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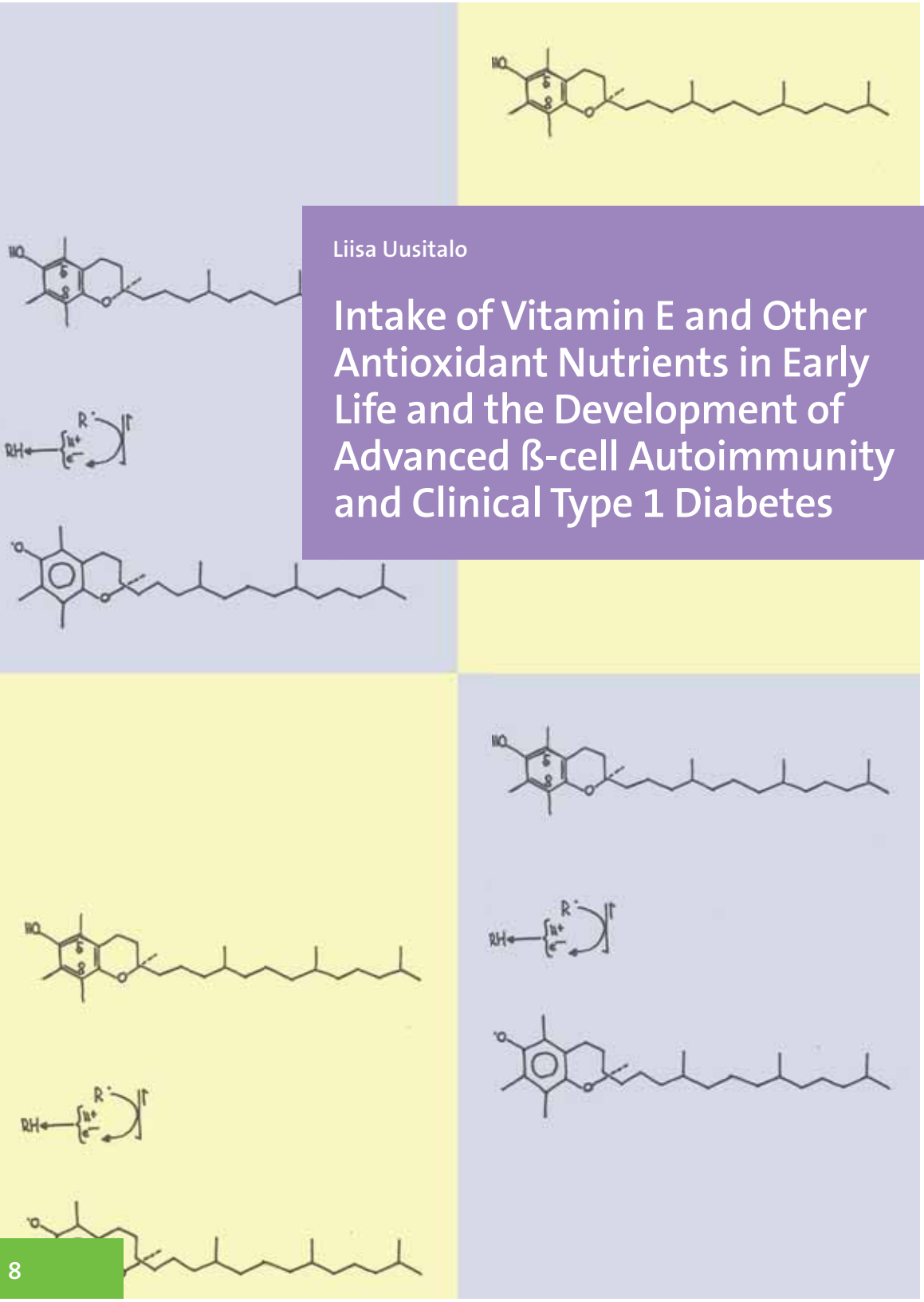
Liisa Uusitalo

Intake of Vitamin E and Other Antioxidant Nutrients in Early Life and the Development of Advanced ß-cell Autoimmunity and Clinical Type 1 Diabetes



Liisa Uusitalo

INTAKE OF VITAMIN E AND OTHER ANTIOXIDANT NUTRIENTS IN EARLY LIFE AND THE DEVELOPMENT OF ADVANCED ß-CELL AUTOIMMUNITY AND CLINICAL TYPE 1 DIABETES

ACADEMIC DISSERTATION

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ABSTRACT

Free radicals, highly reactive oxygen or nitrogen compounds at high concentrations are harmful to human health. They are believed to play a role in the development of all the major degenerative diseases, and may also be involved in the development of type 1 diabetes. Antioxidants are substances with an ability to neutralize free radicals. Antioxidant defense system of the body consists of antioxidative enzymes, other endogenously synthetized compounds and dietary antioxidants. Vitamin E, vitamin C and β-carotene are the classical dietary antioxidants. Diet also provides the trace elements needed as cofactors of antioxidative enzymes. The effect of antioxidant nutrients on the development of type 1 diabetes has been studied mainly in animal models, and research has concentrated on vitamin E and zinc. Protective effects against diabetes have been reported for both. Epidemiological studies on the associations of antioxidant nutrients with type 1 diabetes are rare.

The present study falls into the field of nutritional epidemiology. It is based on epidemiological analyses of the associations of vitamin E and other antioxidant nutrients with the development of type 1 diabetes. The specific research objectives were:

– to analyze whether serum vitamin E concentrations are associated with the risk of pre-type 1 diabetes, defined as repeated positivity for ICA and at least one other disease-predictive autoantibody, and with clinical type 1 diabetes;
– to analyze whether maternal intake of vitamin E or other selected antioxidant nutrients or the consumption of their dietary sources during pregnancy is associated with pre-type 1 diabetes in the child; and
– to analyze the sociodemographic and lifestyle determinants of intake of antioxidant nutrients and consumption of their dietary sources among Finnish pregnant women.

The data are derived from two large Finnish cohort studies: the cohort of the ‘Childhood Diabetes in Finland’ (DiMe) Study, which consists of unaffected siblings of children

with diabetes, and the Type 1 Diabetes Prediction and Prevention (DIPP) Project, which is a birth cohort of children with HLA-conferred susceptibility to type 1 diabetes. The associations of serum vitamin E concentration and the risk of clinical type 1 diabetes were analyzed within the DiMe cohort. Two separate study designs were used. In a nested case-control study, α-tocopherol concentrations of cases with type 1 diabetes (n = 16) were compared with the concentrations of matched controls (n = 81). In the second study design, the association of α-tocopherol with type 1 diabetes was analyzed in a subcohort of seropositive siblings (n = 80). During follow-up, 26 siblings progressed to type 1 diabetes. A conditional logistic regression model was employed in the case-control setting, and a Cox regression model in the cohort setting.

The associations of serum α- and γ-tocopherol concentrations and preclinical type 1 diabetes were analyzed within the DIPP cohort using a nested case-control study design. There were 108 cases and 216 matched controls with at least one serum sample available. For each case-control set, all the repeated serum samples, collected at 1-year intervals starting from the age of 1 year, were analyzed up to the age of seroconversion of the case. A conditional logistic regression model was used to compare vitamin E concentrations of cases and controls.

Maternal antioxidant intake during pregnancy and the consumption of their dietary sources, and their associations with preclinical type 1 diabetes in the child were studied on the DIPP study using a cohort design. The series comprised children with HLA-conferred disease susceptibility who participated in the follow-up for the emergence of diabetes-associated autoantibodies and/or overt type 1 diabetes and their mothers. Dietary intake during pregnancy was assessed using a validated food frequency questionnaire. During follow-up, 165 children of the total of 4,297 children in the cohort were affected with preclinical and/or clinical type 1 diabetes. The associations of maternal diet with pre-type 1 diabetes in the child were analyzed by piece-wise exponential survival models. The associations of sociodemographic and lifestyle characteristics with antioxidant intake among the 3,730 mothers were analyzed by multiple linear regression models, and those with food consumption by multiple logistic regression models.

Serum α-tocopherol concentrations analyzed within the cohort of initially non-diabetic siblings showed an inverse association of borderline statistical significance with clinical type 1 diabetes both in the nested case-control design ($p = 0.08$) and in the subcohort of seropositive children ($p = 0.09$), adjusted for potential confounding factors. In the birth cohort of genetically susceptible children, serum α- or γ-tocopherol concentrations were not significantly associated with the risk of pre-type 1 diabetes. Neither were the maternal intakes of vitamin E, retinol, β-carotene, vitamin C, selenium, zinc or manganese, or the consumption of their main dietary sources during pregnancy associated with the risk of pre-type 1 diabetes in the offspring. There was considerable variation in the intake of

antioxidant nutrients among pregnant women, and all the sociodemographic and lifestyle factors studied (age, parity, own and partner's level of education, smoking during pregnancy, and BMI at the first antenatal visit) were independently associated with antioxidant intake. Young age, low level of education, and smoking during pregnancy were the most important predictors of a low intake of antioxidant nutrients.

As a conclusion, a protective effect of vitamin E against clinical type 1 diabetes seems possible, and further studies are warranted. No evidence was found of a protective effect of vitamin E against preclinical type 1 diabetes. In future research, the associations of dietary vitamin E intake with the development of type 1 diabetes should be analyzed. Methodological studies are needed to identify the optimal methods for the measurement of true long-term vitamin E intake. Maternal intake of antioxidant nutrients during pregnancy was also not associated with pre-type 1 diabetes in the offspring. The absence of association could result from the short period of time when the newborn infant is dependent on the maternal antioxidant intake, and therefore it is advisable to next analyze the associations of the child's own antioxidant intake with the development of clinical and preclinical type 1 diabetes.

Keywords: vitamin E, antioxidants, children, pregnant women, type 1 diabetes, autoantibodies, epidemiology, nutrition

Liisa Uusitalo, E-vitamiinin ja muiden antioksidanttiravintoaineiden saanti varhaisella iällä ja tyypin 1 diabeteksen kehittyminen
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TIIVISTELMÄ

Vapaat radikaalit ovat reaktiivisia happi- tai typpiyhdisteitä, jotka suurina pitoisuuksina ovat haitallisia terveydelle. Vapaiden radikaalien oletetaan vaikuttavan yleisimpien ikääntymiseen liittyvien sairauksien syntyyn, ja ne ovat osallisina myös tyypin 1 diabeteksen kehittymisessä. Antioksidantit ovat yhdisteitä, jotka kykenevät neutraloimaan vapaita radikaaleja. Elimistön antioksidanttipuolustus muodostuu antioksidatiivisista entsyymeistä, muista elimistön itse tuottamista yhdisteistä sekä ravinnon mukana saatavista antioksidanteista. E-vitamiini, C-vitamiini ja beetakaroteeni ovat parhaiten tunnetut ravinnon antioksidantit. Ravinnosta saadaan myös antioksidanttientsyymien kofaktoreina toimivat hivenaineet. Ravinnon antioksidanttien vaikutusta tyypin 1 diabeteksen kehittymiseen on tutkittu etupäässä eläinkokein, ja tutkimus on kohdistunut eniten E-vitamiiniin ja sinkkiin. Molemmilla on raportoitu olevan suojavaikutusta diabetesta vastaan. Epidemiologisia tutkimuksia antioksidanttiravintoaineiden yhteyksistä tyypin 1 diabetekseen on tehty vähän.

Tämä tutkimus kuuluu ravitsemusepidemiologian alaan. Sen perustana ovat epidemiologiset analyysit E-vitamiinin ja muiden antioksidanttiravintoaineiden yhteyksistä tyypin 1 diabeteksen kehittymiseen. Väitöskirjan tavoitteena oli

– selvittää onko seerumin E-vitamiinipitoisuus yhteydessä tyypin 1 diabeteksen esiasteen tai kliinisen tyypin 1 diabeteksen riskiin;
– selvittää onko äidin raskaudenaikainen E-vitamiinin ja muiden antioksidanttiravintoaineiden saanti tai runsaasti näitä ravintoaineita sisältävien ruokien käyttö yhteydessä tyypin 1 diabeteksen esiasteen riskiin lapsella; sekä
– tarkastella antioksidanttiravintoaineiden saantiin sekä runsaasti antioksidantteja sisältävien ruokien käyttöön vaikuttavia sosiodemografisia ja elämäntyyliin kytkeytyviä taustamuuttujia suomalaisilla odottavilla äideillä

Tyypin 1 diabeteksen esiaste on tässä työssä määritelty siten että se edellyttää toistettua positiivisuutta saarekesoluvasta-aineille (ICA) sekä vähintään yhdelle kolmesta muusta tutkitusta autovasta-aineesta ja/tai kliinistä tyypin 1 diabetesta.

Tutkimuksessa käytettiin kahden suomalaisen kohorttitutkimuksen aineistoja. ’Lasten diabetes Suomessa’ (DiMe)- tutkimuksen kohortti koostui diabetekseen sairastuneiden lasten terveistä sisaruksista. ’Diabeteksen ennustaminen ja ehkäisy’ (DIPP) -projekti oli tyypin 1 diabetekselle geneettisesti alttiiden lasten syntymäkohorttitutkimus. Seerumin E-vitamiinipitoisuuden ja kliinisen tyypin 1 diabeteksen riskiä tutkittiin DiMe-tutkimuksen aineistossa käyttäen kahta erillistä tutkimusasetelmaa. Upotetussa tapaus-verrokkitutkimuksessa diabetekseen sairastuneiden lasten (n = 16) α-tokoferolipitoisuuksia verrattiin kaltaistettujen verrokkien pitoisuuksiin (n = 81). Toinen tutkimusasetelma oli kohorttitutkimus, jossa analysoitiin α-tokoferolipitoisuuden yhteyttä kliinisen diabeteksen riskiin autovasta-ainepositiivisilla lapsilla (n = 80). Seurannan aikana 26 lasta sairastui diabetekseen. Tilastollisena mallina käytettiin upotetussa tapaus-verrokkitutkimuksessa ehdollista logistista regressiomallia ja kohorttiasetelmassa Coxin regressiomallia.

Seerumin α- ja γ-tokoferolipitoisuuden yhteyttä tyypin 1 diabeteksen esiasteeseen tutkittiin DIPP-tutkimuksen kohortissa upotettuna tapaus-verrokkitutkimuksena. Aineistossa oli 108 tapausta ja 216 kaltaistettua verrokkia, joilta oli käytettävissä vähintään yksi seeruminäyte. Seeruminäytteitä oli kerätty yhden vuoden iästä alkaen vuoden välein, ja kultakin tapaus-verrokkiryhmältä analysoitiin kaikki näytteet tapauksen serokonversioikään saakka. Tapausten ja verrokkien E-vitamiinipitoisuuksia verrattiin ehdollisen logistisen regressiomallin avulla.

Äitien raskaudenaikaista ruokavaliota ja sen yhteyksiä lapsen esidiabetekseen tutkittiin DIPP-tutkimuksen aineistossa käyttäen kohorttiasetelmaa. Aineisto koostui autovasta-aineseurantaan osallistuneista lapsista ja heidän äideistään. Äitien raskaudenaikaista ruokavaliota tutkittiin validoidulla ruoankäyttökyselylomakkeella (food frequency questionnaire). Seurannan aikana 165:lle kohortin 4 297 lapsesta ilmaantui tyypin 1 diabeteksen esiaste tai kliininen tauti. Äidin ruoankäytön ja antioksidanttien saannin yhteyksiä lapsen esidiabetekseen analysoitiin paloittain eksponentiaalisella eloonjäämismallilla. Sosiodemografisten ja elämäntyyliin kytkeytyvien muuttujien yhteyksiä antioksidanttien saantiin tutkittiin lineaarisella regressiomallilla, ja niiden yhteyksiä ruoankäyttöön logistisella regressiomallilla. Aineistoon kuului 3 730 odottavaa äitiä.

Seerumin α-tokoferolipitoisuudella todettiin olevan kääntеinen, tilastollisesti melkein merkitsevä yhteys tyypin 1 diabeteksen riskiin diabetekseen sairastuneiden lasten terveistä sisaruksista muodostuvassa aineistossa sekä upotetussa tapaus-verrokkiasetelmassa ($p = 0{,}08$) että kohorttiasetelmassa ($p = 0{,}09$), kun mahdolliset sekoittavat tekijät olivat mukana mallissa. Seerumin α- tai γ-tokoferolipitoisuus ei ollut yhteydessä esidiabeteksen riskiin. Äidin raskaudenaikainen E-vitamiinin, retinolin, β-karoteenin, C-vitamiinin, seleenin, sinkin tai mangaanin saanti tai runsaasti näitä ravintoaineita sisältävien ruokien käyttö ei myöskään ollut yhteydessä lapsen esidiabetekseen. Raskaudenaikaisessa antioksidanttien saannissa esiintyi huomattavaa vaihtelua, ja kaikilla tutkituilla sosiodemografi-

silla ja elämäntyyliin kytkeytyvillä muuttujilla (ikä, aikaisempien synnytysten lukumäärä, oma ja puolison koulutustaso, tupakointi raskauden aikana, painoindeksi ensimmäisellä neuvolakäynnillä) oli itsenäinen yhteys antioksidanttien saantiin. Nuori ikä, alhainen koulutustaso ja raskaudenaikainen tupakointi olivat tärkeimmät alhaista antioksidanttiravintoaineiden saantia ennustavat tekijät.

Tutkimuksen tulokset antavat varovaista tukea hypoteesille E-vitamiinin tyypin 1 diabetekselta suojaavasta vaikutuksesta, mutta mahdollinen yhteys tulee vielä varmistaa lisätutkimuksin. Väitöskirjan tulosten perusteella ei näytä siltä, että E-vitamiini suojaisi diabeteksen esiasteelta. Seuraavassa vaiheessa tulisi tutkia ravinnosta saadun E-vitamiinin yhteyttä tyypin 1 diabeteksen riskiin. Pitkäaikaista E-vitamiinin saantia luotettavasti kuvaavien menetelmien kehittämiseksi tarvitaan lisää metodologisia tutkimuksia. Äidin raskaudenaikainen antioksidanttien saanti ei ollut yhteydessä lapsen esidiabetekseen. Yhteyden puuttuminen saattaa johtua siitä, että lapsen antioksidanttistatus on riippuvainen äidin raskaudenaikaisesta saannista vain lyhyen ajan syntymän jälkeen. Seuraavaksi tulisi sen vuoksi tutkia lapsen oman antioksidanttien saannin yhteyttä tyypin 1 diabetekseen ja sen esiasteeseen.

Avainsanat: E-vitamiini, antioksidantit, lapset, raskaus, tyypin 1 diabetes, autovasta-aineet, epidemiologia, ravitsemus

CONTENTS

LIST OF ABBREVIATIONS

ABIS	All Babies in Southeast Sweden
ALX	Alloxan
BB(-DP) rats	BioBreeding (diabetes-prone) rats
BB-DR rats	BioBreeding diabetes-resistant rats
BMI	Body mass index
CDC	Centers for Disease Control
CI	Confidence interval
CV	Coefficient of variation
DAISY	Diabetes Autoimmunity Study in the Young
DASP	Diabetes Autoantibody Standardization Program
DiMe	Childhood Diabetes in Finland -study
DIPP	Type 1 Diabetes Prediction and Prevention -project
FAD	Flavin adenine dinucleotide
FFQ	Food frequency questionnaire
GADA	65 kD isoform of glutamic acid decarboxylase
HLA	Human leukocyte antigen
HPLC	High pressure liquid chromatography
HR	Hazard ratio
IAA	Insulin autoantibodies
IA-2A	Islet antigen 2 autoantibodies
ICA	Islet cell autoantibodies
ip	intraperitoneal
IPGTT	Intraperitoneal glucose tolerance test
ISCED	International standard classification of education
iv	intravenous
IVGTT	Intravenous glucose tolerance test
JDF	Juvenile Diabetes Foundation
ln	natural logarithm
log2	base-2 logarithm
MHC	major histocompatibility complex
NADPH	Nicotinamide adenine dinucleotide phosphate
NOD mice	Nonobese diabetic mice
NS	Non-significant
OR	Odds ratio
SD	standard deviation
STZ	Streptozotocin
T1D	Type 1 diabetes

LIST OF ORIGINAL CONTRIBUTIONS

This doctoral dissertation is based on the following original contributions referred to in the text by their Roman numerals (I–V). In addition to the material presented in the original articles, some previously unpublished data are presented in this dissertation.

