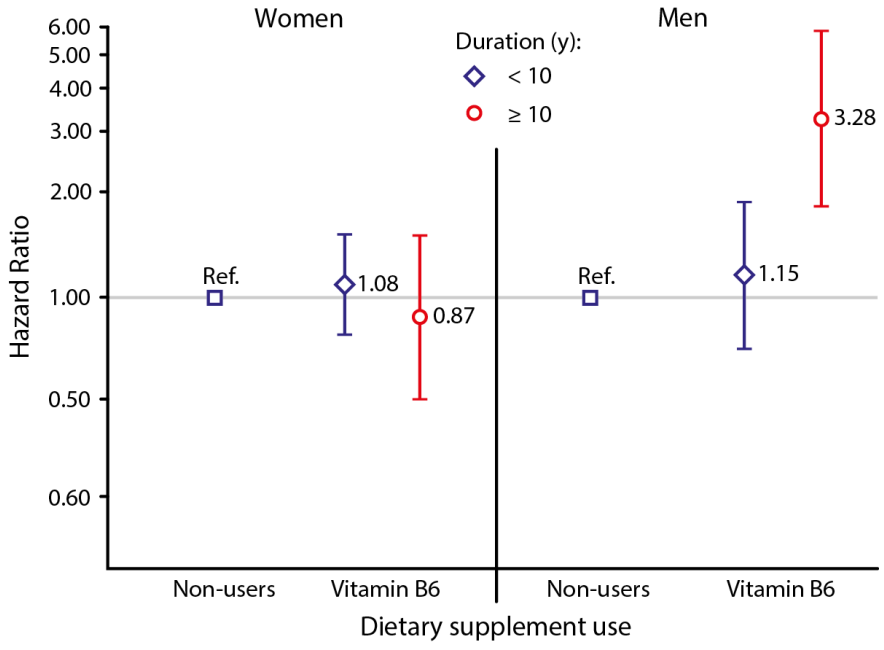

Figure 12. Multivariable-adjusted hazard ratios and 95% confidence intervals for duration of vitamin B6 use and risk of cataract in women and men.

5.4 PHYSICAL ACTIVITY AND CATARACT (PAPER V)

The association between physical activity and risk of age-related cataract was examined in a prospective cohort study of 52,660 participants (23,853 women and 28,807 men), aged 45-83 years at baseline. A total of 11,580 incident cases of age-related cataract were identified during follow-up between 1 January 1998 and 31 December 2011. Baseline characteristics by quartiles of total physical activity are shown in **Table 1, Paper V**.

The dose-response relationship between total physical activity and risk of cataract was examined using a restricted cubic spline model (**Figure 13**). Total physical activity was inversely associated with cataract risk. There was no evidence of significant departure from linearity (*P*=0.22). Every increment of 4 MET*h/day, which corresponds to 1 h of moderate effort, was associated with a 5% reduced risk of cataract (HR=0.95; 95% CI: 0.94-0.97). Similar results were observed separately in women (HR=0.96; 95% CI: 0.94-0.98) and in men (HR=0.94; 95% CI: 0.92-0.97). Participants in the highest quartile of total physical activity had a 13% reduced cataract risk, as compared to those in the lowest quartile (HR=0.87; 95% CI: 0.82-0.92) (**Figure 14**).

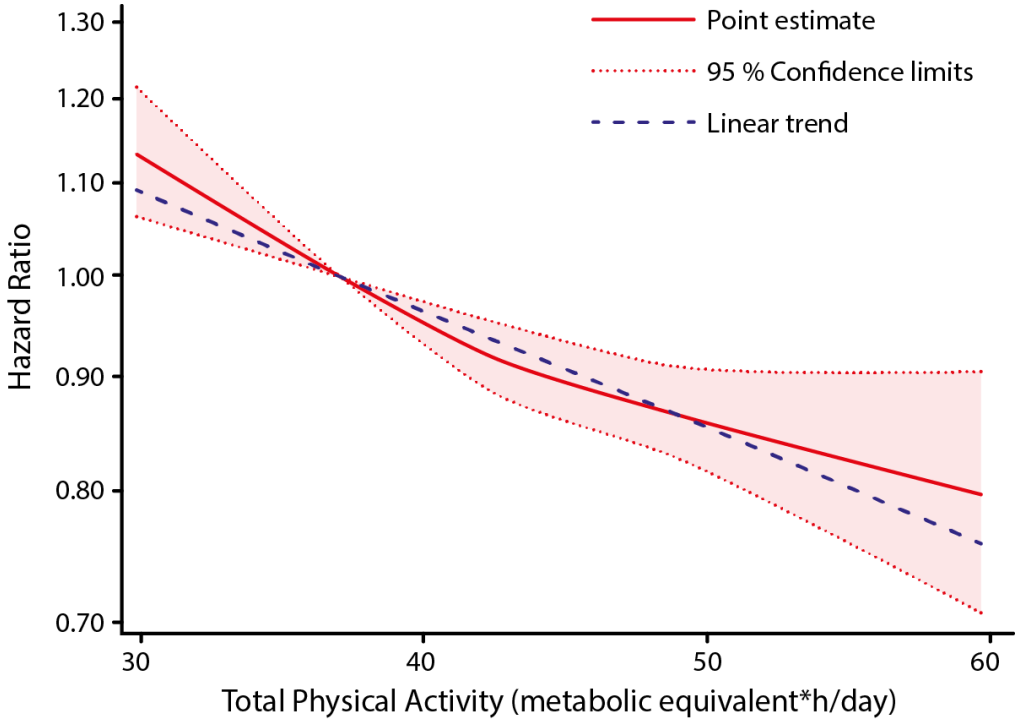

Figure 13. Risk of cataract according to total physical activity. Data were fitted using a multivariable-adjusted Cox regression model. Total physical activity was modeled using restricted cubic splines (the solid line is the point estimate and dashed lines are 95% confidence limits). The long-dashed line represents the linear trend and the reference value is 37 metabolic equivalent*h/day (median value of the lowest quartile of total physical activity).