I Uusitalo L, Knip M, Kenward MG, Alfthan G, Sundvall J, Aro A, Reunanen A, Åkerblom HK, Virtanen SM; Childhood Diabetes in Finland Study Group. Serum alpha-tocopherol concentrations and risk of type 1 diabetes mellitus: a cohort study in siblings of affected children. J Pediatr Endocrinol Metab 2005;18:1409–16.

II Uusitalo L, Nevalainen J, Niinistö S, Alfthan G, Sundvall J, Korhonen T, Kenward MG, Oja H, Veijola R, Simell O, Ilonen J, Knip M, Virtanen SM. Serum α- and γ-tocopherol concentrations and risk of advanced beta cell autoimmunity in Children With HLA-conferred susceptibility to type 1 diabetes mellitus. Diabetologia 2008;51:773–80.

III Virtanen SM, Uusitalo L, Kenward MG, Nevalainen J, Uusitalo U, Kronberg-Kippilä C, Ovaskainen ML, Arkkola T, Niinistö S, Hakulinen T, Ahonen S, Simell O, Ilonen J, Veijola R, Knip M. Maternal food consumption during pregnancy and later risk of advanced β-cell autoimmunity in the offspring. *Submitted.*

IV Uusitalo L, Kenward MG, Virtanen SM, Uusitalo U, Nevalainen J, Niinistö S, Kronberg-Kippilä C, Ovaskainen ML, Marjamäki L, Simell O, Ilonen J, Veijola R, Knip M. Intake of antioxidant vitamins and trace elements during pregnancy and risk of advanced β-cell autoimmunity in the child. Am J Clin Nutr 2008;88:458–64.

V Uusitalo L, Uusitalo U, Ovaskainen ML, Niinistö S, Kronberg-Kippilä C, Marjamäki L, Ahonen S, Kenward MG, Knip M, Veijola R, Virtanen SM. Sociodemographic and lifestyle characteristics are associated with antioxidant intake and the consumption of their dietary sources during pregnancy. Public Health Nutr 2008;11:1379–88.

1 INTRODUCTION

Antioxidants have received considerable attention both in scientific research and in the popular media in recent decades. They are believed to protect the human body from the adverse effects of free radicals, highly reactive compounds of oxygen and nitrogen (Fang et al. 2002). Free radicals are part of the normal physiological functions of the body, but at high concentrations they are harmful. A complicated antioxidant defense system exists in the cells to keep the concentrations of free radicals at a non-harmful level. The defense system includes numerous endogenously synthesized compounds, but several dietary factors are also essential for antioxidant protection. Trace elements selenium, zinc, copper and manganese, needed as cofactors of antioxidative enzymes, must be supplied by the diet (reviewed in Halliwell 1996, Stahl and Sies 1997, Chan et al. 1998, Clark 2002). Diet also provides nutrients that react directly with free radicals in a noncatalytic manner (Halliwell 1995). Of these, vitamin E, vitamin C and carotenoids have attracted the widest scientific attention (Stahl and Sies 1997). In addition, non-nutrient components of the diet, such as flavonoids and catechin may have antioxidative properties (Rice-Evans et al. 1995).

Free radicals, and thus antioxidant nutrients, have been linked to the etiologies of more than a hundred human diseases (Devasagayam et al. 2004). The most prominent of these are degenerative diseases of adult age, but free radicals are also implicated in allergic disorders of childhood (Seaton et al. 1994) and type 1 diabetes, a chronic autoimmune disease with peak incidence in childhood. Inverse associations between maternal intake of antioxidant nutrients and risk of asthma or wheezing in the offspring were observed for vitamin E and zinc (Devereux et al. 2006, Litonjua et al. 2006), and selenium concentrations in cord blood were inversely associated with wheezing (Shaheen et al. 2004). These allergologic findings, together with the fetal origin hypothesis (Barker et al. 1989), provide the scientific rationale to study fetal determinants of type 1 diabetes as well. Indeed, there is some evidence that type 1 diabetes may also already be initiated during the fetal or neonatal period (Helgason and Jonasson 1981, Dahlquist and Källen 1992, Hyöty et al. 1995, Dahlquist et al. 1999, Lindberg et al. 1999, Stene et al. 2004).

Type 1 diabetes results from the destruction of insulin-excreting β-cells in the pancreatic islets. In insulitis leading to β-cell death and type 1 diabetes, islet-infiltrating macrophages release free radicals. β-cell toxic cytokines excreted by macrophages and cytotoxic T cells may also, at least partly, act via excessive formation of oxygen free radicals (Rabinovitch 1992, Rabinovitch and Suarez-Pinzon 1998). Pancreatic islet cells have been observed to be especially susceptible to the toxic effects of free radicals (Asayama et al. 1986).

Type 1 diabetes is the most prevalent chronic disease, after asthma and allergic diseases, among children in industrialized countries (Leslie and Elliott 1994). Its incidence

is increasing globally by 2–5% per year (Daneman 2006). Finland is the leading country in the incidence of type 1 diabetes (Tuomilehto et al. 1999), and the incidence in our country appears faster than a linear increase. The increase is most rapid in children aged 0–4 years (Harjutsalo et al. 2008). During the years 2005–2006, the yearly incidence of type 1 diabetes among children <15 years of age was more than 60/100,000 (Knip and Siljander 2008). Patients with type 1 diabetes need lifelong follow-up and care (Atkinson and Eisenbarth 2001). They are also prone to develop diabetes-associated complications such as retinopathy, renal dysfunction and neuropathy (Brink 2001). The risk of cardiovascular disease may be as much as 10-fold for subjects with diabetes compared with general population (Daneman 2006), and their life expectancy is shorter (Simell et al. 1996). Excess mortality among young people with diabetes has been reported even in a well-developed health care system, diabetic ketoacidosis being the most common diabetes-related cause of death (Dahlquist and Källen 2005).

The development of type 1 diabetes is affected both by genetic and environmental factors. Environmental factors may act either as triggers of the disease process, or as modifiers accelerating or decelerating its natural course. Viral infections and infant feeding are the most widely studied putative environmental risk factors, but no significant predictors of type 1 diabetes have so far been identified (Atkinson and Eisenbarth 2001, Knip et al. 2005).

The present study aims to analyze whether a higher intake of vitamin E and other antioxidant nutrients is associated with a lower risk of developing type 1 diabetes. A systematic review on the research conducted so far on vitamin E, other antioxidant nutrients relevant for the study, and type 1 diabetes is included.

2 REVIEW OF THE LITERATURE

2.1 Free radicals and the antioxidative system

Free radicals are unstable and highly reactive compounds, which may cause cell damage and death by oxidating biomolecules. Free radicals are defined as molecules with an unpaired electron in the outer orbit, but the term is often used in a broader sense to include other reactive species that lead to or result from free radical reactions (Devasagayam et al. 2004). In the present text, the term free radical is used in the latter sense. Free radicals are formed as by-products of normal aerobic metabolism. It has been estimated that up to 5% of the total oxygen consumed is converted into reactive oxygen species (Clark 2002). Concentrations of free radicals in the body may be increased by strenuous physical activity or autoimmune activation of the immune system, and by environmental factors like xenobiotics, ionizing radiation, cigarette smoke and certain pollutants (reviewed in Devasagayam et al. 2004, Fang et al. 2002).

Free radicals are part of the normal physiological functions of the body. They take part in signal transduction (Lander 1997), cell homeostasis (Devasagayam et al. 2004), antimicrobial defenses (Clark 2002) and in gene transcription (Zheng and Storz 2000). At high concentrations, free radicals and related reactive species have deleterious effects. They may damage all the major biomolecules essential for cell structure and function. Cell membranes are susceptible to lipid peroxidation (Devasagayam et al. 2004). Oxidation of proteins may lead to their fragmentation, aggregation and degradation (Davies 1993). Oxidized proteins may gradually accumulate in the cells, and contribute to the adverse effects of aging (Stadtman 1992). Oxidative damage to DNA can result in mutagenesis and carcinogenesis, and oxidation of carbohydrate molecules leads to structural alterations (Devasagayam et al. 2004). Considering the multiple biological effects of free radicals in the cell, it is not surprising that they have been linked to the etiologies of a wide variety of diseases, including cancer, cardiovascular diseases, complications of diabetes, sepsis, eye diseases such as cataract and macular degeneration, and neurologic conditions (reviewed in Clark 2002).

Stahl and Sies (1997) have presented an excellent review of the formation of free radicals. Among the most reactive oxygen species are hydroxyl radical, superoxide anion radical, hydrogen peroxide and singlet oxygen. Superoxide radicals are formed during normal energy metabolism by one-electron reduction of oxygen. Superoxide reacts with nitric oxide radical to form peroxynitrite and further other reactive nitrogen species, or is converted into hydrogen peroxide in a reaction catalysed by superoxide dismutase. Hydrogen peroxide is converted into water by catalase and glutathione peroxidase, or

transforms into hydroxyl radical in a metal-catalysed reaction. Hydroxyl radicals have the shortest half-life among the reactive oxygen and nitrogen species, and react immediately with the surrounding target molecules.

A complicated antioxidant defense system exists in the cells to keep the concentrations of free radicals at a non-harmful level. Components of the antioxidant system may be classified into antioxidative enzymes, free radical scavenging dietary factors, endogenous non-enzymatic antioxidants, and compounds with indirect antioxidative properties. Antioxidative enzymes superoxide dismutase, catalase and glutathione peroxidase are directly involved in the detoxification of free radicals via catalytic action (Stahl and Sies 1997). Enzymes are synthetized by the organism, but the trace elements needed as cofactors must be supplied by the diet. Selenium is a cofactor in glutathione peroxidase (Chan et al. 1998). The superoxide dismutase present in the cytosol contains zinc and copper, while the mitochondrial form of superoxide dismutase contains manganese (Fridovich 1986).

In addition to trace elements that serve as cofactors of antioxidative enzymes, the diet also provides nutrients that react directly with free radicals in a noncatalytic manner (Halliwell 1995). Of these, vitamin E, vitamin C and carotenoids have attracted the widest scientific attention (Stahl and Sies 1997). Vitamin E is a fat-soluble vitamin of plant origin, which consists of eight structurally related compounds (Hensley et al. 2004). The principal vitamers both in the diet and in human tissues are α- and γ-tocopherol (Wagner et al. 2004). Vitamin E is probably the most efficient antioxidant in the lipid phase (Burton et al. 1982). Membrane lipids are susceptible to a chain reaction initiated by free radicals, in which lipid peroxyl radicals react with other lipid molecules to form new lipid radicals. Vitamin E acts as a chain-breaking antioxidant, since it is able to prevent or slow down the propagation of free radical reactions in the cell membranes (reviewed in Halliwell 1996, Devasagayam et al. 2004). Vitamin E also quenches singlet oxygen and reacts with peroxynitrite (Stahl and Sies 1997).

Vitamin C is a powerful water-soluble antioxidant, which is present in the organism at high concentrations. It is able to scavenge superoxide anion radical, hydrogen peroxide, hydroxyl radical, singlet oxygen, and nitrogen oxide species (reviewed in Stahl and Sies 1997). Vitamin C also regenerates tocopheroxyl radicals back to the reduced tocopherol form (Stahl and Sies 1997), and may react with air pollutants in the respiratory tract (Halliwell 1996).

Carotenoids are natural lipophilic pigments, which exist in more than 600 structurally different forms. Carotenoids are classified into carotens and xanthophylls according to their chemical composition. Some of the carotenoids serve as precursors of vitamin A. β-carotene is the major provitamin A in the Western diet (Stahl and Sies 2005), and is the most widely studied of the carotenoids (Clark 2002). Carotenoids are effective scavengers of singlet oxygen, and contribute to the defense against lipid peroxidation by scavenging

peroxyl radicals (reviewed in Stahl and Sies 2005). Preformed vitamin A – retinol and its derivatives retinaldehyde and retinoic acid – is less well known as an antioxidant than its precursor β-carotene, but it is able to trap peroxyl radicals, and acts as a chain-breaking antioxidant in lipid membranes (Livrea and Tesoriere 1998).

Diet also contains non-nutrient components that may have antioxidative effects. For instance, phenolic and polyphenolic substances like flavonoids and catechin are potent antioxidants (Fang et al. 2002). Flavonoids are able to scavenge superoxide, hydroxyl radical and peroxyl radicals. However, their plasma concentrations tend to remain low, and they are rapidly metabolized. Evidence of *in vivo* antioxidant activity of flavoinoids is scarce (Halliwell et al. 2005).

Free radicals are also scavenged by several endogenously synthesized, non-enzymatic compounds. These include glutathione (Stahl and Sies 1997), uric acid, bilirubin and ubiquinol (Livrea and Tesoriere 1998).

In addition, numerous compounds are indirectly involved in the antioxidant defense system. Enzymes and their cofactors are needed to restore endogenous antioxidant levels, and proteins that bind iron and copper prevent the formation of hydroxyl radicals (Stahl and Sies 1997). For example, selenoenzyme thioredoxin reductase catalyses the regeneration of ascorbic acid (Dodig and Cepalak 2004), and transferrin binds iron (Clark 2002). Nicotinamide and riboflavin are indispensable components in NADPH and FAD respectively, which are cofactors of glutathione reductase (Fang et al. 2002). Reduced glutathione recycles vitamin E (Clark 2002). Magnesium, in turn, is needed to regenerate NADPH (Fang et al. 2002).

Accordingly it is evident that the antioxidative defense of the organism consists of a complex network of nutrients and endogenously synthesized components. The antioxidative action of a single substance is dependent on its interplay with other components of the system. Furthermore, antioxidants may also have pro-oxidant effects depending on their chemical and physical environment. For instance, β-carotene may act as a pro-oxidant in high oxygen tension (Clark 2002), and vitamin E if the concentration of vitamin C, reduced glutathione or NADPH is inadequate. Vitamin C acts as a pro-oxidant in the presence of transition metals iron and copper (Clark 2002).

Free radicals, and thereby antioxidant nutrients, have been linked to the etiologies of more than a hundred human diseases (Devasagayam et al. 2004). The most prominent of these are degenerative diseases of adult age, but free radicals are also implicated in type 1 diabetes, an autoimmune disease with peak incidence in childhood.

2.2 Type 1 diabetes and its natural course

Type 1 diabetes results from an autoimmune destruction of insulin-producing β-cells of the pancreatic islets. The initiation of the disease process is believed to result from interplay between genetic predisposition, immunological dysfunction and environmental triggers. There is a preclinical phase of several months or years, which is characterized by the appearance of autoantibodies to islet cell antigens, insulitis, and a gradual loss of β-cells. As the disease progresses, these are accompanied by progressive loss of insulin release and impaired glucose tolerance. Overt type 1 diabetes is diagnosed on the basis of hyperglycemia and specific autoantibody assays (Atkinson and Eisenbarth 2001, Babaya et al. 2005). It is estimated that 80–90% of β-cells have been destroyed at the time of diagnosis (Knip 1997). The final stage is usually a nearly complete loss of insulin secretion (Babaya et al. 2005).

There are at least 10 gene loci associated with type 1 diabetes (Todd et al. 2007). The most prominent risk genes are the major histocompatibility complex (MHC) class II genes on chromosome 6, which are estimated to account for about 45% of genetic susceptibility to the disease. These genes encode molecules expressed on the surface of antigen presenting cells involved in the presentation of antigenic peptides to T lymphocytes. Besides gene alleles associated with an elevated risk of type 1 diabetes, protective alleles also exist (Atkinson and Eisenbarth 2001). In recent decades, the proportion of high-risk genotypes has decreased, and the proportion of protective genotypes has increased among children diagnosed with type 1 diabetes, which suggests that the role of environmental factors as determinants of the disease risk has become relatively more important (Hermann et al. 2003). Within the HLA region, the alleles in the DQB1 locus are important predictors of type 1 diabetes. Their effect ranges from strongly disposing (*0302 and *0201) to strongly protective (*0602). Interactions have been observed between different loci within the HLA region. For instance, haplotypes containing alleles DRB1*04–DQB1*0302 and DRB1*03–DQB1*0201 have a strongly synergistic effect on the risk of type 1 diabetes. Other genes known to be associated with the risk of type 1 diabetes are the insulin gene region, CTLA4 locus and PTPN22 locus (reviewed in Onengut-Gumuscu and Concannon 2005).

The emergence of autoantibodies against islet cell antigens is the first indication of the abnormal activation of the immune system. However, β-cell destruction is thought to be mediated by autoreactive T-cells, and humoral autoimmunity is not assumed to be involved in the initial β-cell damage (Babaya et al. 2005, Daneman 2006). There are four autoantibody specificities predictive of type 1 diabetes. Autoantibodies against insulin (IAA) are often the first autoantibodies that appear in genetically susceptible children (Knip 2002). GADA are autoantibodies to 65 kD isoform of enzyme glutamic

acid decarboxylase (Baekkeskov et al. 1990). IA–2A are autoantibodies to 37 and 40 kD fragments of islet antigen 2, which belongs to protein tyrosine phosphatases (Christie et al. 1990, Rabin et al. 1994). IA–2A are strong predictors of the risk of type 1 diabetes. They are more frequent at clinical onset under 20 than at 20–40 years (Gorus et al. 1997). GADA and IA–2A tend to be persistent (Decochez et al. 2000). Islet cell autoantibodies (ICA) bind to a heterogenous group of cytoplasmic components of islet cells, including GAD65, IA–2 and several unknown antigens (Knip 2002).

The risk of progression from pre-type 1 diabetes to overt disease increases with the number of autoantibodies in the serum. It seems that positivity to one autoantibody is often a harmless and reversible state, whereas the presence of two or more autoantibodies indicates a progressive and irreversible process (Knip 2002). In a cohort of siblings of children with newly diagnosed type 1 diabetes, the risk of progression to overt type 1 diabetes during the subsequent years of those with two or more autoantibodies has been about 55–70% (Kulmala et al. 1998, Kimpimäki et al. 2000, Mrena et al. 2003a). The risk of progression seems to be higher among those with an increased genetic susceptibility (Mrena et al. 2003b). Preclinical type 1 diabetes may be classified into stages according to the number of autoantibodies, where one autoantibody represents early, two autoantibodies advanced, and three or more autoantibodies late prediabetes (Mrena et al. 2003a).