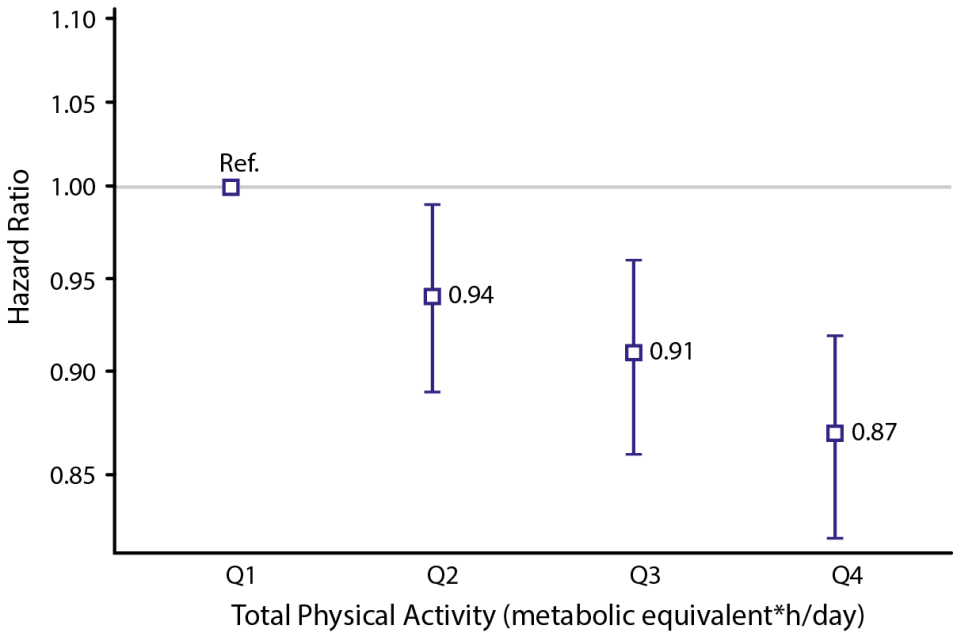

Figure 14. Multivariable-adjusted hazard ratios and 95% confidence intervals for quartiles (Q) of total physical activity and risk of cataract.

Long-term high total physical activity (highest quartile both at age 30 years and at baseline) was associated with a 24% reduced risk of cataract, as compared to long-term low total physical activity (lowest quartile at both time periods) (**Figure 15**).

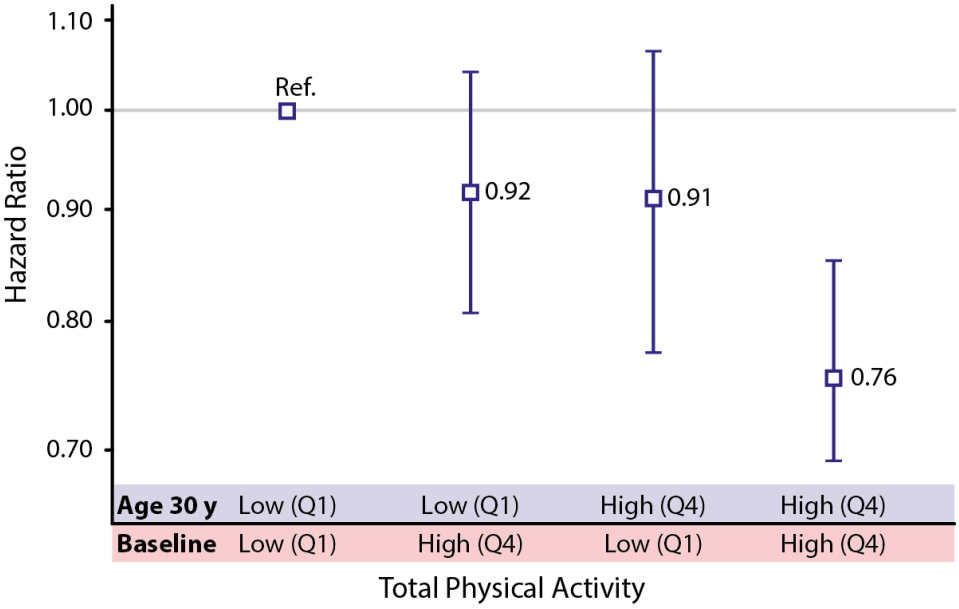

Figure 15. Multivariable-adjusted hazard ratios and 95% confidence intervals for long-term total physical activity and risk of cataract. Long-term total physical activity levels were estimated by combining levels (low=quartile 1, Q1 and high=quartile 4, Q4) at age 30 years and at baseline.

For specific types of physical activity, both walking/bicycling >60 min/day, as compared to hardly ever (modifiable), and heavy manual labor, as compared to mostly sitting (less modifiable), were associated with 12% and 16% reduced risk of cataract, respectively (**Figure 16**). Exercise training and home/housework were not associated with cataract risk.

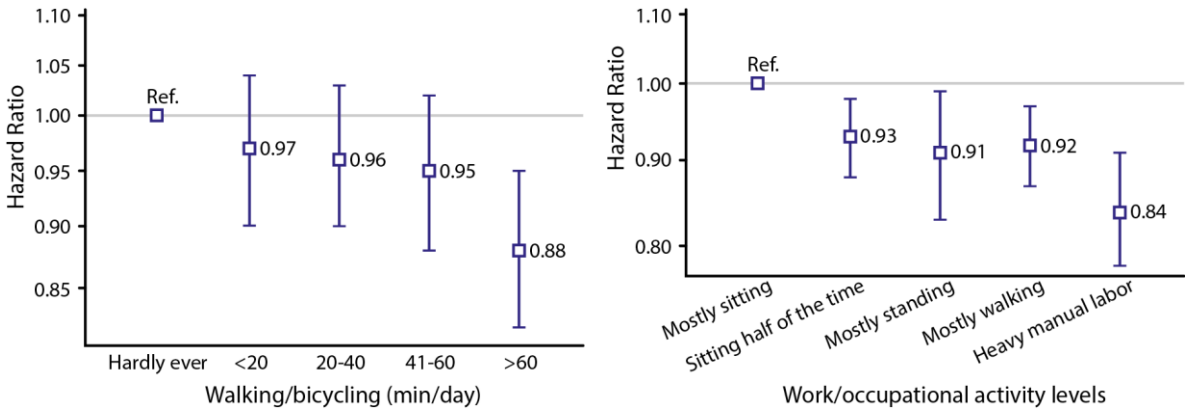

Figure 16. Multivariable-adjusted hazard ratios and 95% confidence intervals for walking/ bicycling and work/occupational activity and risk of cataract.

Conversely, leisure time inactivity >6 h/day (as compared to sitting <1 h/day) was associated with a 27% increased risk of cataract (HR=1.27; 95% CI: 1.07-1.50) (**Figure 17**).

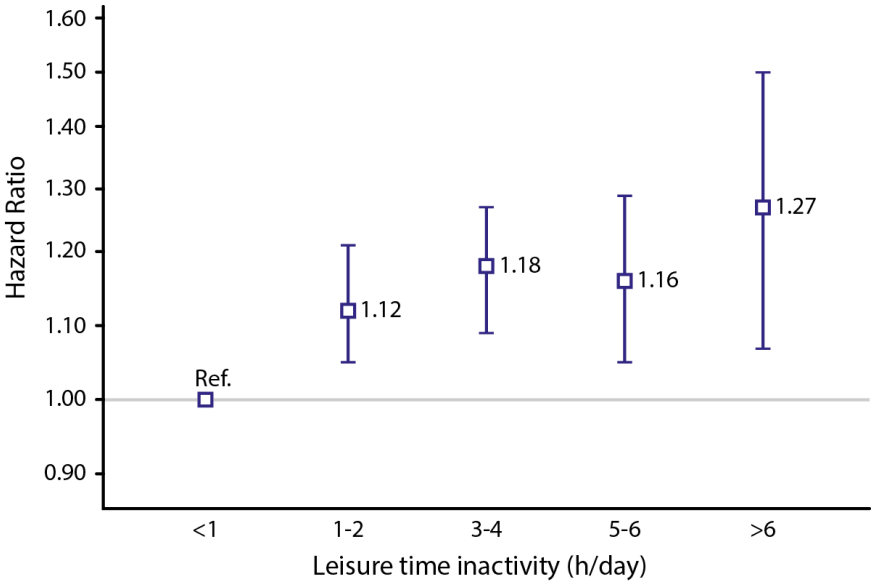

Figure 17. Multivariable-adjusted hazard ratios and 95% confidence intervals for leisure time inactivity and risk of cataract.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

When interpreting results from observational epidemiological studies, several methodological aspects need to be considered, including the study design and random and systematic errors.

6.1.1 Study design

This thesis is comprised of different types of study designs, including cross-sectional (**Paper I**), nested case-control (**Paper II**) and prospective cohort studies (**Papers III-V**). Each study design has strengths and limitations. All observational studies have in common that the results reflect associations rather than direct evidence/effect or causation.

Paper I is a cross-sectional study, in which the exposures and outcomes are assessed at a single point in time. Cross-sectional studies can be used for descriptive purposes and the prevalence of a disease can be estimated, but not the incidence, because there is no information on time aspect/temporality (i.e. whether the exposure precedes the outcome or not). Although it is not possible to directly infer causality, associations observed in cross-sectional studies can lead to the generation of hypotheses for future studies.

Paper II is a nested case-control study, which includes women with incident age-related cataracts as cases, and for each case, one woman without cataract who fulfilled the matching criteria and was randomly selected from the SMC-C (as a control). Matching the cases and controls on confounding variables, such as age and date of urine sample collection, may be a way to increase the efficiency of stratified analysis.[151] Major limitations of case-control studies are that they may introduce selection bias (controls may not be representative of the population from where the cases came) and recall bias (cases may tend to recall their exposures differently than controls). However, the impact of these biases is eliminated in a nested case-control study due to that the cases and controls were from the same study population (SMC-C) and information on exposures was prospectively collected (i.e. the FFQ and urine sample collection was completed before the incidence of cataract).

Papers III-V are large, population-based prospective cohort studies based on the SMC and/or COSM. In prospective cohort studies, the occurrence of a disease can be measured in a population/cohort that is disease-free at baseline and followed over a period of time. The purpose can be to measure and compare disease occurrence among people who are exposed and unexposed. Large cohort studies are suitable to examine multiple exposures and/or multiple outcomes, and from this type of study the disease incidence can be calculated. The prospective design of a cohort study reduces the risk of different biases, such as selection and recall bias. Some limitations of cohort studies are that they are time consuming and expensive (without registers). However, the latter is less of a problem with the Swedish health/disease registers.

6.1.2 Random error

The precision of a risk estimate, defined as the absence of random errors, is indicated by its confidence interval: a narrow interval indicates high precision and a wide interval indicates low precision.[151] In this thesis, 95% confidence intervals were used, which means that if the studies (data collection and statistical analysis) were replicated many times, the correct value should lie within the confidence interval in 95% of the time. A confidence interval that includes the null value (HR=1.00) shows no association, but if it does not include the null value it suggests a statistically significant association. The precision may be improved by increasing the sample size.[151] In this thesis, the main associations observed were statistically significant and the study sizes were relatively large and included many cases of cataract, especially in **Papers III and V**, which improved the precision and reduced the risk of rejecting the null hypothesis when this was true. However, the precision may be lower in some of the subgroup analyses due to a smaller number of cases in the exposure groups.