Environmental factors may act either as triggers of β-cell destruction, or they may modify the rate of its progression. Their effect may depend on the timing and quantity of the exposure. At present, no significant predictors of type 1 diabetes have yet been identified among the environmental factors (Atkinson and Eisenbarth 2001). Suspected candidates have been viral infections, e.g. enteroviruses and congenital rubella infection, infant feeding, especially early introduction of cow's milk or cereal protein, toxins like nitrosoamines, increased linear growth and obesity (reviewed in Atkinson and Eisenbarth 2001, Virtanen and Knip 2003, Babaya et al. 2005, Daneman 2006, Gillespie 2006). According to the hygiene hypothesis, childhood infections and other immunological challenges may protect against allergies and autoimmune diseases (reviewed in Daneman 2006). Among dietary factors, breastfeeding, nicotinamide, zinc and vitamins C, D and E have been proposed as potential protective factors against the development of type 1 diabetes (reviewed in Virtanen and Knip 2003).

2.3 Vitamin E, other selected antioxidant nutrients and the development of type 1 diabetes: a systematic review

Earlier studies on the associations of vitamin E or other antioxidant nutrients analyzed in the present study and the development of type 1 diabetes were identified by a systematic literature search. The search strategy and the articles identified in the search are presented

in Appendix 1. The detailed inclusion and exclusion criteria for the selection of the articles are listed in Appendix 2. The basic principles were as follows:

Inclusion criteria were intake and/or biomarker of vitamin E, vitamin A, β-carotene, vitamin C, selenium, zinc or manganese as explanatory variable, and type 1 diabetes, its animal models, or a variable indicating preclinical type 1 diabetes as response variable. Exclusion criteria included cross-sectional studies comparing the intakes and/or biomarkers of diabetic and non-diabetic study subjects, and studies analyzing the associations of nutrients with complications or management of diabetes.

The systematic search identified original articles, reviews, editorials, conference abstracts and summaries. The role of review articles and editorials in the systematic review was to provide further references to be analyzed. Abstracts were excluded from the review, because they did not usually provide enough information on the details of the study. Articles in languages other than English, Finnish or Swedish were also excluded. In practice, all the included articles were in English.

Many of the articles included in the review consisted of several separate experiments, in which different animal strains, response variables, diabetes-inducing drugs or doses of antioxidants, for example, were used. Each experiment is analyzed as an independent test in the review, and therefore the number of experiments is greater than the number of articles.

2.3.1 Vitamin E and risk of type 1 diabetes

Vitamin E in animal models of spontaneous immune-mediated diabetes

Nonobese diabetic (NOD) mice and BioBreeding diabetes-prone (BB-DP) rats are widely used as animal models of type 1 diabetes. The characteristics of these breeds have been reviewed by Lang and Bellgrau (2004). Susceptibility to immune-mediated diabetes in both of these breeds originates from spontaneous mutations, and in both models T cells are involved in β-cell destruction. All NOD mice develop insulitis, and approximately 70% of females and 40% of males become overtly diabetic by the age of 30 weeks. In BB rats the diabetes incidence is similar for both sexes, and in pathogen-free conditions more than 90% of BB rats develop diabetes. Diabetes tends to appear between 60 and 100 days of age (Crisa et al. 1992).

Studies using these animal models of spontaneous immune-mediated diabetes support the hypothesis of a protective effect of high vitamin E intake. The search procedure identified 11 experiments in which the animal strain was either BB rat (seven experiments) or NOD mouse (four experiments) and the endpoint was diabetes. An inverse association between vitamin E intake and the incidence of diabetes was observed in six of these experiments,

of which BB rats were used in four experiments (Behrens et al. 1986, Scott 1986, Flechner et al. 1990, Murthy et al. 1992), and NOD mice in two (Hayward et al.1992). In the report by Behrens and coworkers (1986), the protective effect was of borderline significance. In another experiment, vitamin E delayed the onset of diabetes in NOD mice, although it did not significantly reduce the overall incidence (Beales et al. 1994).

In five experiments, other substances were provided along with vitamin E (Elliott 1989, Kolb et al. 1989, Flechner et al. 1990, Murthy et al. 1992). A protective effect was observed in four of the six experiments with only vitamin E tested (Behrens et al. 1986, Scott 1986, Hayward et al. 1992, Beales et al. 1994). In most studies, vitamin E concentration in feed (Behrens et al. 1986, Scott 1986, Hayward et al.1992, Murthy et al. 1992) or drinking water (Elliott 1989) was close to 1 g/kg. Assuming an average daily comsumption of 10 g feed/rat and an average weight of 150 g (Murthy et al. 1992), the daily vitamin E intake in supplemented rats tended to be about 67 mg/kg body weight. For comparison, the average vitamin E intake of Finnish men is about 10 mg/day (Paturi et al. 2008), yielding about 0.14 mg/kg body weight in a 70 kg male. A protective effect was reported in four of these seven experiments. Flechner et al. (1990) supplied a dose of 200 IU (=200 mg synthetic α-tocopherol acetate) twice per day along with other nutrients, and found no independent protective effect of vitamin E. In addition to a protective effect of vitamin E supplementation, Hayward et al. (1992) reported a protective effect of a vitamin E deficient diet as well.

Either a standard or low vitamin E diet was used as a control for high vitamin E supply. There were seven experiments in which high vitamin E intake was compared to a standard diet (Elliott 1989, Flechner et al. 1990, Hayward et al. 1992, Murthy et al. 1992, Beales et al. 1994), and a protective effect was observed in four of these. Standard rodent chow contains about 0.03–0.08 g vitamin E/kg (Rao 1997). When a low vitamin E concentration of 0.02 g/kg feed was used as a reference (Behrens et al. 1986, Scott 1986), a protective effect was reported in two of the three experiments. In the work by Kolb and collaborators (1989), the vitamin E concentration of the reference diet was not reported. The results of the experiments did not seem to differ systematically according to the number of animals or the length of follow-up.

Because of the small number of studies, it is difficult to assess whether vitamin E supplementation protects against insulitis. The literature search yielded only four experiments on this topic. It seems possible that vitamin E has less effect on the development of insulitis than on progression to diabetes. In two experiments in NOD mice, high vitamin E intake did not protect against insulitis (Hayward et al. 1992, Beales et al. 1994). Neither was vitamin E deficient diet associated with insulitis (Hayward et al. 1992). However, in BB rats receiving a high vitamin E diet insulitis was less common than in the low vitamin E group (Behrens et al. 1986).

Vitamin E in drug-induced diabetes

Diabetes in rats and mice can be induced by the drugs streptozotocin (STZ) and alloxan (ALX). A single high dose of STZ kills the β-cells, while multiple low doses cause autoimmune diabetes by inducing inflammation (Rossini et al. 1977). The effect of vitamin E on the development of drug-induced diabetes was assessed in three reports (Slonim et al. 1983, el-Hage et al. 1986, Stetinova and Grossman 2000). In all these studies a single dose of either STZ or ALX was used to induce the disease, and none of the authors stated explicitly whether the study was intended to model type 1 diabetes.

In two experiments with ALX-induced diabetes in mice, hyperglycemia was employed as the endpoint variable. In the study by el-Hage et al. (1986), mean blood glucose concentrations were lower in mice given supplementary vitamin E prior to ALX than in those not receiving vitamin E. In the report by Stetinova and Grossman (2000), blood glucose concentrations were also lower in vitamin E-treated mice, but not significantly so. Intravenous glucose tolerance tests were used in five experiments, all on rats (Slonim et al. 1983). Diabetes was induced by STZ in four experiments, and by ALX in one. A protective effect of vitamin E was observed in all five experiments. Rats receiving intraperitoneal injections of vitamin E had lower glucose concentrations in response to a glucose load (four experiments). Rats fed a vitamin E and selenium deficient diet showed higher glucose concentrations in the glucose tolerance test than rats receiving a standard diet (Slonim et al. 1983). In the same study, vitamin E supplementation protected against islet cell damage. The vitamin E doses in these experiments with drug-induced diabetes ranged from a single dose of 10 mg/kg (Stetinova and Grossman 2000) to 400 U (approximately equivalent to 400 mg) divided into three daily doses (Slonim et al. 1983). Vitamin E supplementation was administered orally in el-Hage et al. (1986), and intraperitoneally in the other experiments.

Other animal models assessing the effects of vitamin E on diabetes-related parameters

The effects of vitamin E on islet cells were assessed in normal laboratory rats without using drugs to induce diabetes. Asayama et al. (1986) tested the glucose tolerance and insulin secretory capacity in vitamin E or selenium-deficient rats. Insulin secretory capacity was reduced in vitamin E-deficient rats, and glucose tolerance was lower in rats with combined deficiency.

Epidemiologic studies on vitamin E and type 1 diabetes

Epidemiologic evidence on the associations of vitamin E and risk of type 1 diabetes was scarce. The systematic literature search identified only three studies besides those included in the present study. An inverse association between serum α-tocopherol concentrations and risk of type 1 diabetes was reported in a cohort of Finnish adult men (Knekt et al. 1999). The

odds ratio (OR) for developing type 1 diabetes was 0.12 (95% CI 0.02–0.85) in the highest third of α-tocopherol concentration compared to the lowest third. The number of affected cases was 19, and their mean age at diagnosis was 26 years. The cases were diagnosed with type 1 diabetes 4–14 years after the serum sample had been drawn. In an ecological study in 11 European countries, vitamin E intake of adolescents was not associated with the incidence of type 1 diabetes. However, there were differences in the dietary assessment methods employed in different countries, so the results are not necessarily comparable (Thorsdottir and Ramel 2003). In addition, α-tocopherol concentrations were studied in a cross-sectional setting (Leinonen et al. 1998). Plasma α-tocopherol concentrations of children and adolescents with type 1 diabetes-associated autoantibodies tended to be slightly lower than those of unaffected controls, but the difference was not statistically significant.

2.3.2 Other antioxidant nutrients and risk of type 1 diabetes

Vitamin A and ß-carotene

Data on the associations of vitamin A and diabetes incidence were scarce, and the results were contradictory. There were four studies on animal models, with altogether nine experiments. In five of these nine experiments a protective effect of vitamin A against diabetes was seen (Chertow et al. 1989, Zunino et al. 2007). In two experiments there was no effect (Chertow et al. 1989, Lu et al. 2000), whereas in two experiments vitamin A deficient diet was protective (Driscoll et al. 1996). Five of the experiments come from the study by Chertow et al. (1989). In that study, the response variable used was blood glucose levels in diabetic animals, reflecting the severity of the drug-induced diabetes in rats. Vitamin A was administered as a single intraperitoneal or intravenous injection immediately after STZ or ALX. A protective effect was observed in four of these five experiments. In the other four experiments vitamin A was provided in the diet. Diabetes-prone BB rats were used in three experiments (Driscoll et al. 1996, Lu et al. 2000), and NOD mice in one (Zunino et al. 2007). A protective effect was observed in only one model of spontaneous immune-mediated diabetes (Zunino et al. 2007).

The effect of vitamin A on the development of insulitis was assessed in two experiments on spontaneous immune-mediated diabetes, again with inconsistent results: a deficient diet was protective in BB rats (Driscoll et al. 1996), whereas vitamin A supplementation was protective in NOD mice (Zunino et al. 2007). In diabetes-resistant BB (BB-DR) rats, vitamin A supply was not associated with insulitis (Driscoll et al. 1996). BB-DR rats are a resistant substrain of BB rats (Crisa et al. 1992).

Other types of data on vitamin A and type 1 diabetes are also rare. In an *in vitro* experiment in a rat pancreatic β-cell line, retinoic acid prevented cytokine-induced β-cell destruction (Kang et al. 2004). Serum retinol concentration was not associated with type 1 diabetes in the nested case-control study by Knekt et al. (1999), but since blood concentration is a poor indicator of vitamin A intake (Hunter 1998), this finding is not very informative. Two studies were published on the use of cod liver oil, which, besides vitamin D and n-3 fatty acids, is rich in vitamin A (Stene et al. 2000, Stene et al. 2003). In a case-control study in Vest-Agder county in Norway, maternal use of cod liver oil during pregnancy showed a strong inverse association with type 1 diabetes in the offspring. The association of child's own consumption of cod liver oil during the first year of life with type 1 diabetes was also inverse, although not significantly so (Stene et al. 2000). When the study was repeated in a larger nationwide case-control series, the maternal association was no longer observed, but the child's consumption during the first year of life was significantly associated with a lower risk of type 1 diabetes (Stene et al. 2003). However, as the normal diet usually provides a high supply of vitamin A (Paturi et al. 2008), vitamin A is an unlikely candidate for the possible etiological factor in cod liver oil supplements.

The systematic literature search did not identify any studies on the associations of β-carotene and the development of type 1 diabetes.

Vitamin C

In the systematic literature review, only one study was identified assessing the effect of vitamin C on the development of type 1 diabetes in laboratory animals. Al-Zuhair and Mohamed (1998) induced diabetes in rats with interferon α, and observed a protective effect of vitamin C supplementation against hyperglycemia. The authors presented the study as a model of type 1 diabetes.

Two case-control studies had been published on the associations of vitamin C intake and type 1 diabetes, one from Australia and the other from Sweden. The reported consumption of vitamin C supplements for a period of at least one month was inversely associated with diabetes (Glatthaar 1988), whereas the consumption of foods rich in vitamin C was not associated with the risk of diabetes (Dahlquist et al. 1990). In a cross-sectional study, plasma vitamin C concentrations were not associated with autoantibody status (Leinonen et al. 1998).

In addition, a number of animal studies in which dehydroascorbic acid was assessed as a diabetogenic agent have been published (Patterson 1949, Patterson 1950, Patterson 1951, Merlini and Caramia 1965, Domke and Weis 1983). However, as dehydroascorbic acid is the oxidized form of ascorbic acid, its role in the antioxidative system is likely to be quite different from that of the reducing agent ascorbic acid, and these results are therefore not presented in this review.

Selenium

The effect of selenium on the development of spontaneous immune-mediated or drug-induced diabetes was assessed in four animal experiments. NOD mice were used in two experiments (Elliott 1989, Hwang et al. 2007). In two experiments diabetes was induced by STZ either in mice (Mukherjee et al. 1998) or in rats (Barbosa et al. 2008). In Hwang et al. (2007) selenium was injected intraperitoneally, while it was supplied orally in the other three experiments. An inverse association between selenium supplementation and blood glucose concentration was observed in one study (Mukherjee et al. 1998), whereas the other studies did not indicate any association. In one experiment, vitamin E was provided along with selenium (Elliott 1989).

Futhermore, the effect of selenium deficiency was tested using normal laboratory rats without drug-induced diabetes (Asayama et al. 1986). The insulin secretory capacity was significantly lower in the selenium-deficient group, and glucose tolerance was significantly impaired in rats with combined selenium and vitamin E deficiency.

The two epidemiologic reports published did not support the hypothesis of a protective role of selenium against type 1 diabetes. In a study among Swedish children, the groundwater concentration of selenium at the place of residence was not associated with the risk of diabetes (Haglund et al. 1996). However, the intake of minerals from drinking water tends to represent only a small fraction of total intake (Stene et al. 2002), and the result does not exclude an inverse association between dietary selenium intake and type 1 diabetes. Serum selenium concentration was not associated with diabetes in the nested case-control study by Knekt et al. (1999).

Zinc

Zinc has received considerable attention in diabetes research, probably not mainly for its antioxidant properties, but because it has long been known to play an important role in insulin synthesis, storage and secretion (reviewed in Chausmer 1998, Taylor 2005). Recently, a zinc transporter protein present in the membranes of insulin secretory granules, Slc30A8, has been identified as an autoantigen in type 1 diabetes (Wenzlau et al. 2007). There were 32 animal experiments, from 15 research papers, assessing the effects of zinc supply on the development of diabetes.

Models of spontaneous immune-mediated diabetes were used in five experiments, three in diabetes-prone BB rats and two in NOD mice. A protective effect of zinc was reported in two experiments. BB rats fed a diet supplemented with 1,000 µg/g zinc had a lower incidence than a control group consuming a diet with 50 µg/g zinc (Tobia et al. 1998). The supplementation with 1,000 µg/g feed yielded a daily zinc intake of approximately 60 mg/kg body weight. The average zinc intake of Finnish men is about 14 mg/d (Paturi et

al. 2008), yielding about 0.2 mg/kg body weight in a 70 kg male. A continuous supply of zinc-enriched drinking water for parent and offspring NOD mice was inversely associated with diabetes in the offspring (Schott-Ohly et al. 2004). In the other three experiments no associations were reported. There was no significant difference in diabetes incidence of BB rats receiving either 1 μg/g or 50 μg/g zinc in diet (Tobia et al. 1998). Zinc supplementation in drinking water to the breeding pair or to their offspring only did not protect the offspring from diabetes (Schott-Ohly et al. 2004). Zinc supplementation of 180 μg/g feed along with vitamin A supplementation did not reduce the diabetes incidence in BB rats (Lu et al. 2000).

A model of drug-induced diabetes was used in 27 experiments, 20 in mice and seven in rats. STZ was used in 17 experiments, ALX in nine, and dithizone was used in one experiment (Mikhail and Awadallah 1977). An inverse association between zinc and the endpoint used (diabetes/hyperglycaemia/blood glucose concentration/blood glucose concentration in oral glucose tolerance test) was observed in 15 experiments (Awadallah et al. 1979, Yang and Cherian 1994, Apostolova et al. 1997, Ohly et al. 1998, Ohly et al. 2000, Ho et al. 2001, Schulte im Walde et al. 2002). Furthermore, a protective effect was reported in three experiments, but the statistical significance of the finding was not reported (Mikhail and Awadallah 1977, Tadros et al. 1982). A positive association was observed in an experiment comparing the effects of intraperitoneal infusion of ZnEDTA and CaEDTA on diabetes incidence (Kim et al. 2000). CaEDTA is a chelator of zinc, while ZnEDTA was used as a control reagent. In eight experiments no association was shown (Apostolova et al. 1997, Minami et al. 1999, Kim et al. 2000, Ohly et al. 2000, Ho et al. 2001). Multiple low-dose STZ treatment was employed in eight experiments (Ohly et al. 1998, Ohly et al. 2000, Ho et al. 2001), and a protective effect was observed in six of them. Zinc was provided orally in 11, and by intraperitoneal, intravenous or subcutaneous injection in 14 experiments. A protective effect was observed more often with injected than orally administered zinc (11/14 and 5/11 experiments respectively). In the majority of studies the animals received a standard rodent diet, which tends to contain approximately 50–60 μg zinc per gram (Rao 1997).

The effects of zinc on insulitis or islet cell destruction were evaluated in 15 experiments. In ten experiments, a drug-induced diabetes model was employed. ALX was applied in five experiments, STZ in four, whereas dithizone was used in one experiment. Diabetes-prone BB rats were used in two experiments, and NOD mice in one. Two experiments were conducted on Chinese hamsters without inducing diabetes by drugs (Boquist and Lernmark 1969). It was common not to test the viability of islet cells statistically; in 10 experiments an inverse association between zinc and insulitis/islet cell destruction was reported without presenting statistical significances (Mikhail and Awadallah 1977, Tadros et al. 1982, Tobia et al. 1998, Ho et al. 2001). A protective effect was observed in

one experiment (Schott-Ohly et al. 2004), and in four there was no effect (Boquist and Lernmark 1969, Tobia et al. 1998, Minami et al. 1999).

Futhermore, it has been reported that glucose tolerance was similar in zinc-deficient and zinc-supplemented normal laboratory rats without drug-induced diabetes. The number of animals was very small, however, and the association was not tested statistically (Macapinlac et al. 1966). Hove et al. (1937) reported some differences in glucose tolerance curves, but similar glucose concentrations in blood and urine of zinc-deficient and non-deficient rats. In an *in vitro* study, zinc ions induced cell death in insulinoma cells and isolated human islet cells in a dose-dependent manner (Kim et al. 2000).