6.1.3 Systematic error

Systematic error includes different types of biases that can be classified into three categories: selection bias, information bias and confounding.[151] The internal validity of a study is related to the biases and the external validity refers to generalizability of the results.

6.1.3.1 Selection bias

The selection of subjects into a study can lead to selection bias if there are errors in the selection procedures or if some factors influence the participation. In cohort studies, selection bias may occur if an association observed for an exposure and an outcome differs between study participants and non-participants. The presence of selection bias is difficult to assess since exposure and outcome information is usually unknown for non-participants.[151] Self-selection, or the "healthy worker effect", is a type of selection bias, which means that the individuals who agree to participate in a study are different in some aspects from those who do not want to participate, for example by being more health conscious and therefore more interested to participate. It may lead to a lower observed disease rate than expected. On the other hand, it may also be possible that individuals with poorer health want to participate, since they are more worried and at higher risk of developing a disease, which may lead to a higher observed disease rate. The net effect of the bias would be unknown, since it is difficult to quantify the biases. Selection bias may also occur due to differences between exposed and unexposed with regard to loss of follow-up. In this thesis, the studies are based on large, population-based prospective cohorts with a high response rate for the women in the SMC of 74% and a response rate for the men in the COSM of 49%. The prospective design of the studies reduces the risk of selection bias. Moreover, follow-up of the SMC and COSM is almost complete, because of the linkage to various high-quality population-based Swedish health/disease registers.

6.1.3.2 Information bias

Information bias refers to errors in the measurement of a variable, leading to misclassification of exposure and/or outcome. For example, one person can be categorized incorrectly due to erroneously information collected. Misclassification can be classified into differential or non-differential for both exposures and outcomes. Misclassification is affected by the sensitivity and specificity of a method used to identify or measure the exposure and/or outcome. If the misclassification of an exposure is unrelated to the occurrence of an outcome, then it is non-differential. On the other hand, if the exposure misclassification differs between those with and without the outcome, then it is differential. The same applies for outcome misclassification related to the exposure. Non-differential misclassification of a dichotomous variable tends to dilute the observed association towards the null/no-effect value.[151] Differential misclassification can lead to either overestimation or underestimation of an association. A typical type of information bias that leads to differential misclassification is recall bias, which occurs in case-control studies in circumstances where the cases tend to recall exposure information differently than the controls. The risk of recall bias is, however, minimized in the papers included in this thesis, due to the prospective design where exposure information was collected before the identification of outcome/incidence of cataract.

In **Papers I and II,** biomarkers of antioxidants and oxidative stress were measured, which is a more objective way to assess exposures than using self-administered questionnaires and may reduce the risk of misclassification, if laboratory analyses are performed carefully. There are, however, some factors that may lead to misclassification in biomarker measurement, such as the timing of sample collection and storage, stability of the measured biomarkers, and potential laboratory errors. To reduce the risk of misclassification, the samples in the studies were collected at the same time (in the morning) and stored at -80 °C. Enzyme activity (e.g. EC-SOD activity) can be difficult to measure, since assays may be sensitive to a number of factors, such as temperature, time and pH. Therefore, it is important to be as consistent and careful as possible during the laboratory analysis (e.g. control samples for each plate were analyzed in the EC-SOD assay). Samples and standards were analyzed in duplicate to decrease the risk of non-differential misclassification, and the inter- and intra-assay coefficients of variations were calculated. The biomarkers chosen for systemic oxidative stress and inflammation were considered to be reliable and stable over time, and were analyzed using previously validated methods.[8,137]

The exposures in **Papers III-V** (dietary supplement use and physical activity) were assessed using a self-administered questionnaire completed by the study participants at recruitment. People may recall and estimate their habits differently (inter-person variability). However, due to the prospective design of the studies (data collection precedes the identification of outcome), potential misclassification of the exposures is likely to be non-differential (i.e. unrelated to the incidence of age-related cataract). However, cataract may develop slowly and there is a possibility that participants may change their diet and lifestyle habits due to some early symptoms, which may lead to differential misclassification. For example, if people with

early eye symptoms start to take dietary supplements, the observed association may be due to reverse causality. In **Papers III-V**, the first 4-7 years of follow-up were excluded in the Cox proportional hazards models in sensitivity analysis and the risk estimates were similar to those in the main analyses, suggesting that reverse causality is unlikely to explain the observed findings. The sensitivity and specificity of dietary supplement use have been examined in a validation study of men living in central Sweden and were shown to be relatively high for vitamin C (0.7 and 0.9), vitamin E (0.8 and 0.99) and multivitamins (0.7 and 0.98).[143] The physical activity questions in the questionnaire have also been validated against 7-day activity records in both cohorts and appear to estimate total physical activity satisfactory (Spearman's rank correlation: 0.6).[145,146]

There could also be misclassification of the outcome (cataract diagnosis and/or cataract extraction). Since lens opacities can occur without symptoms and there is a lack of standardized eye examinations in the cohorts, an underestimation of cases is possible. This under-ascertainment of cases is likely to be non-differential (unrelated to the exposures). However, there is also the possibility that participants who are more health conscious may seek medical care earlier for visual complaints and are also more likely to use dietary supplements or be physically active. On the other hand, participants with poorer overall health may also seek medical care earlier and may use dietary supplements more often due to suboptimal/limited nutritional intake. This would affect the risk estimates in either direction. However, since everyone has the same affordable access to cataract extractions in the Swedish health care system (patient charge of <$50/surgery), seeking medical help may not depend on socioeconomic factors.

6.1.3.3 Confounding

When interpreting results from observational studies, one has to consider if the observed association may be explained by a third factor, a confounder, which is a variable that is related to both the exposure and the outcome of interest, without being an intermediary step in the causal pathway.[151] The possible effects of confounding may include that the whole or part of an association is accounted for by the confounding factor(s), leading to overestimation or underestimation of the true association. Confounding can be handled in different ways, for example by including potential confounders as covariates in a statistical model, using restrictions (excluding participants with certain characteristics at baseline) or through randomization (in experimental studies). Randomization of the exposure variable (used in RCTs) is an efficient way to prevent known and unknown confounding. However, the efficacy of the randomization may depend on the size of the study population. If the study population is sufficiently large, the randomization process should produce experimental groups with almost the same distribution of characteristics.[151]

In this thesis, confounding was controlled for by including covariates in statistical models and through restrictions. Since those who used dietary supplements and/or had a high physical activity levels may be more health conscious, this could influence our observed associations. To address this issue, the statistical models were adjusted for several potential confounders, including demographic, medical, lifestyle and dietary factors. The main results remained similar when comparing the crude and adjusted risk estimates. Furthermore, in the cohort studies (**Papers III-V**), participants with a history of CVD, diabetes and cancer were excluded, to avoid potential confounding by these diseases on diet and lifestyle-related changes.

Although attempts have been made to control for confounding in our observational studies, the possibility that the findings may be influenced by residual confounding cannot be ruled out. Residual confounding is the confounding that remains after adjustments have been made, and it can be caused by unmeasured factors/confounders, imprecise data or misclassification of the confounding variables.

6.1.3.4 Generalizability

In this thesis, the papers are based on two large, population-based, prospective cohort studies of middle-aged and elderly women and men from central Sweden. The participants were recruited from the general population living in specific geographic areas. They appear to well represent the Swedish population of middle-aged and elderly persons during the study period, since distributions of several demographic and lifestyle factors, such as age, smoking habits, body mass index and education level, are similar to the Swedish population.[156,157] The results in this thesis may therefore be generalizable to middle-aged and elderly women and men in Sweden and other similar countries. However, the generalizability may be limited in younger populations and populations with other ethnicities.

6.2 MAIN FINDINGS AND INTERPRETATIONS

6.2.1.1 Relationship between exogenous and endogenous antioxidants (Paper I)

Results from **Paper I** showed that high levels of exogenous/dietary antioxidants including plasma carotenoids and fruit and vegetable intake were associated with lower plasma EC-SOD activity (endogenous antioxidant enzyme) in healthy women. However, no association was observed in women with a history of chronic diseases, such as CVD, diabetes or cancer.

Few previous studies have investigated the association of fruit and vegetable intake or blood concentrations of carotenoids with plasma EC-SOD activity. One cross-sectional study observed a non-significant inverse association between plasma EC-SOD activity and carotenoids in a healthy population.[158] Another study found a weak positive correlation; however, the sum of serum carotenoids was measured at baseline and EC-SOD activity was collected and measured 15 years later.[159]

The biological mechanism behind the inverse association observed in healthy women is unclear. It could be speculated that high levels of exogenous/dietary antioxidants may lead to a compensatory down-regulation of EC-SOD activity. Experimental studies have shown that plasma carotenoids may be involved in different transcription systems, thereby influencing the expression of antioxidants.[50,160] In humans, high concentrations of blood carotenoids have also been related to lower oxidative damage.[158] However, more studies are needed to examine the mechanisms by which carotenoids may potentially influence EC-SOD activity.

In women with a history of CVD, diabetes or cancer, no inverse association between the antioxidants was observed, suggesting that plasma EC-SOD activity may not be down-regulated with increasing exogenous/dietary antioxidants in the presence of disease. This may potentially be due to higher systemic oxidative stress and inflammation levels. Moreover, the mean plasma EC-SOD activity in the highest quartiles of plasma carotenoids and fruit and vegetable intake was statistically significantly higher in non-healthy women than in healthy women, which suggests a potential up-regulation of the enzyme.[161] Animal studies have shown a protective role of EC-SOD in vascular function and blood pressure regulation.[162-167] However, human studies have shown inconsistent results for EC-SOD activity in association with CVD. Some studies observed an up-regulation of EC-SOD activity in CVD patients as compared to healthy controls,[47,161,168-170] while others observed no difference,[49,168] or decreased[48] activity. Human studies have also observed statistically significantly higher serum EC-SOD activity in patients with type II diabetes than healthy controls.[168-170]

6.2.1.2 Systemic oxidative stress, inflammation and age-related cataract (Paper II)

This nested case-control study (**Paper II**) indicates that higher levels of urinary 8-iso-$PGF_{2\alpha}$ (a biomarker of systemic oxidative stress), but not 15-keto-dihydro-$PGF_{2\alpha}$ (a biomarker of systemic inflammation), were associated with increased risk of age-related cataract in women. These results are in line with previous studies in humans showing increased oxidative stress levels both in the lens[65,171] and systemically.[75-79] However, previous studies have only measured 8-iso-$PGF_{2\alpha}$ in blood and not in urine, and no studies have assessed the association of 15-keto-dihydro-$PGF_{2\alpha}$ and risk of cataract. Urinary 8-iso-$PGF_{2\alpha}$ may be a more suitable biomarker for studying chronic diseases, since it reflects a longer-term exposure than blood.[8,137] Only one case-control and one cross-sectional study have previously investigated levels of 8-iso-$PGF_{2\alpha}$ (in plasma) and risk of cataract.[75,79] The cross-sectional study (n=27 participants with early cataract and n=13 participants without cataract) did not observe any difference in plasma 8-iso-$PGF_{2\alpha}$ levels, although there was increased levels of another lipid peroxidation biomarker, hydroxyoctadecadienoic acid, in participants with cataract.[75] The case-control study (n=102 cases and n=102 controls) observed statistically significantly higher levels of plasma 8-iso-$PGF_{2\alpha}$ in cataract patients as compared to controls, matched on age and gender.[79] Other case-control studies have observed increased blood levels of other biomarkers for lipid peroxidation and oxidative stress, such as malondialdehyde, in cataract patients as compared to age-matched controls.[76-78]