The association of zinc concentration in drinking water and type 1 diabetes was analyzed in four studies. In a large Swedish analysis with nearly 3,000 cases, a significant inverse association between groundwater zinc concentration of the home parish and diabetes was observed (Haglund et al. 1996). In a case-control study from Norway with individually analyzed tap water samples, the risk of diabetes was significantly reduced in the highest quarter of tap water zinc concentration, after adjustment for potential confounding factors (Stene et al. 2002). In an ecological analysis from England, there was a non-linear association between the zinc concentration of domestic drinking water and the incidence rate of childhood type 1 diabetes, with a reduced risk in the middle third of zinc concentration (Zhao et al. 2001). In a Finnish ecological analysis, no association between groundwater zinc and childhood type 1 diabetes was observed (Moltchanova et al. 2004). However, these associations are likely to be confounded by some other factor associated with water quality, and probably do not reflect zinc intake as such (Stene et al. 2002).

Manganese

Experimental evidence on the associations of manganese and type 1 diabetes is scarce. There were four animal experiments from three reports assessing the effects of injected manganese on ALX or dithizone-induced hyperglycemia in rats. A protective effect was reported in each of these experiments (Mikhail and Awadallah 1977, Awadallah et al. 1979, Tadros et al. 1982), although the statistical significance of the finding was given in only one of the studies (Awadallah et al. 1979). In contrast, manganese injections did not prevent insulitis in the three experiments with ALX- or dithizone-induced diabetes in rats (Mikhail and Awadallah 1977, Tadros et al. 1982). Again, statistical tests were not conducted. Mikhail and Awadallah (1977) noted that the findings may indicate a hypoglycemic effect of manganese counteracting the hyperglycemic effect of the drug used to induce diabetes.

In the Norwegian case-control study already discussed in the context of zinc (Stene et al. 2002), the manganese concentration in drinking water was not associated with type 1 diabetes (Stene et al. 2002).

2.3.3 Summary

The effect of antioxidant nutrients on the development of type 1 diabetes has been studied mainly in animal models, and research has concentrated on vitamin E and zinc. Overall, evidence from animal models indicates a possible protective role of vitamin E against type 1 diabetes. Protective effects were observed in most studies, and many of them employed diabetes-prone BB rats or NOD mice. The relevance of these models of spontaneous immune-mediated diabetes is clearer than that of drug-induced diabetes models. The models of drug-induced diabetes used in vitamin E studies may not have much resemblance to human type 1 diabetes; the disease was induced by a single drug dose, which kills the β-cells without causing an autoimmune reaction.

In the case of zinc, drug-induced models of diabetes were used more often than models of spontaneous immune-mediated diabetes, and there were also experiments using multiple low doses of the drug. Protective effects were reported in many studies, and their proportion seemed to be greatest in studies with multiple low doses of a diabetes-inducing drug or with injected rather than orally administered zinc. On the whole, it seems possible that zinc also has a protective effect against type 1 diabetes.

There were a few animal experiments on the associations of vitamin A and diabetes. The results were somewhat contradictory. Protective effects were observed mainly in drug-induced models of diabetes. In two of the four experiments using models of spontaneous immune-mediated diabetes, a vitamin A deficient diet was reported to be protective. This could be explained by an immunosuppressive effect of vitamin A deficiency, as has been suggested in the case of vitamin E deficient diet (Hayward et al. 1992). Animal data on selenium and manganese are scarce. Most of the experiments did not indicate any effect of selenium on diabetes. In the case of manganese, the inverse association with blood glucose concentration reported in some studies could result from a hypoglycemic effect of manganese, and not necessarily indicate protection against diabetes.

Animal data on vitamin C and β-carotene are practically non-existent. Vitamin C was analyzed in only one animal model of diabetes, and studies on β-carotene were not identified in the systematic search.

Although vitamin E and zinc may be potential protective agents against type 1 diabetes in animal models, the findings should be viewed with caution. The results of animal experiments can not be readily extrapolated to humans. Humans and rodents are biologically quite different species, and they live in totally different environmental conditions. The baseline diets of laboratory rodents and humans differ vastly, and the doses of antioxidant nutrients supplied to the experimental animals are huge compared to the normal range of dietary intake in man. Moreover, the disease models are likely to differ from human type 1 diabetes, and may also vary by rodent strain. Therefore, the role of

animal models is mainly to serve as a starting point for epidemiologic or clinical research in humans.

Epidemiologic studies in human populations on the associations of antioxidant nutrients with type 1 diabetes are few. Vitamin E showed an inverse association with type 1 diabetes in a cohort study. Vitamin C seemed protective in one of the two case-control studies, while no inverse associations have been reported for vitamin A, β-carotene, selenium or manganese. The promising results for vitamin E and zinc from animal experiments, the scarcity of epidemiologic analyses, and the relative lack of research on other antioxidant nutrients in the context of type 1 diabetes indicated a clear need for further epidemiologic studies assessing the associations of antioxidant nutrients with the risk of type 1 diabetes.

3 AIMS OF THE PRESENT STUDY

The present study is based on epidemiologic analyses of the associations of vitamin E and other antioxidant nutrients with the development of type 1 diabetes. Besides etiological targets, the study also had the descriptive purpose of analyzing the sociodemographic and lifestyle determinants of antioxidant intake. The study aimed to answer the following specific research questions:

– Are serum vitamin E concentrations associated with the risk of preclinical or clinical type 1 diabetes?
– Is maternal intake of vitamin E and other selected antioxidant nutrients or the consumption of their dietary sources during pregnancy associated with pre-type 1 diabetes in the offspring?

In addition, the study aimed to

– Analyze the sociodemographic and lifestyle determinants of intake of antioxidant nutrients and consumption of their dietary sources among Finnish pregnant women

4 SUBJECTS AND METHODS

Although type 1 diabetes is one of the most common chronic diseases in childhood, the risk of developing the disease is small among general population. Therefore, to ensure a sufficient number of affected cases, the cohorts used for studying the risk factors of type 1 diabetes must represent individuals with an elevated risk of the disease. In the present study, answers to the study questions were searched for by exploiting data from two such data sets. Both were prospective cohorts from Finland. The first one was a nationwide cohort of unaffected siblings of children with newly diagnosed type 1 diabetes (DiMe). The other was a birth cohort of genetically susceptible children from the hospital districts of Oulu and Tampere (DIPP).

4.1 Subjects and overview of the study cohorts and design

4.1.1 Childhood Diabetes in Finland (DiMe) Study (Study I)

The Childhood Diabetes in Finland (DiMe) Study is a nationwide project which aims at evaluating the role of genetic, environmental and immunological factors in the development of type 1 diabetes (Tuomilehto et al. 1992). All newly diagnosed children with type 1 diabetes aged 14 years or under were invited to participate in the study. Recruitment began in September 1986 in 13 hospitals, and in early 1987 it expanded to cover the whole of Finland. The recruitment continued until 30 April 1989. As part of the DiMe Study, a prospective cohort was formed from unaffected siblings of children with diabetes, aged 3–19 years (n=722). The siblings entered the study at the time of the diagnosis of the affected child, and were followed-up for 2–4 years with clinic visits and blood sampling at intervals of 3 to 12 months. Siblings who tested positive either for ICA or IAA were invited to an intravenous glucose tolerance test (IVGTT) every 6 months. Information on the incident cases of clinical type 1 diabetes among the cohort was acquired from the National Central Drug Registry. The data in the register is based on approvals for free-of-charge medication for diabetes at the Social Insurance Institution (Reunanen et al. 2000).

We used two separate study designs in Study I. In a nested case-control study (study design 1), siblings who were diagnosed with type 1 diabetes by 31 December, 1998, were compared with controls who had remained unaffected and negative for diabetes-associated autoantibodies. There were 16 cases from whom at least one frozen serum sample collected prior to diagnosis was available. Alpha-tocopherol and cholesterol concentrations were measured from the earliest available serum sample. Up to six controls per each case, matched for sex, age (±12 months) and the date of the serum sample (±14 months), were

randomly picked from the cohort. There were altogether 81 controls, ranging from 1–6 per case.

The second study design in Study I (Study design 2) was a subcohort of seropositive siblings. All siblings who had IVGTT samples available for analysis (n = 80) were followed-up for type 1 diabetes from the date of the first available serum sample until 31 December, 2001. Alpha-tocopherol and cholesterol concentrations were measured from all the samples collected during follow-up. The median follow-up time was 11.9 years (range 41 days–14.7 years), and the median number of serum samples per individual was 4 (range 1–12). The intervals between the samples varied considerably between individuals. During follow-up, 26 siblings progressed to type 1 diabetes.

4.1.2 Type 1 Diabetes Prediction and Prevention Project (Studies II–V)

The Type 1 Diabetes Prediction and Prevention (DIPP) Project is a birth cohort of children with increased genetic risk of type 1 diabetes. In the university hospitals of Turku, Oulu and Tampere, cord blood samples from all newborn infants were screened for HLA-DQB1 alleles shown to be associated with risk of type 1 diabetes in Finnish population (Ilonen et al. 1996), with the informed consent of the parents. The families of children with a HLA-DQB1-conferred genetic susceptibility to type 1 diabetes (HLA-DQB1*02/0302 heterozygous and DQB1*0302/x-positive individuals, x standing for homozygosity or a neutral allele) were invited to a prospective follow-up study. The children were monitored for the emergence of type 1 diabetes-associated autoantibodies, growth, diet and viral infections with clinical visits at the age of 3, 6, 12, 18 and 24 months, and thereafter once a year.

ICA were used as the primary screening tool for β-cell autoimmunity (Kupila et al. 2001). When a child seroconverted to positivity for ICA for the first time, all his/her preceding and subsequent samples were analyzed for IAA, GADA and IA–2A, and the follow-up continued at intervals of 3 months thereafter. The endpoint variable, advanced β-cell autoimmunity, was defined as repeated positivity for ICA and at least one other autoantibody, and/or clinical type 1 diabetes.

The DIPP Nutrition Study was implemented within the framework of DIPP in the university hospitals of Oulu and Tampere. Dietary intake during pregnancy and lactation was elicited from the mothers by two separate food frequency questionnaires. Children's nutrition was monitored using a questionnaire on the introduction of new foods up to 2 years of age, 3-day food records and a structured questionnaire at the ages of 3, 6, 12, 18 and 24 months and thereafter yearly, and serum and hair samples for nutrient analyses collected at one year intervals starting from the age of one year.

The data sets used in Studies II–V are presented in Figure 1. Study II was designed

as a nested case-control study. The cases and controls came from a cohort of the at-risk children born between 1 October, 1996 and 1 July, 2004 in Oulu University Hospital and between 20 October, 1997 and 6 July, 2004 in Tampere University Hospital (n=5787, 76% of the children invited). The emergence of autoantibodies was monitored until 30 September, 2004, and clinical diabetes until 20 November, 2004. There were 119 cases with repeated positivity for ICA and at least one other autoantibody and 45 cases of overt type 1 diabetes, yielding a total number of 128 cases with advanced β-cell autoimmunity. There were 20 cases with no vitamin E samples drawn until the age of seroconversion, and these cases were excluded from the analyses. Two matched controls were selected for each case. First all possible controls who fulfilled the matching criteria were identified from the cohort. The matching criteria included birth date within 3 months, the same gender, the same hospital of birth, and the same genotype (*HLA–DQB1*02/0302* heterozygous, defined as high risk or *DQB1*0302/x*, defined as moderate risk). The controls were not allowed to come from the same family as the case. The controls could come from the same family. The control children had to be observed up to the visit when the case turned out to be a case and they had to have been seronegative and non-diabetic up to that point. From the series of all possible control children fulfilling the matching criteria, two controls were randomly selected. If no serum sample was available from the selected control for vitamin E analysis, an extra control was randomly selected. The controls of each case were selected totally independently, so that the same subject could be a control for more than one case, and a subject, who was already a control for one or more cases, could him- or herself become a case at a later point in time.

The study cohort of Studies III–IV comprised 4,297 children with genetic HLA-conferred susceptibility to type 1 diabetes (79.4% of invited) born between 20 October, 1997 and 31 December, 2002 at the university hospitals of Oulu and Tampere, who participated in the follow-up for the emergence of diabetes-associated autoantibodies and/or overt type 1 diabetes. The data included 39 twin pairs and 1 triplet. The emergence of autoantibodies was monitored until 31 August, 2006. During the follow-up, 144 children were repeatedly positive for ICA plus at least one other antibody (Table 1), and 74 had progressed to clinical type 1 diabetes, yielding a total of 165 cases with advanced β-cell autoimmunity. Dietary data were available from the mothers of 3,723 of these children (86.6%).

The data of Study V included 3,730 mothers of at-risk children born between 20 October, 1997 and 31 December, 2002 at the university hospitals of Oulu and Tampere (Table 2, Figure 2.).

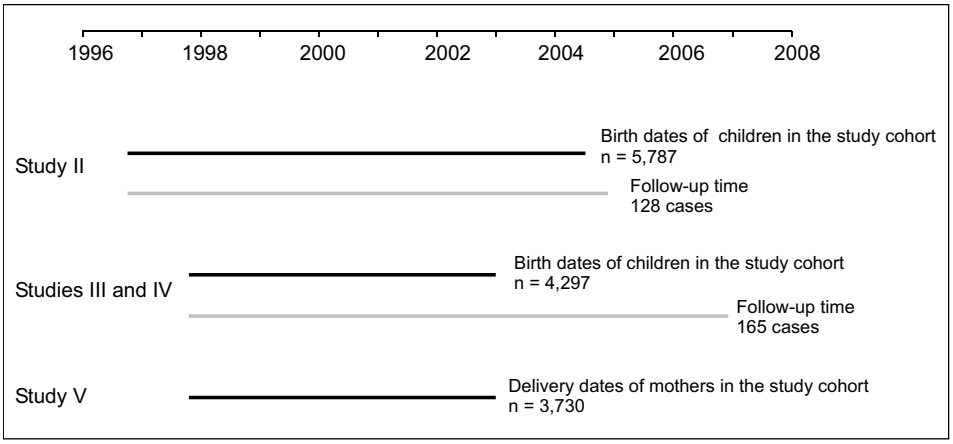


Figure 1. DIPP Nutrition Study: Illustration of the data sets of Studies II–V.

Table 1. Autoantibody specificities among cases with repeated positivity for ICA and at least one other type 1 diabetes-associated autoantibody from the birth cohort of 4,297 children with HLA-conferred susceptibility to type 1 diabetes, followed-up until August 31, 2006.

Autoantibodies detected at least twice	n	%	n (%) of reverted [1]	n (%) presenting clinical type 1 diabetes[2]
ICA + IAA	15	10.4	8 (53.3)	5 (33.3)
ICA + GADA	10	6.9	9 (90.0)	0 (0.0)
ICA + IA–2A	4	2.8	0	4 (100)
ICA + IAA + GADA	14	9.7	0	6 (42.9)
ICA + IAA + IA–2A	16	11.1	0	12 (75.0)
ICA + GADA + IA–2A	6	4.2	0	4 (66.7)
ICA + IAA + GADA + IA–2A	78	54.2	0	42 (53.8)
Other[3]	1	0.7	1 (100)	0 (0.0)
Total	144	100	18 (12.5)	73 (50.7)

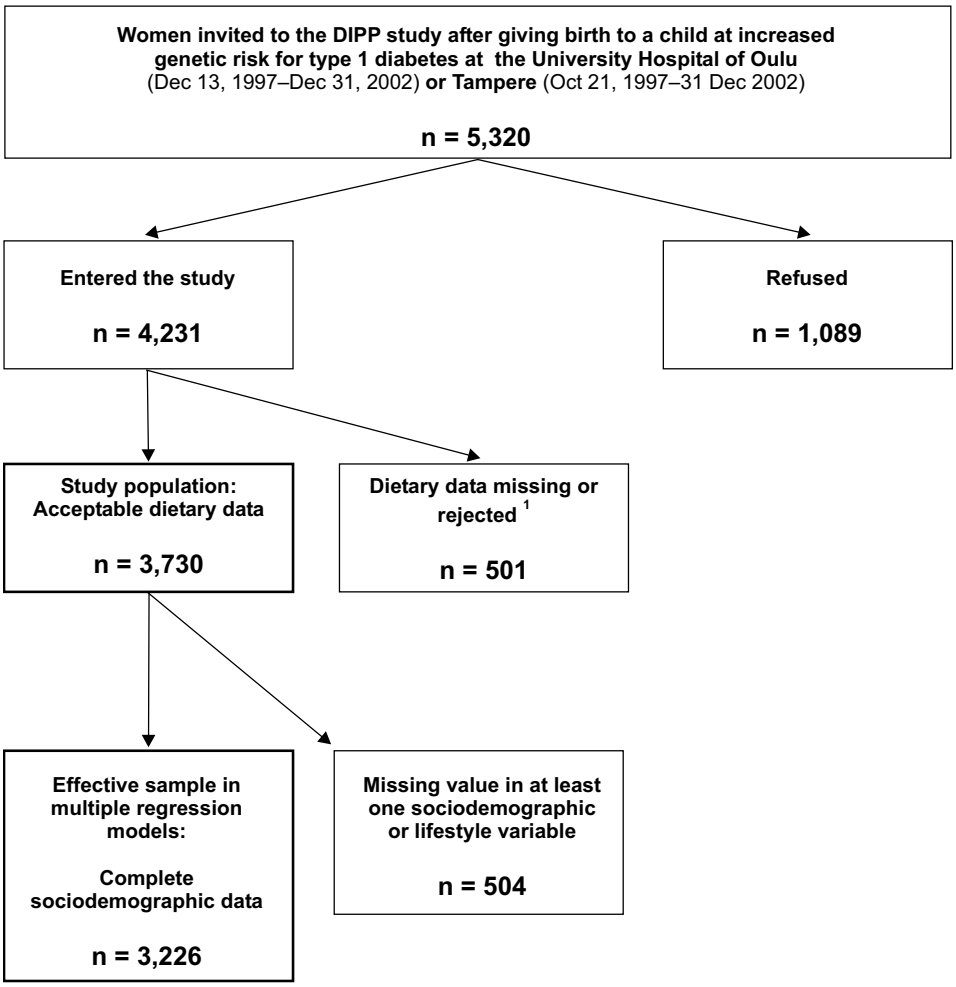
[1] Positive for ≤1 autoantibody specificity in a later serum sample, followed-up until 1.11.2007. Cases with evidently temporary reversion and cases who developed clinical type 1 diabetes excluded.

[2] Followed-up until 1.11.2007

[3] Repeated positivity for ICA, positive for IAA in one serum sample and positive for GADA in another serum sample

Table 2. Characteristics of pregnant women who delivered a child at increased genetic risk for type 1 diabetes at the University Hospital of Oulu or Tampere, Finland, 1997–2002.