In this study, urinary 15-keto-dihydro-$PGF_{2\alpha}$ was not associated with age-related cataract. This is in line with two cross-sectional studies that observed no association between systemic inflammation biomarkers (as measured by serum C-reactive protein, an acute-phase protein) and cataract in humans.[81,82] However, one of the studies observed a positive association between the inflammatory biomarker serum interleukin 6 and prevalence of nuclear cataract.[81] Moreover, a case-control study nested within the Physicians Health Study I observed a positive association between C-reactive protein levels and cataract in apparently healthy male physicians (n=834 of which 143 men developed cataract).[80] However, since different biomarkers have been used to measure systemic oxidative stress and inflammation, and the lack of standardized methods, the comparability between studies may be limited.[172]

The potential mechanisms underlying the observed findings may be linked to some of the risk factors for cataract, such as age, diabetes and use of corticosteroids.[5] Since these factors are often associated with increased generation of ROS and lipid peroxidation products *in vivo*, especially in the lens,[65,171] they may also be related to systemic oxidative stress and increased urinary 8-iso-$PGF_{2\alpha}$ levels.

6.2.1.3 Dietary supplement use and cataract (Papers III and IV)

Results from the large, population-based prospective cohort studies of dietary supplement use (**Papers III and IV**) suggest that the use of single, high-dose supplements of vitamin C, vitamin E or B vitamins, but not multivitamins (estimated to usually contain nutrient doses close to RDI), was associated with increased risk of cataract. The results also suggest that for vitamin C supplements, the risk may be even higher in older men, corticosteroid users, and long-term users. The positive association observed between B vitamins and cataract seemed to be stronger in younger participants (<60 y).

The results for high-dose vitamin C supplements confirm findings from a previously published study of women from the SMC (n=24,593).[102] However, observational studies have in general shown inconsistent results regarding the use of dietary supplements, including vitamin C, vitamin E, B vitamins and multivitamins (previously reviewed by Mares[90,91] and Seddon,[92] and summarized in **Table 2**).[84,87,93-102] RCTs have mostly shown no effect of high-dose dietary supplements, given either individually or in a combination, for cataract prevention, except one study[103] that reported a deceleration in cataract progression for a combination of antioxidants (previously reviewed by Mares,[90,91] Mathew[104] and Chew,[105] and summarized in **Table 3**).[103,106-113] These results are in line with several other large RCTs investigating the effect of single, high-dose vitamin/antioxidant supplements for prevention of other chronic diseases that also have failed to show any protective effect.[117-121] Indeed, some RCTs have even reported harmful effects of high-dose antioxidant supplements for CVD,[122] some types of cancers[123-125] and mortality.[126] On the other hand, three RCTs have reported a modestly decreased risk of total and nuclear cataract for multivitamins containing doses close to RDI. One of the trials (the Linxian Cataract Studies) was based on a study

population with micronutrient deficiencies at baseline.[114] The other two trials were conducted among generally well-nourished study populations (the Italian-American Clinical Trial of Nutritional Supplements and Age-Related Cataract and the Physicians' Health Study II).[115,116]

One interesting and important question to discuss is why antioxidants from dietary sources seem to be protective against chronic diseases (mainly observed in observational studies), while the use of dietary supplements have mainly failed to show protective effects (inconsistent results in observational studies and mostly no effects in RCTs)? There are several possible explanations for these observations. First, from a biological point of view, antioxidant-rich sources, such as fruit and vegetables, may include a variety of nutrients/components that act in a complex manner (e.g. synergistically). Therefore, intake of a single nutrient separated from the food source and provided at relatively high concentrations may not contribute to the same physiological effects. Moreover, since different exogenous/dietary antioxidants may have different antioxidant properties and may be involved in redox cycling of other antioxidants, providing only one single antioxidant (e.g. one isoform of a vitamin) may disturb the redox balance and in some cases lead to pro-oxidative effects (e.g. for vitamin C and E). Furthermore, the bioavailability of a nutrient may be different depending on intake from either a natural food source or in dietary supplement form. Second, from a methodological point of view, differences between studies in multiple aspects related to study design make the comparability between studies limited.

The following factors may influence the comparability of results between studies:

- **Study design:** observational or intervention?
- **Type of supplement(s) used:** single or multiple?
- **Dosage:** RDI or high-dose?
- **Duration:** short-term or long-term?
- **Antioxidant status at baseline:** deficiency or adequate nutritional status?
- **Health status at baseline:** primary or secondary prevention?
- **Outcome:** cataract diagnosis or cataract extraction (information on subtypes)?

It is important to consider these factors when interpreting results from different studies. Observational studies measure associations rather than causation and the possibility of residual confounding cannot be ruled out, which may influence the observed associations between dietary supplement use and risk of cataract. However, this is generally not an issue in a well-designed RCT, which is considered as the "golden standard" (providing most convincing evidence). The type, dose, and duration of supplement use are other aspects to consider when interpreting results from different studies. For example, there is a large variation in the dose and duration of vitamin C and vitamin E supplements used in RCTs (summarized in **Table 8**). Of note, some trials have used vitamin doses that exceeded the "safe"/upper tolerable limits as recommended by the European Food Safety Authority.[173]

Moreover, baseline antioxidant status may also be important, since the supplementation of antioxidants may to be more efficient if given to a population with baseline deficiencies, which was the case for the Linxian Cataract Studies (RCTs).[111,112] The health status of the study population may also influence the results, for example the populations of two other large RCTs were based on physicians and nurses, which are considered to generally be more health conscious and well-nourished. Only 3-4% of participants were current smokers among the physicians,[112] as compared to 24% among the men in the COSM. Furthermore, the outcome used to assess cataract differs between studies. In our studies, cataract diagnosis and cataract extraction were defined as the outcome, but we did not have information on grade or subtypes of cataracts, which some studies had, while other studies only included cataract extraction or one subtype. Another factor is difficulty in translating results from animal experiments into recommendations for humans. Since it is difficult to obtain and study cataract lenses from humans, several experimental animal models have been developed, but the human lens differs from animal experiment lenses in several aspects, including levels of oxidation with age, endogenous antioxidant enzymes, vitamin C synthesis, protein content, etc.[53]

Table 8. The dose and duration of vitamin supplements used in RCTs for cataract prevention

Antioxidants	Dose (range)[a]	Duration (range)[a]	Recommended Daily Intake[b]	Upper Tolerable Limit[b]
Vitamin C	120-750 mg/day	3-8 years	75 mg/day	-
Vitamin E	30-600 mg/day	3-10 years	8-10 mg/day	300 mg/day[c]

[a] From references 103, 107, 110, 112 and 114 (vitamin C); 103, 106, 107 and 109-114 (vitamin E).
[b] Recommendations for adults: women/men (Source: Nordic Nutrition Recommendations 2012).[20]
[c] From supplements (Source: European Food Safety Authority 2003).[173]

The biological mechanisms behind our findings are not fully understood. The mechanisms could be related to disturbed redox homeostasis and pro-oxidative properties of single, high-dose vitamins/antioxidants, which have been shown in experimental studies using large doses of vitamin C, vitamin E or B vitamins.[32,33,41,53,174-178] The use of a single high-dose antioxidant supplement, such as for vitamin E supplements where the commonly used isoform is α-tocopherol, may reduce the uptake of other natural forms of vitamin E at high concentrations.[179] The statistically significant interaction observed between use of vitamin C and older age, as well as corticosteroids, may involve an inflammatory mechanism and increased oxidative stress.[80] Both aging and the use of corticosteroids are risk factors for cataract.[180] The use of corticosteroids may also indicate an underlying illness associated with increased oxidative stress and inflammation.[181] However, the association between B vitamins supplements and risk of cataract seemed to be stronger in the younger age groups. The mechanism for this association is unclear. We can only speculate that it could be related to a relatively high prevalence of inadequate B vitamin status, which has been observed in some elderly populations.[10]

The results from **Paper III and IV** add to the accumulating evidence from other recent studies also showing potentially harmful effects of single, high-dose antioxidant supplements in the general population. They underscore the need to consider with caution use of high-dose supplements or unregulated supplements with uncontrolled dosages. However, the use of multivitamins was not associated with cataract risk in our study. Three RCTs have suggested a potential protective effect of multivitamins for nuclear cataract development, but this effect was countered by a potentially harmful effect for posterior subcapsular cataract development, as observed in one of the trials.

6.2.1.4 Physical activity and cataract (Paper V)

In this thesis, results showed that high total physical activity levels and specific types of physical activity, including walking/bicycling and work/occupational activity, were statistically significantly associated with decreased risk of age-related cataract in women and men. The inverse association seemed to be even stronger with long-term consistently high total physical activity levels, both during young adulthood (age 30 years) and later on in life. Leisure time inactivity was positively associated with risk of cataract.

These population-based results are in line with the three previous studies that have examined the association between physical activity and risk of cataract in selected physically active populations[133,134] or at a hospital setting.[135] Two prospective cohort studies based on the National Runners' and Walkers' Health Studies, observed that moderate (walking) and vigorous (running) physical activity, but not other moderate-intensity exercise, were associated with similar decreased risk of cataract in women and men.[133,134] Of note, the recruitment of participants in these cohorts was conducted among subscribers of activity-targeted publications and participants at footrace events, and therefore included more physically active participants than a general population. Moreover, a small case-control study (n=110 cataract cases and n=50 age-matched hospital controls) observed a 4-7 fold higher odds ratio for cataract in patients with low physical activity as compared to high physical activity.[135] This study was conducted in a hospital setting and the ORs were adjusted only for age.[135] To our knowledge, our study was the first to prospectively examine the association between physical activity, including total and specific subtypes and duration, and risk of cataract in a population-based study.

Potential biological mechanisms behind the findings may involve reduced oxidative stress and inflammation levels, as well as improved insulin resistance and lipid profiles.[127-129] Long-term, high physical activity levels in elderly people have been associated with higher total antioxidant capacity and lower oxidative damage.[129] During physical activity/muscle contractions, there is a mild pro-oxidative state, which triggers some of the beneficial effects of exercise, including signaling processes for muscle adaptation and increased expression of antioxidant enzymes.[182] Moreover, physical activity may also reduce inflammation (as measured by C-reactive protein)[183] and improve the lipid profile by elevating high-density

lipoprotein-cholesterol and lowering triglyceride concentrations.[184] Hypertriglyceridemia has been positively associated with risk of posterior subcapsular cataract.[185] Other beneficial health effects of physical activity include improved insulin resistance[3] and decreased risk of diabetes and hypertension,[130] which are suggested risk factors for cataract.[5] On the other hand, high levels of physical inactivity may lead to increased ROS production, endothelial dysfunction and atherosclerosis in mice,[131] and increased protein oxidation and lipid peroxidation in humans.[186] The inverse association observed for long-term total physical activity and the lack of association for leisure time exercise with cataract risk suggest that being physically active on a regular daily basis as reflected by long-term total physical activity, in contrast to short weekly episodes of exercising/training, may contribute to cataract prevention. Home/housework was not associated with risk of cataract, potentially due to a large variation in the different types of tasks and activity levels performed.

In summary, the results in **Paper V** are in accordance with the inverse association observed between physical activity and risk of many other chronic diseases, such as CVD and type II diabetes, and further add to the knowledge of health benefits of physical activity.

7 CONCLUSIONS

Results from the papers included in this thesis showed that:

- High levels of exogenous/dietary antioxidants, namely plasma carotenoids and intake of fruit and vegetables, were associated with lower plasma EC-SOD activity (endogenous antioxidant enzyme) in healthy women. This association was, however, not observed in women with a history of CVD, diabetes or cancer.