Variable	% (n)
Age, years	
<25	19.3 (720)
25–29.9	34.1 (1,273)
30–34.9	29.7 (1,108)
≥35	16.8 (628)
Missing	0.0 (1)
Parity	
0	45.3 (1,691)
1	31.7 (1,181)
2	13.8 (515)
≥3	8.5 (317)
Missing	0.7 (26)
Degree of urbanization	
Urban	76.4 (2,848)
Semi-urban	9.8 (367)
Rural	13.1 (489)
Missing	0.7 (26)
Place of residence	
Region of Tampere	58.7 (2,188)
Region of Oulu	41.3 (1541)
Vocational education	
None	6.3 (236)
Vocational school or course	29.0 (1,082)
Upper secondary vocational education	40.6 (1,513)
Academic	21.4 (800)
Missing	2.7 (99)
Vocational education of the partner	
None	5.4 (203)
Vocational school or course	39.5 (1,472)
Upper secondary vocational education	29.3 (1,092)
Academic	21.1 (788)
Missing	4.7 (175)
Smoking during pregnancy	
No	86.2 (3,217)
Yes	10.3 (383)
Missing	3.5 (130)
BMI at the first antenatal visit	
<21.6	23.4 (873)
21.6–23.5	23.6 (879)
23.6–26.2	23.5 (877)
>26.2	23.5 (877)
Missing	6.0 (224)
All	100.0 (3,730)



[1] Compared with those who provided dietary data, women ≥ 35 years old, with 2 or more children, from the northern region, living in a rural community, or having no vocational education were overrepresented, and those expecting their first child or with academic education underrepresented in this group ($p \leq 0.001$)

Figure 2. DIPP Nutrition Study: Study population of pregnant mothers (Study V).

4.1.3 Ethical aspects

In the DIPP Project, newborn infants are screened for genetic susceptibility to type 1 diabetes and followed-up for the emergence of type 1 diabetes-associated autoantibodies. In the DiMe Study, the participants were siblings of children with type 1 diabetes. As there is currently no known means of prevention or cure for type 1 diabetes, questions have been raised about whether screening for the disease is ethically justified (Ross 2007). The American Diabetes Association discourages screening outside the context of defined research studies (American Diabetes Association 2003). In medical sciences, there are increasing efforts to predict and prevent diseases, instead of just curing them. Accordingly, the pathogenic process of type 1 diabetes is of great interest to researchers, and epidemiologic studies on human populations are not feasible without genetic or immunological screening (Weber and Roth 1997). The scientific advantages of screening are unquestionable, but the benefits for an individual study subject of participating the study project are less evident. Some individual benefits, however, may be expected: children who participated in a prospective follow-up study were less often hospitalized and had milder metabolic abnormalities at diagnosis (Barker et al. 2004).

On the negative side, information about an elevated risk for type 1 diabetes may have adverse psychological effects on the parents, and later in life also in the child. The psychological impact of a screening result is likely to depend on the characteristics of the disease, the rate of its progression, and the prognosis (Simonen et al. 2006). In the case of type 1 diabetes, the risk of developing clinical disease is only 3–8% according to the genotype (Nejentsev et al. 1999), while the emergence of type 1 diabetes-associated autoantibodies confers a higher risk (Kulmala et al. 1998). Psychological reactions of participating families have been assessed in the context of screening studies. Information on elevated genetic risk may cause some concern, distress or depression in the parents (Yu et al. 1999, Simonen et al. 2006, Kerruish et al. 2007), although overall anxiety or stress seem not to be increased (Yu et al. 1999, Johnson et al. 2004, Simonen et al. 2006, Kerruish et al. 2007). Increased anxiety was observed in some subgroups of mothers, but it tended to dissipate with time (Johnson et al. 2004). A positive test result for ICA antibodies induced anxiety both in the child and his or her parents (Galatzer et al. 2001, Johnson et al. 2001). Anxiety was stronger in the parents than in the child, and children's reactions were milder than those of children diagnosed with clinical type 1 diabetes (Galatzer et al. 2001). After a few months, anxiety had decreased (Galatzer et al. 2001) or subsided (Johnson et al. 2001). Even if screening results may worry the parents, they seem to think that it is good to know about the child's risk (Gustafsson Stolt et al. 2004, Simonen et al. 2006). Parents of children participating in the DIPP Study reported that they were pleased that they participated (Simonen et al. 2006).

The study design and procedures of the present study were approved by the local ethics committees. The families provided written informed consent. The families were informed about the outcome of the autoantibody tests done during the study. No individual dietary data were given to the participating families.

4.2 Methods

4.2.1 Genetic methods

Study I: The determination of genetic risk group was based on HLA-DR alleles. HLA typing was performed using conventional HLA serology (Tuomilehto-Wolf et al. 1989). All HLA A, B, C and DR specifities recognized by the Nomenclature Committee of the WHO in 1984 (Albert and Mayr 1984) were included in the test panel. HLA-DR phenotypes were grouped into three categories, in descending order according to the risk of type 1 diabetes: DR3/DR4, DR4/non DR3 or DR3/nonDR4, and nonDR3/nonDR4.

Studies II–V: The identification of children with HLA-DQB1-conferred disease susceptibility was based on a rapid genotyping method for alleles known to be associated with risk for (DQB1*0302 and DQB1*0201) or protection from type 1 diabetes (DQB1*0602 and DQB1*0301) (Ilonen et al. 1996, Kupila et al. 2001). In brief, a part of the second exon of the HLA-DQB1 gene was amplified using a primer pair with a biotinylated 3' primer. The biotinylated PCR products were then transferred to streptavidin-coated microtitration plates, denatured and hybridized with sequence-specific probes labeled with lanthanide chelates: europium (Eu), terbium (Tb) or samarium (Sm). Two hybridization mixtures were used, one containing probes hybridizing with DQB1*0602 and *0603, DQB1*0603 and *0604 and a consensus sequence, and the other containing probes specific to the DQB1*0201, *0301 and *0302 alleles. After appropriate incubations and washings, the specific hybridization products were detected using three-color time-resolved fluorescence of the lanthanide chelates.

4.2.2 Immunological methods

ICA were quantified by a standard indirect immunofluorescence method on sections of frozen human pancreas from a blood group O donor (Bottazzo et al. 1974). The end-point dilution titre of positive samples was recorded and the results expressed in Juvenile Diabetes Foundation (JDF) units. The detection limit was 2.5 JDF units. In Studies II–V, all samples initially positive for ICA were retested for confirmation. The disease sensitivity of the ICA assay in our laboratory was 100% and the specificity 98% in a standardization workshop round (Greenbaum et al. 1992).

In Study I, IAA were quantified with a radiobinding assay modified from Palmer et al. (1983), as described by Kimpimäki et al. (2000). The samples were treated with acid-charcoal beforehand to remove endogenous insulin. Polyethylene glycol was used to separate the free and bound insulin fractions after incubation for 20 h with mono-^{125}I(Tyr^{A14}) human insulin (Novo Research Institute, Bagsvaerd, Denmark). The results were expressed in nanounits per ml, where 1 nU/ml corresponds to a specific binding of 0.01%. The subject was considered to be IAA positive if the specific insulin binding exceeded 68 nU/ml (99th percentile in 102 nondiabetic children under the age of 5 years). The sensitivity of the IAA assay was 26%, and its specificity was 97% based on 140 samples included in the 1995 Multiple Autoantibody Workshop (Verge et al. 1998).

In Studies II–V, IAA were analyzed with a microassay (Williams et al. 1997, Ronkainen et al. 2001). The IAA values representing the specific binding were expressed in relative units based on a standard curve run on each plate using the MultiCalc™ software program (PerkinElmer Life Sciences Wallac, Turku, Finland). The limit for IAA positivity was set at 1.55 relative units, which represents the 99th percentile among more than 370 non-diabetic Finnish children and adolescents. The disease sensitivity of the IAA assay was 44% and specificity 98% in the Centers for Disease Control (CDC) sponsored Diabetes Autoantibody Standardization Program (DASP) Workshop 2002. Samples with IAA, values between the 97.5th and 99.5th percentiles were reanalyzed to confirm the antibody status.

GADA and IA–2A were quantified with specific radiobinding assays (Petersen et al. 1994, Savola et al. 1998a, Savola et al. 1998b). The values were expressed in relative units based on a standard curve constructed from a dilution of positive and negative samples. The limits for GADA and IA–2A positivities were set at 5.36, and 0.43 relative units respectively, representing the 99th percentile among more than 370 non-diabetic Finnish children and adolescents. The disease sensitivity and specificity of the GADA and IA–2A assays were 82% and 98%, and 62% and 100% respectively, in the CDC sponsored DASP Workshop 2002. In Studies II–V, samples with IAA, GADA, or IA–2A values between the 97.5th and 99.5th percentiles were reanalyzed to confirm the antibody status. Transplacentally transferred autoantibodies (Hämäläinen et al. 2000), i.e. autoantibodies present at birth but eliminated during infancy, were excluded from the analyses.

4.2.3 Determination of vitamin E and cholesterol concentrations

In Study I, only serum samples collected after overnight fasting in connection with the IVGTT were available from the cases, whereas fasting was not required of the controls. In Study II, nonfasting blood samples were collected by venipuncture. Samples were protected from light during processing. They were kept at room temperature for 30–60

min to clot. After centrifugation, an aliquot of at least 1 ml serum from 1-year-old children and 2 ml from those aged 2 onwards was separated and stored at –70° C prior to analysis. For the analyses in Studies I and II, samples were transported on dry ice to the National Public Health Institute. Serum α- and γ-tocopherol concentrations were measured at the Biomarker Laboratory with a micromethod on 50 µl aliquots with reversed-phase HPLC with detection by fluorescence at 292/324 nm (Anttolainen et al. 1996). The precision between series was 5.0 coefficient of variation (CV) % for α-tocopherol and 6.7 CV% for γ-tocopherol. Serum cholesterol concentrations were analyzed manually at the Laboratory of Analytical Biochemistry by Olli-C photometer (Thermo Fisher Scientific Oy, Vantaa, Finland) using an enzymatic, colorimetric (CHOD-PAP) method. The laboratory personnel were unaware of the case-control status of the samples. In Study II, each case-control group was analyzed in the same batch. Within each case-control group, the samples were in random order in relation to the case-control status.

4.2.4 Measurement of food use and nutrient intake

Dietary intake during pregnancy (Studies III–V) was assessed postnatally by a self-administered, semiquantitative 181-item food frequency questionnaire (FFQ). The FFQ was designed to assess the entire diet over the last month before the beginning of maternity leave, i.e. the eighth month of pregnancy. The FFQ was validated specifically for the target population of the DIPP Nutrition Study, as described (Erkkola et al. 2001). The mothers received the FFQ by mail, and returned it at the first visit to the Study Center when the baby was 3 months old. A trained study doctor or nurse checked the FFQ and, if required, completed it in collaboration with the mother. All the returned FFQ forms were checked by a trained nutritionist. The respondents were asked to report the frequency of use in terms of natural units (e.g. one apple), common household measures (e.g. one glass of milk) or portions (e.g. a portion of lasagne), for which typical portion sizes identified in earlier Finnish dietary studies were used in dietary calculations. The frequency part of the questionnaire included categories ‘not at all’, ‘times per month’, ‘times per week’ and ‘times per day’, and the respondent was advised to either tick the box ‘not at all’ or write down the appropriate number (times of use) in one of the other boxes. If there were ≥10 food items with missing frequency, the FFQ was rejected (n = 52, 1.4% of all FFQs). Other missing items were imputed with null, i.e. coded as non-use (Parr et al. 2008). FFQ data were entered into a data file at a commercial data entry service. Daily intake of energy and nutrients was calculated using the Finnish food composition database (National Public Health Institute 2007) by in-house software of the National Public Health Institute.

The use of dietary supplements was assessed by a separate question on the FFQ form. The respondent was asked to write down the type, brand name and manufacturer's name of all the dietary supplements used during the entire pregnancy, the amount of each supplement used per day or per week, and the weeks of pregnancy during which each supplement was used. The nutrient contents of the dietary supplements registered as drugs were obtained from the Finnish pharmacopoeia. Information on other dietary supplements was acquired from the National Food Administration, the manufacturer and/or the Internet. In Study IV, average daily intakes of antioxidant nutrients from dietary supplements during the entire pregnancy were calculated as separate variables by in-house software of the National Public Health Institute. In Study V, the supplements which contained antioxidant nutrients were grouped into the following types: (1) Vitamin A or AD supplements or cod liver oil, (2) β-carotene supplements, (3) Vitamin C supplements, (4) Vitamin E or vegetable oil supplements, (5) Multivitamin and mineral supplements, (6) Mineral supplements with manganese, selenium or zinc. However, due to the small number of users in all supplement groups except for multivitamin and mineral supplements, a combined variable representing the use of any of these types of dietary supplements was used in the statistical model.

4.2.5 Collection of sociodemographic and perinatal characteristics

Study I: Information on parental age and length of education, urbanization degree of the place of residence and number of children in the family was collected using a structured questionnaire at the time of diagnosis of the index child. Maternal education, place of residence and number of children in the family were coded as dichotomous variables of less than 13 years of education vs. 13 or more, urban vs. rural area, and one or two children vs. more.

Studies II–V: Information on general education, vocational education and age of both parents was registered by a structured questionnaire completed after the delivery. In preliminary single covariate analyses, vocational education was more closely associated with dietary factors than general education, and was used in the multiple covariate models. Information on home municipality, the number of earlier deliveries (parity), mother's height and weight measured at the first antenatal examination, mother's smoking during pregnancy, gestational age and weight and height of the newborn was obtained from the Medical Birth Registries of the Oulu and Tampere University Hospitals.

Vocational education was coded as none, vocational school or course (up to level 3 in ISCED 1997 (UNESCO 1997), upper secondary vocational education (levels 3 and 4), and academic (levels 5 and 6). Maternal age was categorized into four groups. Home municipality was coded as urban, semi-urban or rural according to the classification of Statistics Finland (2007). Body mass index (BMI) was calculated by the formula

kg/m^2. Smoking during pregnancy was registered on a three-class scale (non-smoker/quit smoking during the first trimester/smoker). As the number of quitters was small, they were aggregated with smokers.

4.2.6 Statistical methods

Tranformations and coding of the variables

Alpha-tocopherol concentrations are related to the concentrations of cholesterol and triglycerides, and should therefore be adjusted for cholesterol and/or triglycerides to avoid confounding (Hunter 1998). In Study I, α-tocopherol concentrations were adjusted for cholesterol concentrations using the residual method (Stryker et al. 1988). The distribution of cholesterol concentration was skewed, and we used ln-transformed values in the regression model. In Study II, potential confounding by cholesterol was controlled for by adding cholesterol concentration as a covariate in the etiological models. In the case-control design of Study I, cholesterol-adjusted α-tocopherol concentrations were categorized into three groups using the tertiles of controls as cut-off points. In Study II, concentrations of α- and γ-tocopherol and cholesterol were aggregated into low (values less than then 25th percentile), intermediate (within the interquartile range) and high (above the 75th percentile) values.

Several of the food groups showed clear non-normality, even after logarithmic transformation. In Study III, these food variables were used either as dichotomized or quarters in the analysis depending on the distribution. Food variables with a distribution that was normal or close to normal after logarithmic transformation were adjusted for energy by the residual method (Willett 1998), and used as continuous in the analysis. In Study V, all the food variables were coded as binary variables, using the highest quartile as the cut-off point.

The intakes of all the antioxidant nutrients from foods had distributions that were close to normal after logarithmic transformation. They were adjusted for energy intake by the residual method (Willett 1998). In Study IV, the total intake of each antioxidant was calculated by adding up the intakes from foods and supplements. The distributions of total intakes of all the antioxidants were also close to normal after logarithmic transformation. Logarithmic values were used in the statistical analyses. The total intakes were not adjusted for energy. Derived categorical variables were formed both from energy-adjusted intakes from foods and from total intakes from foods and supplements by using quartile values as cut-off points.

In energy adjustments according to the residual method, the variables are often transformed using the natural logarithm (ln) to improve normality and homoscedasticity

(Willett 1998). However, when these ln-scale residuals are used as explanatory variables in a statistical model applied to analyze the associations between dietary intake and an endpoint variable, the results may be difficult to interpret. In Studies III and IV, the interpretation of the hazard ratio (HR) produced by the survival model would have been 'the increase in risk per a 2.7-fold increase in energy-adjusted intake of the food/nutrient'. To achieve an interpretation of the hazard ratio that is intuitively more appealing, we used base-2 logarithmic ($\log_2$) transformation for the dietary variables instead of ln-transformation in Studies III and IV. When a $\log_2$-transformed food or nutrient is used as an explanatory variable in the survival model, the interpretation of the hazard ratio is 'the increase in risk per twofold increase in energy-adjusted intake of the food/nutrient'.

In Studies II–V, BMI at the first antenatal examination and gestational age were categorized into quarters. All the categorical sociodemographic and perinatal characteristics were coded as binary indicator variables for model fitting purposes.

Statistical models

Study I: In the case-control design, we used a conditional logistic regression model with type 1 diabetes as the dependent variable and categorized α-tocopherol concentrations as the explanatory variable. Genetic risk group, maternal age and education, place of residence and number of siblings were added into the model as covariates. Stata 5.0 (StataCorp, College Station, Texas, USA) was used in the analyses. In the cohort design, the association between α-tocopherol concentrations and risk of type 1 diabetes was analyzed by Cox regression model using SPSS 9.0 (SPSS, Chicago, Illinois, USA). To reduce the effect of intra-individual variation, the mean of each individual's α-tocopherol concentrations in the repeated serum samples was used as the explanatory variable. Sex, genetic risk group, age and maternal education were added into the model as covariates.

Study II: A conditional likelihood analysis of a logistic regression model was used within the nested case-control design to estimate potential associations between seroconversion and serum α- and γ-tocopherol concentrations. The explanatory variables were analyzed both as continuous and categorical. As more than 40% of the seroconversions took place before the second year of age, the first year serum values were employed in the model. A model which allowed an interaction with the time of seroconversion was fitted as well. Moreover, the effect of an average category of individual α-tocopherol and γ-tocopherol concentrations over time on the response was estimated. Adjusted multiple logistic regression models included serum cholesterol and background characteristics (education of the mother and the father, maternal age, duration of gestation, diabetes in a first-degree relative, number of earlier deliveries, maternal smoking during pregnancy) in addition to α- or γ-tocopherol concentrations. The analyses were conducted on the observed data and, to reduce potential bias associated with missing explanatory variables and to allow subjects

with incomplete sets of explanatory variables to be included, multiple imputation was used. The sequential imputation regression method (Raghunathan et al. 2001) as implemented in the IVEware software was used to generate five sets of imputed missing values and the final estimates and their standard errors were calculated according to Rubin's rules (Rubin 1987). α- and γ-tocopherol concentrations were imputed for 11 cases and 17 controls. SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA) was used in the analyses.

Studies III–IV: The endpoint of advanced β-cell autoimmunity is interval censored. To accommodate this structure, piece-wise exponential survival models were used to analyze the associations of maternal diet with the endpoint, with constant hazard in the intervals 0–0.99, 1–1.99, 2–2.99 and ≥3 years. The models were fitted using maximum likelihood in SAS PROC NLMIXED, with standard errors of estimates derived from the observed information matrix. SAS version 9.1.3 was used in the analyses. The possible confounding by background characteristics was controlled for by adding the background variables as covariates in the statistical models. Two sets of covariates were used, one including diabetes in a first-degree relative and genetic risk group, and the other including also sex, gestational age, maternal age, maternal parity, maternal education and maternal smoking during pregnancy, degree of urbanization of the home municipality, and region of birth (Oulu vs. Tampere area). In Study III, the proportionality of the hazards was tested by adding interaction terms of the exposure variables with time interval to the models; in Study IV, the associations of antioxidant nutrients with early endpoints (advanced β-cell autoimmunity appearing before the age of 2.5 years) were checked by Cox regression models using SPSS 15.0 (SPSS Inc., Chicago, IL). In Study IV, the associations of antioxidant nutrients with the risk of clinical disease were also analyzed. Because there was no interval censoring in the diagnosis of clinical diabetes, the associations could be analyzed by Cox regression models using SPSS 15.0. Furthermore, Cox regression analysis was used to check the associations of categorized antioxidant intakes with advanced β-cell autoimmunity. In etiological analyses the effective sample size was 3,723, including 138 cases with advanced β-cell autoimmunity, 55 cases with clinical type 1 diabetes and 57 cases with advanced β-cell autoimmunity appearing before the age of 2.5 years.

Study V: The associations of social variables with antioxidant intake were analyzed by multiple linear regression models, with the ln-transformed energy-adjusted intake of each antioxidant as the dependent variable and the sociodemographic and lifestyle factors as the explanatory variables. The predicted intakes were calculated by exponentiating the values of the linear predictor produced by the model (0.01 subtracted). Several of the food groups showed clear non-normality, even after a logarithmic transformation [ln(x+0.01)]. Derived binary variables were therefore used as outcomes in multiple logistic regression, with an 'event' defined as a variable in the highest observed quartile. The use of antioxidant supplements was analyzed with a similar multiple logistic regression model,

the dependent variable being the use of any dietary supplement with antioxidant nutrients at any time during the pregnancy. All the explanatory variables were categorical, and coded as binary indicator variables. Explanatory variables measured on an ordinal scale (age, parity, education and BMI) were tested for linear trend by treating them as continuous variables in the multiple regression models, one at a time. Regression models were tested for model assumptions and fit. Of the 3,730 subjects, 504 (13.5 %) were excluded from the statistical models because of missing information in at least one explanatory variable. Thus the effective sample size was 3,226 (60.6 % of those invited to participate in the study). Statistical analyses were performed using SPSS for Windows, version 9.0 (SPSS Inc., Chicago, IL).

5 RESULTS

5.1 Serum vitamin E concentrations and the development of type 1 diabetes

The associations between serum vitamin E concentrations with the development of type 1 diabetes were assessed in the cohorts of the DiMe (Study I) and DIPP studies (Study II). In Study I, the explanatory variable was serum α-tocopherol concentration, and the endpoint variable was overt type 1 diabetes. In Study II, also γ-tocopherol concentrations were analyzed, and advanced β-cell autoimmunity was used as the endpoint variable.

5.1.1 Serum vitamin E concentrations (I, II)

The mean serum α-tocopherol concentrations of cases and controls in Study I (Design 1) were 3.82 mg/l (SD 0.77) and 3.97 mg/l (SD 1.11) respectively. In the cohort of seropositive children (Design 2), the mean α-tocopherol concentration in the first serum sample was 3.59 mg/l (SD 1.23). The mean α-tocopherol concentrations in the serum samples from the control children of Study II ranged from 6.69 mg/l (SD 1.93) at the age of 6 years to 7.98 mg/l (SD 1.94) at the age of 1 year. The α-tocopherol concentrations of cases and controls were similar, and remained stable over the entire age range from 1 to 6 years (Figure 3).

The mean γ-tocopherol concentrations of controls in Study II ranged from 0.49 mg/l (SD 0.23) at the age of 1 year to 0.87 mg/l (SD 0.42) at the age of 5 years. The concentrations of γ-tocopherol tended to be slightly lower at the age of one year compared with the older age groups (Figure 4).

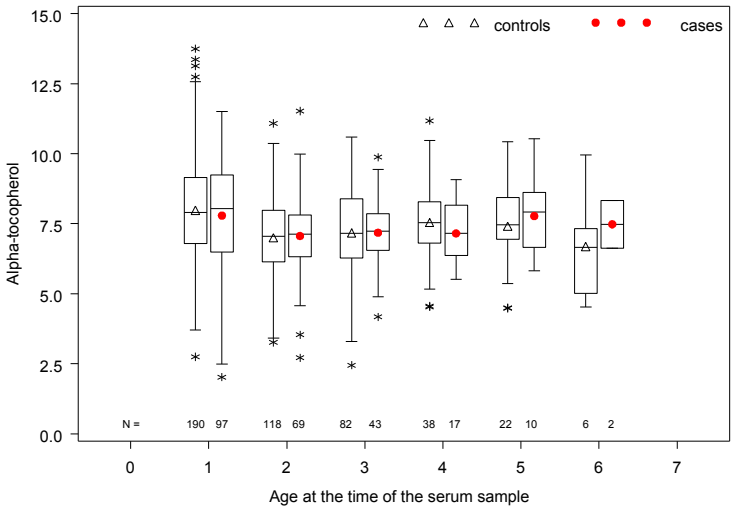


Figure 3. Serum α-tocopherol concentrations (mg/l) of cases and controls by age groups. The central bar in the boxplot represents the median concentration, the box represents the interquartile range, the whiskers represent the smallest and largest non-outlier concentrations, and the asterisks represent outliers. An outlier is defined as an observation that lies more than 1.5*the interquartile range lower than the first quartile or 1.5*the the interquartile range higher than the third quartile.

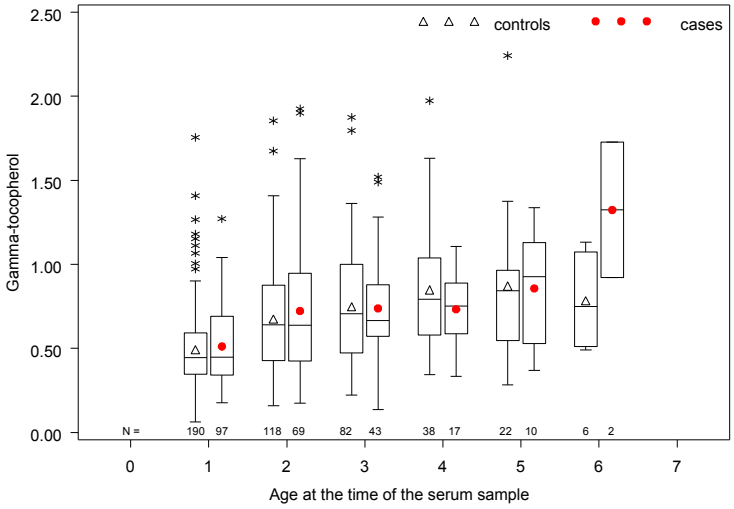


Figure 4. Serum γ -tocopherol concentrations (mg/l) of cases and controls by age groups. The central bar in the boxplot represents the median concentration, the box represents the interquartile range, the whiskers represent the smallest and largest non-outlier concentrations, and the asterisks represent outliers. An outlier is defined as an observation that lies more than 1.5*the interquartile range lower than the first quartile or 1.5*the the interquartile range higher than the third quartile.

5.1.2 Associations of serum α-tocopherol concentrations with the risk of type 1 diabetes (I)

In the nested case-control design (Study design 1), cholesterol-adjusted serum α-tocopherol concentration showed an inverse association of borderline statistical significance with the risk of type 1 diabetes. The odds ratio for high vs. low or intermediate concentration of α-tocopherol was 0.26 (95% CI 0.05–1.27, $p=0.10$). Adjustment for length of mother's education strengthened the association slightly (Table 3). Further adjustment for HLA-DR-conferred risk, mother's age, number of children in the family and place of residence did not change the magnitude of the association. The results were similar for α-tocopherol concentration not adjusted for serum cholesterol.

In the cohort design (study design 2), serum α-tocopherol concentration was not significantly associated with the risk of diabetes. The hazard ratio per 1 mg increase in cholesterol-adjusted α-tocopherol concentration was 0.73 (95% CI 0.49–1.09, $p=0.13$). The association reached borderline statistical significance, when it was adjusted for HLA-DR-conferred risk, sex, age and length of mother's education (Table 3). The results were similar for α-tocopherol unadjusted for cholesterol.

Table 3. Associations of serum α-tocopherol concentrations with the risk of type 1 diabetes (Study I) and advanced β-cell autoimmunity (Study II).

α-tocopherol concentration	OR / HR	95% CI	*p*-value	# of cases	# of unaffected[1]
Study I					
Design 1[2]:					
Low or intermediate[3]	1.00	–	–	14	54
High[4]	0.22	0.04–1.19	0.08	2	27
Design 2:					
Per mg/l increase[5]	0.67	0.43–1.06	0.09	26	54
Study II[6]					
Per mg/l increase in first year sample	0.94	0.82–1.08	0.40	97	199
Values in first year sample					
Low	1.51	0.81–2.81	0.19	28	46
Intermediate[7]	1.00	–	–	43	105
High	1.29	0.70–2.36	0.42	26	48
Overall values					
Low	1.18	0.63–2.20	0.61	30	52
Intermediate[7]	1.00	–	–	55	108
High	0.80	0.44–1.47	0.48	23	56

[1] Controls in Study I, Design 1 and in Study II; members of the cohort who were not affected by type 1 diabetes during the follow-up in Study I, Design 2

[2] Controls were matched for sex, age and the date of the serum sample

[3] Low or intermediate (0 to 67th percentile); high (67th to 100th percentile)

[4] Adjusted for serum cholesterol concentration and length of mother's education

[5] Adjusted for serum cholesterol concentration, HLA-DR conferred risk group, sex, age and length of mother's education

[6] Controls were matched for HLA-DQ conferred risk group, delivery hospital, sex and birth date (±3 months)

[7] Low (0 to 25th percentile), intermediate (25th to 75th percentile) and high (75th percentile to 100th percentile)

5.1.3 Associations of serum α- and γ-tocopherol concentrations with the risk of advanced β-cell autoimmunity (II)

Serum α-tocopherol concentration was not significantly associated with the risk of advanced β-cell autoimmunity (Table 3). α-tocopherol concentration at the age of 1 year was not a significant predictor of seroconversion irrespective whether it was analyzed as a continuous or categorical quartile variable. Neither was the overall concentration of α-tocopherol in all samples up to the seroconversion associated with the endpoint. Multiple imputation of missing values or adjustment for serum cholesterol concentration, education level of mother and father, mother's age, duration of gestation, diabetes in a first degree relative, number of earlier deliveries and maternal smoking during pregnancy did not change the magnitude of the associations or improve the precision of the results. Alpha-tocopherol concentration at the age of 1 year analyzed as a continuous variable showed a borderline interaction with the time of seroconversion ($p=0.09$).

Serum γ-tocopherol concentration was not significantly associated with advanced β-cell autoimmunity, either. OR per 1 mg/l increase in γ-tocopherol concentration in the first year sample was 1.10 (95% CI 0.70–1.74, $p=0.67$). OR for high vs. intermediate γ-tocopherol concentration in the first year sample was 1.35 (0.75–2.41, $p=0.31$), and OR for low vs. intermediate concentration was 1.23 (0.66–2.27, $p=0.52$). For high vs. intermediate overall γ-tocopherol concentrations, OR was 1.07 (0.61–1.87, $p=0.82$), and for low vs. intermediate overall concentrations it was 1.05 (0.58–1.90, $p=0.87$). Multiple imputation of missing values or adjustment for the background variables did not change the magnitude of the associations or improve the precision of the results. However, there was a significant interaction between high vs. intermediate concentrations of γ-tocopherol at the age of 1 year and the time of seroconversion ($p=0.02$).

5.2 Vitamin E intake during pregnancy

5.2.1 Intake and dietary sources of vitamin E (IV, V)

The median daily intake of vitamin E of the pregnant women from food was 13.4 mg (25^{th} percentile 10.9, 75^{th} percentile 16.5). The mean daily intake from dietary supplements was 1.1 mg (SD 4.9, range 0.0–191). On average, intake from dietary supplements covered 7.3% of total vitamin E intake.

Most of the dietary vitamin E was supplied by high-fat foods (Table 4). Vegetable oils and margarine were important sources, as were fats for cooking and industrial use and high-fat potato products (e.g. French fries and potato chips). Other major sources of vitamin E were cereals and vegetables. Among cereals, the main sources of vitamin E were wheat and rye. Poultry and pork were the main contributors to vitamin E intake

within the meat group. Fruit vegetables like tomato, cucumber and sweet pepper as well as nuts and seeds were the major vegetable source of vitamin E.

Table 4. Dietary sources of vitamin E among 3730 pregnant women, who delivered an infant with genetic susceptibility to type 1 diabetes at the University Hospital of Oulu or Tampere, Finland, 1997–2002.

Ingredient group	Proportional supply (%) of vitamin E intake	
Vegetable oils	18.5	
Cereal products	16.4	
– wheat		9.1
– rye		5.5
Other ingredients [1]	13.3	
– fats for cooking and industrial use		7.3
– potato products		2.2
Vegetables	9.6	
– fruit vegetables; e.g. cucumber, sweet pepper, tomato		3.7
– nuts and seeds		2.6
– leaf vegetables; e.g. lettuce		1.3
Margarine	8.6	
Meat, meat products	6.4	
– poultry		2.6
– pork		1.8
Fruit	5.4	
Fish and shellfish	5.1	
Milk, milk products	4.5	
– cheese		1.9
Egg	3.4	
Butter	3.4	
Berries	2.8	
Juice	2.3	
Potato	0.4	
Total	100	

[1] e.g. alcoholic drinks, coffee, tea, soft drinks, industrial and animal fats, snacks, sweets, biscuits

5.2.2 Social characteristics as predictors of vitamin E intake and consumption of its dietary sources (V)

Among all the social background characteristics in the study, age and own vocational education were the most important predictors of vitamin E intake during pregnancy (Table 5) Vitamin E intake increased linearly with age and education. The partner's education was also positively associated with vitamin E intake, but not as strongly as own education. Smokers had a lower intake of vitamin E than non-smokers. Women who lived in a rural municipality had lower intake than those who lived in urban or semi-urban municipalities. Vitamin E intake was higher in the region of Tampere, situated at south-western Finland,

than in the region of Oulu, situated in farther north. Intake of vitamin E was not associated with parity or with BMI at the first antenatal visit. When the effects of all the social characteristics were simultaneously accounted for, the difference between lowest and highest predicted intake was 37.3%.

Table 5. Social factors as predictors for energy-adjusted vitamin E intake among 3,730 pregnant women, who delivered an infant with genetic susceptibility to type 1 diabetes at the University Hospital of Oulu or Tampere, Finland, 1997–2002. Analyzed by a multiple linear regression model with all explanatory variables controlled simultaneously.

Explanatory variables	Vitamin E (mg)	% difference [1]
Age, years		
<25 [2]	13.9 §	
25–30	14.1 *	1.4
30–35	14.5 ***	4.3
>35	15.0 ***	7.9
Parity		
0 [2]	13.9	
1	13.7	–1.4
2	14.0	0.7
≥3	13.8	–0.7
Degree of urbanization		
Urban [2]	13.9	
Semi-urban	13.9	0.0
Rural	13.4 ***	–3.6
Place of residence		
Region of Tampere [2]	13.9	
Region of Oulu	13.7 *	–1.4
Vocational education		
Academic [2]	13.9 §	
Upper secondary vocational education	13.5 ***	–2.9
Vocational school or course	13.5 **	–2.9
None	13.0 ***	–6.5
Vocational education of the partner		
Academic [2]	13.9 §	
Upper secondary vocational education	13.7	–1.4
Vocational school or course	13.5 **	–2.9
None	13.7	–1.4
Smoking during pregnancy		
No [2]	13.9	
Yes	13.4 ***	–3.6
BMI at the first antenatal visit		
1. quarter [2]	13.9	
2. quarter	13.8	–0.7
3. quarter	13.7	–1.4
4. quarter	13.7	–1.4

[1] Compared with the reference category of each explanatory variable

[2] Reference category

Average intake is significantly different from the reference category; * $p =< 0.05$ ** $p =< 0.01$ *** $p =< 0.001$

§ p for linear trend $=< 0.05$

Table 6. Social factors as predictors for consumption of dietary sources of vitamin E among 3,730 pregnant women, who delivered an infant with genetic susceptibility to type 1 diabetes at the University Hospital of Oulu or Tampere, Finland, 1997–2002. Each column is based on a single multiple logistic regression model with all explanatory variables controlled simultaneously.

Explanatory variables	Odds ratio for being in the highest quarter of food consumption			
	Vegetable oils	Cereal products	Vegetables	Margarine
Age, years				
<25 [1]	1.00 §	1.00	1.00 §	1.00
25–29.9	1.52**	1.31*	1.09	0.75*
30–34.9	1.95***	1.36*	1.35*	0.72*
≥35	2.10***	1.38*	1.69***	0.73*
Parity				
0 [1]	1.00	1.00	1.00	1.00 §
1	1.15	0.84	0.94	1.27*
2	1.15	0.83	1.18	1.22
≥3	1.14	1.34	1.24	1.45*
Degree of urbanization				
Urban [1]	1.00	1.00	1.00	1.00
Semi-urban	0.95	1.24	1.04	1.28
Rural	0.80	1.13	0.90	0.60***
Place of residence				
Region of Tampere [1]	1.00	1.00	1.00	1.00
Region of Oulu	0.69***	1.25**	0.77**	1.20*
Vocational education				
Academic [1]	1.00	1.00	1.00	1.00 §
Upper secondary vocational education	0.85	1.05	0.84	1.50**
Vocational school or course	0.78	1.11	0.92	1.55**
None	0.84	1.20	1.09	1.30
Vocational education of the partner				
Academic [1]	1.00	1.00	1.00 §	1.00
Upper secondary vocational education	0.91	1.01	0.85	0.91
Vocational school or course	1.05	0.85	0.64***	1.04
None	0.95	1.01	0.55**	0.90
Smoking during pregnancy				
No [1]	1.00	1.00	1.00	1.00
Yes	1.13	0.82	0.70*	1.39*
BMI at the first antenatal visit				
1. quarter [1]	1.00	1.00	1.00	1.00
2. quarter	0.91	0.83	0.91	1.06
3. quarter	1.06	1.01	1.19	1.14
4. quarter	0.97	0.83	1.13	0.93

[1] Reference category

§ p for linear trend =< 0.05

Odds ratio differs significantly from the reference category; * p =< 0.05 ** p =< 0.01 *** p =< 0.001

Age was positively associated with the use of vegetable oils and vegetables (Table 6). The youngest pregnant women were less likely to be high users of cereals, but more likely to have a high consumption of margarine than the older age groups. Maternal education showed no significant positive associations with any of the main dietary sources of vitamin E, whereas the partner's education was positively associated with the woman's vegetable consumption. Smokers were less likely to consume a lot of vegetables, but used more margarine than non-smokers. Margarine was consumed less in rural than in semi-urban and urban municipalities. In the Oulu region, situated in northern Finland, heavy use of vegetable oils and vegetables was less common, but heavy use of cereals and margarine was more common than in the Tampere region. Women who already had children tended to use more margarine than the women who were expecting their first child. BMI was not significantly associated with the consumption of vegetable oils, cereals, vegetables or margarine.

5.2.3 Intake of vitamin E, consumption of its dietary sources during pregnancy and the risk of advanced β-cell autoimmunity in the child (III, IV)

Maternal intake of vitamin E from food or the combined intake from food and supplements was not associated with pre-type 1 diabetes in the offspring during the median follow-up time of 4.4 years (Table 7). Hazard ratios were non-significant and close to unity. The results were similar irrespective whether only basic adjustment for genetic risk and diabetes in a first-degree relative or a broad adjustment for several sociodemographic and perinatal variables (sex, gestational age, maternal age, maternal parity, maternal education and maternal smoking during pregnancy, degree of urbanization of the home municipality, and region of birth) was applied.