- Systemic oxidative stress (as measured by urinary 8-iso-prostaglandin $F_{2\alpha}$) was associated with increased risk of age-related cataract in women. However, no association was observed between systemic inflammation (as measured by urinary 15-keto-dihydro-prostaglandin $F_{2\alpha}$) and risk of cataract.

- In a generally well-nourished population, the use of high-dose vitamin C or vitamin E supplements was associated with increased risk of cataract in men. However, the use of low-dose multivitamins was not associated with risk of cataract.

- The use of high-dose B vitamins supplements was associated with increased risk of cataract in women and men.

- High levels of total physical activity, especially long-term, as well as specific types such as walking/bicycling and work/occupational activity, were associated with decreased risk of cataract in women and men. On the other hand, high leisure time inactivity was associated with increased risk of cataract.

In summary, these results suggest that maintaining low levels of systemic oxidative stress by having a healthy lifestyle, for example eating an antioxidant-rich diet including plenty of fruits and vegetables instead of taking dietary supplements and being physically active on a regular basis may contribute to the prevention of age-related cataract in the general population.

8 FUTURE RESEARCH

The results from this thesis have added small pieces of evidence to the complex field of oxidative stress research and contributed to the knowledge about the association between oxidative stress and age-related cataract. To further deepen and extend our understanding of the interplay between antioxidants, reactive species and oxidative stress, and for prevention of cataract, future studies could benefit from improved methodology for exposure and outcome assessments.

Since the relationship between exogenous/dietary antioxidants and endogenous antioxidants may differ depending on the health status of the individual, it may be of importance to examine the associations between different antioxidants, as well as biomarkers of oxidative stress, separately in populations/ subgroups depending on health status to better understand their interplay. In future observational studies, it would be interesting to examine the association between different exogenous/dietary and endogenous antioxidants and systemic oxidative stress in people "at risk" of developing or who already have age-related cataract. This could contribute to our understanding of whether specific antioxidants levels may be altered and which potential antioxidant and oxidative stress biomarkers are most relevant to cataract development.

The choice of appropriate biomarkers for measuring oxidative stress and inflammation status is an important aspect to consider. Are the chosen biomarkers reflecting acute or chronic oxidative stress/inflammation? Are the biomarkers useful for prediction or progression of a disease? In this thesis, the risk of cataract was examined using urinary 8-iso-prostaglandin $F_{2\alpha}$ and 15-keto-dihydro-prostaglandin $F_{2\alpha}$ as biomarkers of systemic oxidative stress and inflammation. Although these are generally considered stable and reliable biomarkers, there may be other more sensitive biomarkers that could better reflect the systemic oxidative stress or inflammation associated with cataract. Since different studies have used different biomarkers of oxidative stress and inflammation, it is difficult to compare the results between the studies. Moreover, it may also be difficult to compare results from studies using the same biomarker, because the laboratory methods used may differ, as well as the type of biological samples used (e.g. urine or blood samples). Therefore, to better enable comparison between studies and to confirm our observed association of urinary 8-iso-prostaglandin $F_{2\alpha}$ and risk of cataract, there is a need for standardized methods for measuring oxidative stress status *in vivo*, including reference values (ranges) according to characteristics, such as age and type of samples analyzed.

In this thesis, the use of multivitamins (with doses close to RDI) was not associated with risk of cataract. Moreover, the use of single, high-dose antioxidant supplements was associated with increased risk of cataract. These studies were based on generally well-nourished populations and therefore, a potential explanation for the lack of a protective association may be that increasing the intakes of single antioxidants may not provide further health benefits

among people who already have optimal nutritional status. Potential beneficial effects of multivitamins (with doses close to RDI) have been reported in some other studies and in populations with baseline micronutrient deficiencies. Therefore, future observational and experimental studies may consider examining the use of low-dose (close to RDI) multivitamins (or a combination of other low-dose antioxidants) for the prevention of chronic diseases, such as cataract, in "high-risk" populations, for example those with low antioxidant intake and that are at risk of developing or already have micronutrient deficiencies. To identify the populations that would benefit the most from dietary supplements, it may be important to measure systemic oxidative stress levels *in vivo* in addition to antioxidant status. In future observational studies, to improve exposure assessment of antioxidant status, it could be useful to combine information on antioxidant intake from self-administered FFQs with related biomarkers (that change with dietary intake) preferably using repeated/multiple measurements reflecting long-term status. For future RCTs, which is the type of study that can provide the most convincing evidence, focus could be on the optimal composition, dose and duration of dietary supplements to prevent eye diseases and maintain health. In addition, measurements of *in vivo* oxidative stress biomarkers before and after an intervention may also be valuable to address whether the supplement(s) given has changed oxidative stress levels, which may in turn be related to the risk of cataract.

The protective association observed between physical activity and risk of cataract in our Swedish population-based prospective cohorts needs to be confirmed in other populations. Future studies may include large, prospective cohort studies using validated questionnaires and/or instruments to measure physical activity and taking into account which type, intensity and duration of physical activity may contribute the most to cataract prevention.

The ascertainment of cataract cases should also be improved in future studies. For the studies included in this thesis, cataract diagnosis and/or cataract extraction were used as the outcome. Cataract grades and subtypes were not examined, due to lack of standardized eye examinations and incomplete information on cataract subtypes. Therefore, it would be valuable in future studies to include regular and standardized eye examinations to enable better comparability between studies and to also include information on the grade and subtypes of cataract.

In the future, studies could also examine dietary and lifestyle patterns rather than individual risk factors, which may give a more comprehensive picture of the association between diet and lifestyle-related factors and cataract prevention. Furthermore, studies taking into account gene-environment interactions are also needed. For example, genetics may influence how people respond to dietary antioxidants and physical activity.

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