Maternal intake of vitamin E from food or the combined intake from food and supplements was not associated with the risk of clinical type 1 diabetes (Table 7) or with the early endpoint of advanced β-cell autoimmunity appearing before the age of 2.5 years (data not shown). The hazard ratios were close to one, but the confidence intervals were wider than in the main analyses because of the smaller number of cases. Vitamin E categorized into quarters of intake was not associated with pre-type 1 diabetes or clinical diabetes, either (data not shown).

The consumption of major dietary sources of vitamin E during pregnancy was not associated with the risk of advanced β-cell autoimmunity in the child (Table 7). Hazard ratios for the use of total dietary fats, cereal products and vegetables were insignificant and close to one. However, the consumption of low-fat margarine and butter was inversely associated with the risk of pre-type 1 diabetes, and low-fat margarine remained significant when its consumption was adjusted for the sociodemographic and perinatal factors. None of these foods and food groups showed significant interactions with time interval.

Table 7. Associations of maternal vitamin E intake and consumption of its dietary sources during pregnancy with the development of type 1 diabetes among 3,723 genetically susceptible children born at the University hospital of Oulu or Tampere, Finland, 1997–2002.

	Hazard ratio (95% CI)[1]	
	Prediabetes[2,3]	Overt diabetes[4]
Vitamin E		
Intake from foods[5]	1.13 (0.58–2.21)	1.25 (0.27–5.73)
Intake from foods + supplements	1.04 (0.74–1.46)	0.98 (0.46–2.10)
Dietary sources of vitamin E		
Dietary fats	0.84 (0.61–1.15)	–
– Vegetable oils	0.92 (0.69–1.22)	–
– Vegetable margarines, yes vs. no	1.21 (0.86–1.69)	–
– Low-fat margarines, yes vs. no	0.58 (0.38–0.89)	–
– Butter	0.83 (0.70–0.98)	–
Cereal products	1.09 (0.72–1.65)	–
– Wheat	1.04 (0.79–1.37)	–
– Rye	1.04 (0.92–1.18)	–
Vegetables	0.92 (0.75–1.14)	–

[1] Adjusted for genetic risk and familial diabetes

[2] Defined as repeated positivity for ICA and at least one other diabetes-associated autoantibody and/or clinical type 1 diabetes.

[3] Produced by piecewise exponential survival models. Indicates the change in risk per a twofold increase in intake. For the dichotomous variables, HR indicates the risk associated with use vs. non-use of the food item.

[4] Produced by Cox regression models. Indicates the change in risk per a twofold increase in intake.

[5] Adjusted for energy by the residual method

Table 8. Daily intake of selected antioxidant nutrients among 3,730 pregnant women, who delivered an infant with genetic susceptibility to type 1 diabetes at the University Hospital of Oulu or Tampere, Finland, 1997–2002.

	From food			From supplements
	25th percentile	Median	75th percentile	Mean (% of total)
Retinol (μg)	443	662	1,128	18.1 (1.9)
β-carotene (μg)	2,231	3,407	5,140	261 (5.7)
Vitamin C (mg)	116	172	250	23.3 (10.5)
Selenium (μg)	63.9	77.4	93.5	6.3 (7.9)
Zinc (mg)	13.4	16.2	19.6	2.0 (10.6)
Manganese (mg)	4.94	6.44	8.29	0.3 (4.8)

5.3 Intake of other antioxidant nutrients during pregnancy

5.3.1 Intake and dietary sources of other antioxidant nutrients (IV, V)

The distributions of the daily intakes of antioxidant nutrients were skewed, and therefore quartiles instead of means of intake from food are presented in Table 8. The percentage of total nutrient intake provided by dietary supplements ranged from 1.9% for retinol to 10.6% for zinc.

The main sources of retinol were meat and milk products (Figure 5). Offal supplied 43% and cheese 13% of the total dietary retinol intake. β-carotene was provided mainly by root vegetables (65%) (Figure 6). Juice was the major source of vitamin C, followed by fruit vegetables (e.g. cucumber, sweet pepper and tomato, 10%) and citrus fruits (10%) (Figure 7). The major sources of selenium and zinc were meat, milk and cereal products (Figures 8 and 9). Manganese was provided mainly from cereals (Figure 10).

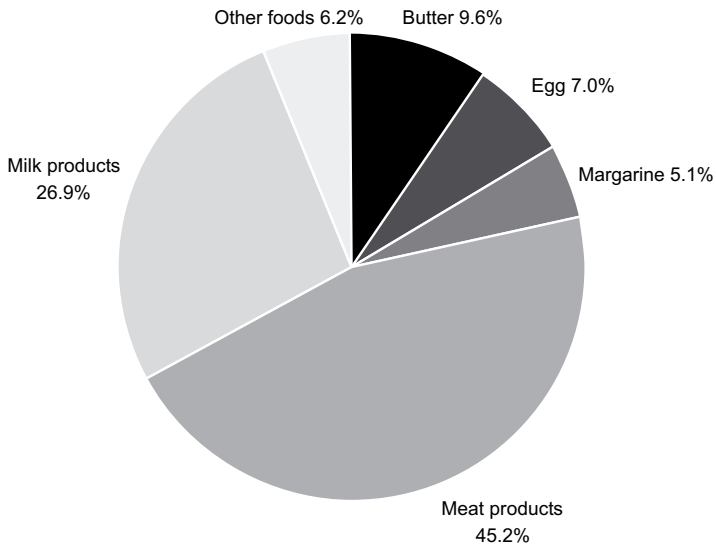


Figure 5. Dietary supply of retinol from food groups.

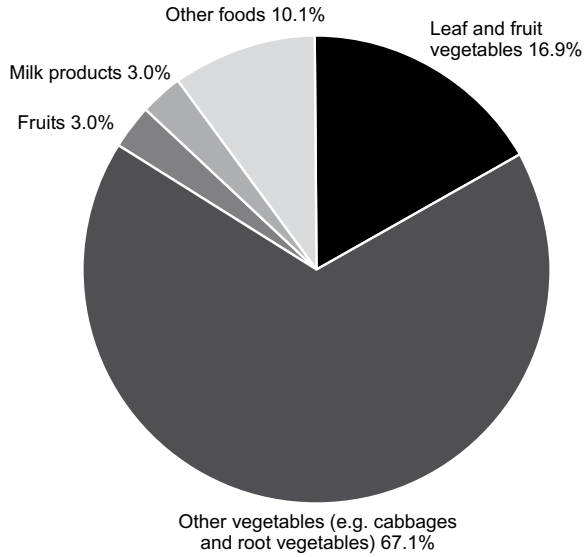


Figure 6. Dietary supply of β-carotene (β-carotene equivalents) from food groups. Leaf and fruit vegetables = vegetables usually eaten fresh, e.g. lettuce, cucumber, sweet pepper, tomato. Other vegetables = vegetables usually eaten cooked, e.g. cabbages, roots and tubers, onions, peas and beans, mushroom.

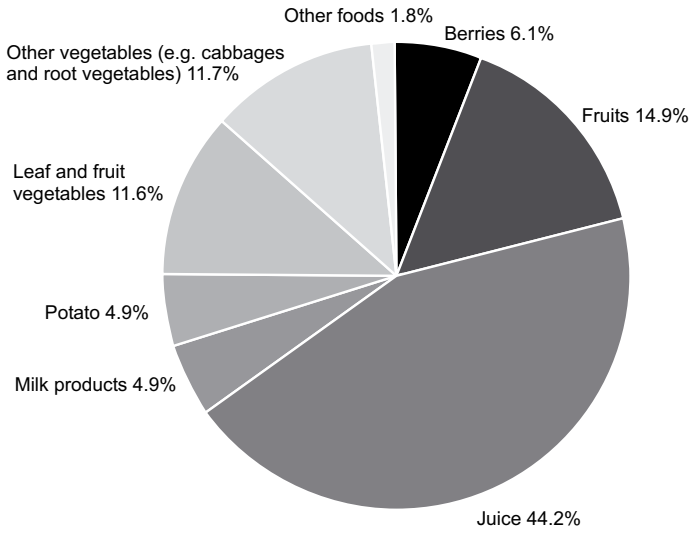


Figure 7. Dietary supply of vitamin C from food groups. Leaf and fruit vegetables = vegetables usually eaten fresh, e.g. lettuce, cucumber, sweet pepper, tomato. Other vegetables = vegetables usually eaten cooked, e.g. cabbages, roots and tubers, onions, peas and beans, mushroom.

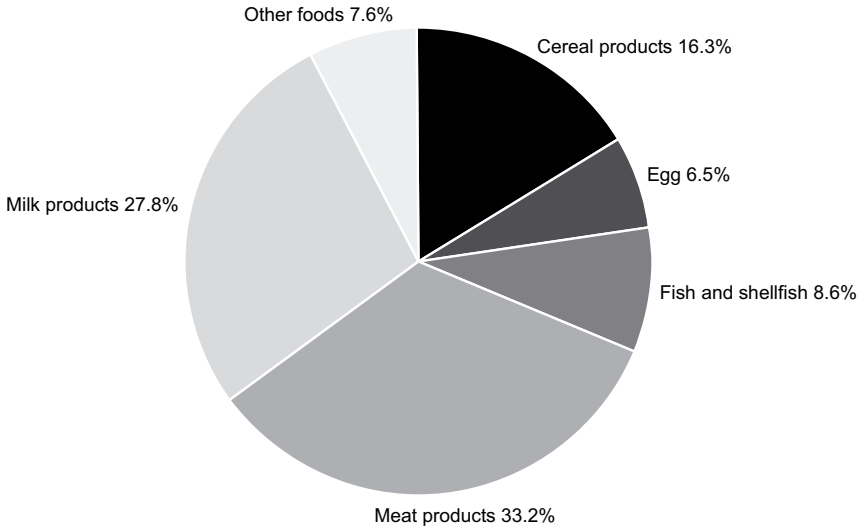


Figure 8. Dietary supply of selenium from food groups.

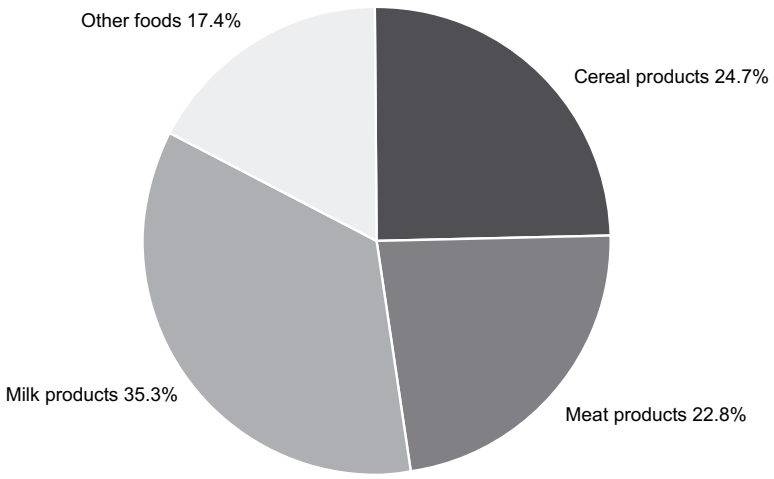


Figure 9. Dietary supply of zinc from food groups.

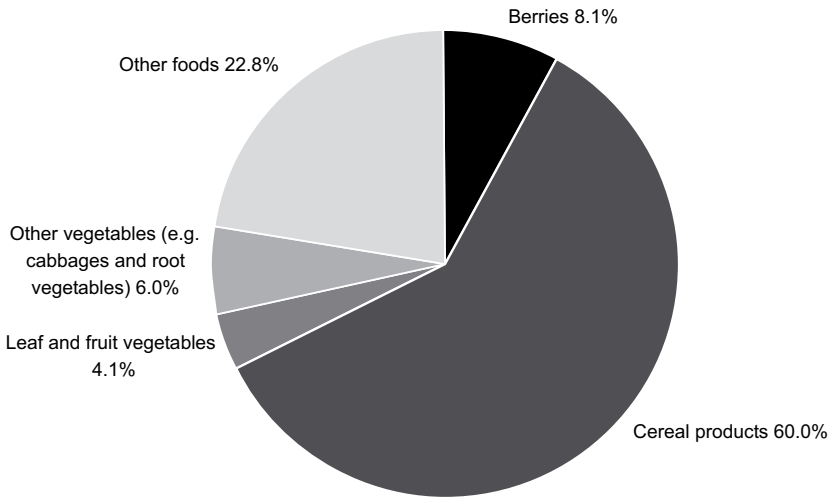


Figure 10. Dietary supply of manganese from food groups. Leaf and fruit vegetables = vegetables usually eaten fresh, e.g. lettuce, cucumber, sweet pepper, tomato. Other vegetables = vegetables usually eaten cooked, e.g. cabbages, roots and tubers, onions, peas and beans, mushroom.

5.3.2 Social characteristics as predictors of the intake of other antioxidant nutrients and consumption of their dietary sources (V)

Retinol intake during pregnancy was positively associated with parity, and inversely associated with the level of the pregnant mother's own vocational education (Table 9). Women who had smoked during pregnancy had a higher retinol intake than non-smokers. Intake of β-carotene was positively associated with age and with the partner's education. Smoking during pregnancy predicted a lower intake of β-carotene. Intake of vitamin C decreased with parity, and tended to be smaller in the groups with lower education. The partner's education was a stronger predictor of vitamin C intake than the woman's own education. Smoking was associated with a lower vitamin C intake.

The intakes of selenium, zinc and manganese correlated positively with age and education. The associations with the own education were stronger than with the partner's education. Women with a higher BMI at the first antenatal examination had higher intakes of selenium and zinc, but lower intake of manganese. Smokers had a lower intake of zinc and manganese than non-smokers.

Table 9. Social factors as predictors for intake of selected energy-adjusted antioxidant nutrients among 3,730 pregnant women, who delivered an infant with genetic susceptibility to type 1 diabetes at the University Hospital of Oulu or Tampere, Finland, 1997–2002. Each column is based on a single multiple linear regression model with all explanatory variables controlled simultaneously.

Explanatory variables	Retinol (µg)	β-carotene (µg)	Vitamin C (mg)	Selenium (µg)	Zinc (mg)	Manganese (mg)
Age, years						
<25 [1]	630	3,866 §	193	76.2 §	15.7 §	6.21 §
25–30	589*	3,892	194	77.1	15 .9	6.55 ***
30–35	607	4,088	191	79.0 ***	16.1 **	6.91 ***
>35	595	4,615 ***	197	79.0 ***	16.1 **	7.27 ***
Parity						
0 [1]	630 §	3,866	193 §	76.2	15.7	6.21
1	724 ***	3,817	176 ***	76.4	15.4 **	5.84 ***
2	763 ***	4,043	177 **	76.3	15.6	6.15
≥3	863 ***	3,909	161 ***	77.0	15.9	6.39
Degree of urbanization						
Urban [1]	630	3,866	193	76.2	15.7	6.21
Semi-urban	622	3,793	190	74.8 *	15.8	6.50 **
Rural	680 *	3,787	185	75.7	16.0 **	6.61 ***
Place of residence						
Region of Tampere [1]	630	3,866	193	76.2	15.7	6.21
Region of Oulu	609	3,847	192	75.8	16.1 ***	6.66 ***
Vocational education						
Academic [1]	630	3,866	193	76.2 §	15.7 §	6.21 §
Upper secondary vocational education	671 *	3,587 **	187	75.4	15.7	6.02 *
Vocational school or course	703 ***	3,617	182 *	74.6 *	15.4	5.81 ***
None	616	3,740	182	73.3 **	15.1 **	5.58 ***
Vocational education of the partner						
Academic [1]	630	3,866 §	193	76.2 §	15.7	6.21 §
Upper secondary vocational education	619	3,634 *	186	75.6	15.7	6.24
Vocational school or course	644	3,374 ***	178 **	74.9 *	15.6	5.93 **
None	679	3,093 ***	194	74.0 *	15.2 *	5.77 **
Smoking during pregnancy						
No [1]	630	3,866	193	76.2	15.7	6.21
Yes	699 **	3,087 ***	179 **	75.1	15.3 **	5.54 ***
BMI at the first antenatal visit						
1. quarter [1]	630	3,866	193	76.2 §	15.7 §	6.21 §
2. quarter	623	3,804	196	76.6	15.8	6.17
3. quarter	633	3,908	201	77.0	15.8	6.14
4. quarter	622	4,020	193	78.9 ***	16.0 *	6.02 *

[1] Reference category

Average intake is significantly different from the reference category; * p =< 0.05 ** p =< 0.01 *** p =< 0.001

§ p for linear trend =< 0.05

The consumption of offal during pregnancy was positively associated with age, parity and smoking, and inversely associated with own education (Table 10). High total consumption of meat and meat products was more common among women who already had children, smokers and women with a higher BMI. The use of milk and milk products was inversely associated with age and level of own education. Heavy consumption of cereal products was more common among the older age groups. The consumption of vegetables and fruits was positively associated with age and the level of the partner's education. Also, vegetable consumption was inversely associated with smoking, and fruit consumption with parity. Juice consumption was inversely associated with age, parity and living in a rural municipality. Heavy juice consumption was more common among smokers than non-smokers.

The consumption of dietary supplements with antioxidant nutrients was positively associated with age and the own level of education, and inversely associated with BMI at the first antenatal examination. Women expecting their first child were more likely to use antioxidant supplements than those with of one or two previous deliveries. (Data not shown).

5.3.3 Intake of other antioxidant nutrients, consumption of their dietary sources and the risk of advanced β-cell autoimmunity in the offspring (III, IV)

The intakes of retinol, β-carotene, vitamin C, selenium, zinc or manganese during pregnancy were not associated with the risk of advanced β-cell autoimmunity in the offspring (Table 11). All the hazard ratios, indicating the change in risk per a twofold increase in intake, were nonsignificant and close to unity. The magnitudes of the hazard ratios were similar for energy-adjusted intakes from food and for total intakes from food and dietary supplements not adjusted for energy. The results were essentially the same, irrespective of whether they were adjusted only for genetic risk group and presence of diabetes in a first-degree relative or also for the broad range of sociodemographic and perinatal characteristics presented in Chapter 5.2.3.

The intake of other antioxidant nutrients was not associated with clinical type 1 diabetes either (Table 11) or with the early endpoint of advanced β-cell autoimmunity appearing before the age of 2.5 years (data not shown). The hazard ratios were close to one, but the confidence intervals tended to be wider than in the main analysis because of the smaller number of cases. Analyzing the antioxidants categorized into quarters of intake did not change the results (data not shown).

The consumption of any food group which was a major dietary source of other antioxidant nutrients was not associated with the risk of pre-type 1 diabetes in the child (data not shown). The hazard ratios indicating a twofold increase in the consumption were close to unity, and the 95% confidence intervals tended to be reasonably narrow. The estimates ranged from 0.92 (95% CI 0.72–1.17) for milk and milk products to 1.20 (0.92–1.56) for root vegetables and potato. None of the food groups showed an interaction with time.

Table 10. Social factors as predictors for consumption of major dietary sources of antioxidant nutrients among 3,730 pregnant women, who delivered an infant with genetic susceptibility to type 1 diabetes at the University Hospital of Oulu or Tampere, Finland, 1997–2002. Each column is based on a single multiple logistic regression model with all explanatory variables controlled simultaneously.

Explanatory variables	Odds ratio for being in the highest quarter of food consumption						
	Offal	Total meat products	Milk products	Cereal products	Vegetables	Fruits	Juice
Age, years							
<25 [1]	1.00 §	1.00	1.00 §	1.00	1.00 §	1.00 §	1.00 §
25–29.9	1.23	1.01	1.11	1.31*	1.09	1.27	1.00
30–34.9	1.51**	1.21	0.83	1.36*	1.35*	1.43**	0.73*
≥35	1.46*	1.00	0.62**	1.38*	1.69***	1.45*	0.64**
Parity							
0 [1]	1.00 §	1.00 §	1.00	1.00	1.00	1.00 §	1.00 §
1	1.42***	1.55***	0.84	0.84	0.94	0.68***	0.88
2	1.52**	1.49**	0.80	0.83	1.18	0.68**	0.80
≥3	1.55**	1.60**	0.95	1.34	1.24	0.44***	0.76
Degree of urbanization							
Urban [1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Semi-urban	0.87	0.90	1.25	1.24	1.04	1.12	0.99
Rural	1.08	0.80	1.00	1.13	0.90	1.03	0.63**
Place of residence							
Region of Tampere [1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Region of Oulu	0.90	0.86	1.41***	1.25**	0.77**	0.74***	1.11
Vocational education							
Academic [1]	1.00 §	1.00	1.00 §	1.00	1.00	1.00	1.00
Upper secondary vocational education	1.32*	1.15	1.19	1.05	0.84	0.99	1.09
Vocational school or course	1.55**	1.13	1.42*	1.11	0.92	1.03	1.08
None	1.26	0.99	1.37	1.20	1.09	1.01	1.14
Vocational education of the partner							
Academic [1]	1.00	1.00	1.00	1.00	1.00 §	1.00 §	1.00
Upper secondary vocational education	1.01	1.09	1.01	1.01	0.85	0.80	0.96
Vocational school or course	0.91	0.98	1.05	0.85	0.64***	0.65***	0.93
None	1.17	1.08	0.95	1.01	0.55**	0.74	1.52*
Smoking during pregnancy							
No [1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Yes	1.38*	1.38*	1.03	0.82	0.70*	0.89	1.34*
BMI at the first antenatal visit							
1. quarter [1]	1.00	1.00 §	1.00	1.00	1.00	1.00	1.00
2. quarter	1.07	0.88	1.02	0.83	0.91	0.95	1.08
3. quarter	1.03	1.16	1.16	1.01	1.19	0.94	1.30*
4. quarter	1.11	1.20	1.21	0.83	1.13	0.91	1.01

[1] Reference category

Odds ratio differs significantly from the reference category; * $p =< 0.05$ ** $p =< 0.01$ *** $p =< 0.001$

§ p for linear trend =< 0.05

Table 11. Associations of maternal intake of selected antioxidant nutrients during pregnancy with the development of type 1 diabetes among 3,723 children with genetic susceptibility to type 1 diabetes born at the University Hospital of Oulu or Tampere, Finland, 1997–2002.

	Hazard ratio (95% CI) [1]			
	Intake from foods, adjusted for energy		Intake from foods + supplements, log_2-transformed	
	Prediabetes [2,3]	Overt diabetes [4]	Prediabetes [2,3]	Overt diabetes [4]
Retinol	0.94 (0.76–1.15)	0.90 (0.56–1.44)	1.05 (0.84–1.32) [5]	1.05 (0.62–1.75) [5]
β-carotene	1.06 (0.88–1.28)	1.29 (0.84–1.96)	1.07 (0.90–1.27)	1.29 (0.88–1.91)
Vitamin C	0.96 (0.76–1.22)	1.14 (0.66–1.97)	0.97 (0.80–1.18)	1.19 (0.76–1.87)
Selenium	1.01 (0.47–2.21)	0.72 (0.12–4.20)	1.04 (0.72–1.50)	0.93 (0.41–2.08)
Zinc	0.84 (0.38–1.89)	1.24 (0.19–8.04)	0.99 (0.70–1.39)	1.05 (0.49–2.28)
Manganese	0.99 (0.69–1.42)	1.45 (0.62–3.39)	1.02 (0.77–1.36)	1.15 (0.60–2.22)

[1] Adjusted for genetic risk and familial diabetes..

[2] Defined as repeated positivity for ICA and at least one other diabetes-associated autoantibody and/or clinical type 1 diabetes.

[3] Produced by piecewise exponential survival models. Indicates the change in risk per a twofold increase in intake.

[4] Produced by Cox regression models. Indicates the change in risk per a twofold increase in intake.

[5] Vitamin A, RE. Because the amount of vitamin A in dietary supplements was expressed as retinol equivalents, also the intake of vitamin A in foods is calculated as retinol equivalents in the combined variable.

6 DISCUSSION

There was a sound scientific rationale to set out to assess the possible protective role of vitamin E and other selected antioxidant nutrients against type 1 diabetes in an epidemiologic setting. Free radicals play a role in the pathogenesis of type 1 diabetes (Asayama et al. 1986, Rabinovitch 1992, Rabinovitch and Suarez-Pinzon 1998), and antioxidants have been protective in several animal models of the disease (Behrens et al. 1986, al-Zuhair et al. 1998, Mukherjee et al. 1998, Tobia et al. 1998). The issue of a possible protective role of dietary vitamin E and other selected antioxidant nutrients was addressed in two well-defined cohorts. Few associations were observed between intake of antioxidant nutrients and the development of type 1 diabetes. The number of endpoints, and hence the statistical power, varied between analyses. In most analyses, the statistical power was adequate enough to exclude a strong inverse association.

6.1 Strengths and weaknesses of the study

6.1.1 Study size and endpoint variables

A clear strength of the present work is the large size of the DIPP cohort. Our study population of more than 3,700 pregnant women compares well with the study populations in earlier surveys on antioxidant intake during pregnancy (Wynn et al. 1994, Erkkola et al. 1998, Rogers et al. 1998, Mathews et al. 2000, Bodnar and Siega-Riz 2002, Freisling et al. 2006). Due to the large size of the study population, it was possible to assess simultaneously the effects of a broad set of sociodemographic and lifestyle characteristics on antioxidant intake. The number of endpoints in the etiological analyses within the DIPP cohort using pre-type 1 diabetes as the endpoint was 108 in the analyses of serum vitamin E concentrations, and 138 in the analyses of maternal diet. These numbers may seem small to those investigating diseases with a higher incidence. However, cohort studies on the associations of diet and type 1 diabetes have so far had a small number of endpoints, and our figures rank well compared to them. The Diabetes Autoimmunity Study in the Young (DAISY) reported 27 (Lamb et al. 2008), 34 (Norris et al. 2003) and 58 endpoints (Norris et al. 2007). The BABYDIAB Study reported 85 endpoints (Ziegler et al. 2003), and the All Babies in Southeast Sweden (ABIS) Study 64 or 266, depending on the cutpoint value used for autoantibody positivity (Holmberg et al. 2007). In our analyses within the DiMe cohort the number of endpoints was small, which was reflected in the wide confidence interval of the risk estimate. In the DIPP cohort, too, the number of endpoints of clinical type 1 diabetes or preclinical diabetes occurring before the age of 2.5 years was

considerably smaller than in the main analyses. Roughly speaking, the CIs of the OR or HR tended to be about 0.20–1.10 in DiMe analyses, 0.70–2.0 in DIPP serum analyses, and 0.70–1.30 in DIPP main analyses on maternal diet.

Another major strength in the present work was the use of advanced pre-type 1 diabetes and clinical disease as the endpoint variables in the etiological analyses. The ultimate interest in etiological research in type 1 diabetes is the prediction and prevention of clinical disease. Advanced prediabetes, defined as seropositivity to at least two diabetes-associated autoantibodies, is a good proxy measure for future clinical type 1 diabetes. Advanced prediabetes tends to persist, and the majority of genetically susceptible children with advanced prediabetes are likely to progress to the clinical stage (Knip 2002, Mrena et al. 2003b). In practice, the vast majority of the case children in the present analyses had three or four autoantibody specificities, representing late prediabetes (Mrena et al. 2003a). During an extended follow-up of the autoantibody positive cases until November 2007, reversion to no or early prediabetes was rare, and more than half had developed clinical type 1 diabetes. In earlier cohort studies assessing diet and the development of type 1 diabetes, early prediabetes of seropositivity to at least one autoantibody has often been used as the endpoint (Norris et al. 2003, Ziegler et al. 2003, Holmberg et al. 2007, Norris et al. 2007, Lamb et al. 2008). However, it has been suggested that early prediabetes is often a non-progressive and reversible state (Knip 2002).

6.1.2 Study design

The study data were derived from prospective cohort studies, and the serum samples and maternal dietary data were collected prior to the development of the endpoint. Therefore, the existence of selection bias of subjects and recall bias in the reporting of food consumption is unlikely. Those responsible for entry and checking of the maternal dietary data or for the analysis of the serum samples were unaware of the endpoint status of the study subjects, which minimizes the risk of bias in the interpretation of the individual results. The families were not informed about whether the child belonged to the moderate risk or high risk group, unless they specifically requested the information.

6.1.3 Representativeness of the study population

In the descriptive analysis of the diet during pregnancy, the representativeness of the study population is essential for the interpretation of the results. The study population of pregnant women comprised mothers of children with HLA-conferred susceptibility to type 1 diabetes from two hospital districts in Finland, Oulu and Tampere. Virtually all children in Finland are born in public hospitals (Vuori E, STAKES, personal communication), and

therefore in terms of their social position the women invited to the study are likely to be an unbiased sample of all new mothers in the catchment areas of the two university hospitals. However, the study population is not representative of pregnant mothers over the whole of Finland. Because there were many dietary differences between the two geographical research areas, further regional differences are expected.

The participation rate of the invited families in the DIPP study was good, but selection by sociodemographic factors is likely. Its effect was not assessed in the present study. The response rate of the FFQ among the mothers was good overall, but there was some selection according to sociodemographic characteristics. The FFQ requires average literacy and numerical skills, and may be too demanding for the most disadvantaged respondents (Turrell and Najman 1995). The respondents of the FFQ within each socioeconomic group are likely to be more interested in a healthy diet than the non-responders. Our study sample was biased towards women with more education and fewer children, groups which tended to have healthy diets. Thus, the true gradient in dietary choices by education level and parity is likely to be even steeper than our results suggest. By contrast, the response rate was lowest in women aged 35 years or older, who tended to have the most healthy diets. Accordingly, our results may overestimate the true differences between the age groups.

6.1.4 Methods of dietary assessment

Food frequency questionnaire

The dietary intake of pregnant women was assessed by a food frequency questionnaire after delivery. A self-administered FFQ is a practical tool for estimating dietary intake in large epidemiological studies (Willett 1998). Besides, it was necessary to use a retrospective method of dietary measurement since the mothers were recruited for the study after delivery. The mothers completed the FFQ form about two months after delivery, which may have caused some inaccuracy in reporting dietary habits during pregnancy. The reference period of the FFQ was the eighth month of pregnancy, prior to the beginning of maternity leave. Food consumption is likely to vary during the pregnancy, and our results are not formally representative for the entire period. However, the eighth pregnancy month may be considered an ideal time to assess the typical diet during pregnancy, since nausea common in early pregnancy has probably already ended, and the employed mothers were still at work and followed their normal meal pattern which may have included some meals eaten outside the home.

The FFQ was validated specifically for the purposes of the present study project. In the validation study the FFQ was also completed after delivery, and two 5-day food records collected during the eighth month of pregnancy were used as the reference method.

The validation study proved the FFQ to be an acceptable tool for ranking the mothers according to their dietary intake during pregnancy; the average correlation between the two methods was 0.47 for foods, and 0.37 for nutrients (Erkkola et al. 2001). Adjustment for energy improved the correlations for nutrients, and correction for attenuation improved the correlations for foods and nutrients. Unfortunately, the correlation between the two methods in the validation study validity of the FFQ was poor for vitamin E and oils, the main source of vitamin E. It seems that the FFQ does not effectively detect the fat content of ready-made industrial foods and foods eaten outside the home. However, a cruder measure of validity – overall percentage of subjects categorized in the same or adjacent fifth of intake according to the two methods – was not strikingly low for vitamin E (70%) or oils (54%), indicating that there was not a total lack of validity in the estimated intakes of these dietary elements.

The intake figures produced by the FFQ tended to overestimate the true intake (Erkkola et al. 2001). Therefore, the absolute values must be interpreted cautiously – for instance, it is not prudent to compare them to recommended dietary intakes. However, as the method of dietary measurement was the same for all participants, comparisons within the study population according to different background characteristics are feasible.

Vitamin E measurements

The serum samples of the DiMe sibling cohort were not originally collected for purposes of vitamin E analyses, which was a major weakness in the present work. Storage of serum samples at –20°C may cause a substantial decrease in α-tocopherol concentration (Ocke et al. 1995), whereas α-tocopherol is well preserved at –70°C (Comstock et al. 1995). The serum samples from the DiMe Study had been stored in –20°C for up to 13 years, which probably accounts for the low concentrations observed. The low quality of the serum samples may have caused inaccuracy in the results and attenuated the observed associations between α-tocopherol concentration and type 1 diabetes. The rate of degradation of α-tocopherol has been shown to vary between individuals (Ocke et al. 1995), and it is possible that some unknown factor affecting the rate of degradation may have confounded the result. For instance, the total antioxidant capacity of the serum could be one potential candidate for such a confounding factor. However, a bias is unlikely, as there is no reason to believe that the rate of degradation varied in a systematic manner between case and control subjects.

On the other hand, the fact that only serum samples taken after overnight fasting were available from the autoantibody positive cases for vitamin E analysis, whereas fasting was not required from the unaffected controls, introduced a source of potential systematic bias. However, diurnal variations in plasma vitamin E concentration seem to be small, and the concentration was in fact higher after overnight fasting (Nierenberg and Stukel

1987). Thus, it is highly unlikely that the inverse association between serum α-tocopherol concentration and the risk of type 1 diabetes was a result of systematic bias. For assurance, this potential source of error was dealt with by conducting a separate analysis among a subcohort of autoantibody positive members of the main cohort. In this subcohort, all the serum samples had been collected after overnight fasting.

The number and timing of serum samples varied widely between the study subjects within the subcohort, which caused difficulties in the statistical analyses. For instance, time-dependent covariates or individual trends could not be included in the statistical model. To utilize the information on the repeated serum samples collected during the follow-up of the seropositive subcohort, the mean of α–tocopherol concentration in the repeated samples was calculated and used as the explanatory variable in the statistical model, which may not be an orthodox solution in a statistical model evaluating survival time. However, the relative lack of epidemiologic studies on the associations of vitamin E and type 1 diabetes made the serum samples from the DiMe cohort too valuable not to be analysed despite these difficulties; only Knekt and coworkers (1999) had published a study on this subject before, and their samples had also been stored at –20°C.

By contrast, a major strength in the DIPP vitamin E analyses was that serum samples collected specifically for the purposes of antioxidant analyses were available. The serum samples were frozen at –70°C, and were of good quality judged by the vitamin E concentrations. Because serum was collected at regular intervals of one year, it was possible to assess the overall effect of vitamin E concentrations in repeated samples.

6.2 Associations of vitamin E and preclinical type 1 diabetes

The association of vitamin E with clinical type 1 diabetes was assessed using frozen serum samples from the DiMe Study. Despite the small number of cases, inverse associations of borderline significance were observed both in the nested case-control design including cases with diabetes and their matched, seronegative and non-diabetic controls, and in the subcohort of children with preclinical type 1 diabetes. The associations were of similar magnitude to those in an earlier report by Knekt et al. (1999) of a significant inverse association between serum vitamin E and type 1 diabetes among adult Finnish men, and lent some support to the hypothesis of a protective effect of vitamin E against developing clinical type 1 diabetes.

The associations of vitamin E with preclinical type 1 diabetes, based on the presence of type 1 diabetes-associated autoantibodies in serum, were analyzed using both serum samples and maternal food frequency data from the DIPP Study. Serum α- and γ-tocopherol concentrations of cases with pre-type 1 diabetes and matched seronegative, non-diabetic controls were compared in a nested case-control design. Neither α- or γ-tocopherol

concentration in the serum sample collected at one year of age, nor the overall α- or γ-tocopherol concentration up to the age of seroconversion of the case were associated with the risk of preclinical type 1 diabetes. The only significant finding was an interaction between high vs. intermediate concentration of γ-tocopherol at the age of 1 year and the time of seroconversion, which could indicate short-term protection of γ-tocopherol against developing advanced β-cell autoimmunity.

Correspondingly, maternal dietary intake of vitamin E during pregnancy was not associated with the risk of preclinical type 1 diabetes in the child. Neither was maternal vitamin E intake associated with early endpoints appearing before the age of 2.5 years or with clinical type 1 diabetes. Because of the poor validity of the FFQ for vitamin E (Erkkola et al. 2001), the lack of association could result from attenuation caused by unreliable intake estimates. The main food sources of vitamin E – dietary fats, cereal products and vegetables – were not associated with pre-type 1 diabetes in the child either, except for low-fat margarines, which showed an inverse association. The validity of the FFQ was acceptable for cereal products and vegetables, but poor for vegetable oils, the most important source of vitamin E. Therefore, a protective effect of maternal vitamin E intake during pregnancy against pre- type 1 diabetes cannot be ruled out despite the finding of no association in the present study.

The finding of no association between serum vitamin E concentrations and pre-type 1 diabetes in the DIPP cohort was unexpected, since significant (Knekt et al. 1999) and borderline (Study I) inverse associations were found in the two earlier analyses on the Mobile Clinic Study (Knekt et al. 1999) and the DiMe Study (Study I). The discrepancy of the results could be explained by false inverse association in the Mobile Clinic and DiMe Studies, or false null association in the DIPP Study. The inverse results in the first two studies may be due to chance, as the number of cases was small and accordingly the confidence intervals of the measure of association were wide in both analyses. Also, the serum samples from both the Mobile Clinic Study and the DiMe Study had been stored frozen at –20°C for several years, and their quality is thus likely to be inferior to the quality of the DIPP samples. Indeed, vitamin E concentrations were low especially in the DiMe samples. This may have caused inaccuracy in the results, even if one would expect that the association was attenuated towards null. In consideration of this, confounding by some factor in serum which slows down the degradation of vitamin E upon storage seems possible.

Even if no significant association was observed in the DIPP analysis, confidence intervals did not exclude a moderate inverse association of serum vitamin E concentrations with the risk of pre-type 1 diabetes. The results could also be attenuated by the considerable intraindividual variation in serum vitamin E concentrations (Tangney et al. 1987). We had only one serum sample from each individual per year, and for over 40% of cases there was

only one serum sample available overall. Furthermore, the validity of serum vitamin E concentrations as an indicator of dietary intake is uncertain, especially in children (Byers et al. 1993, Hercberg et al. 1994, Ortega et al. 2005, Drewel et al. 2006, Kim et al. 2006).

However, it can also be argued that the findings may reflect a real phenomenon of vitamin E protecting against overt type 1 diabetes, but not against the preclinical stage of the disease. The development of type 1 diabetes begins with genetic disposition and environmental triggers, which are followed by autoimmunity, loss of β-cell mass, and finally overt diabetes (Babaya et al. 2005). In many subjects, the insulitis present in the prediabetic stage will spontaneously recover and not progress to clinical disease. The environmental risk factors may be different for insulitis and for clinical disease (Ludvigsson 2006). In NOD mice, for example, small modifications of the immune system can prevent progression to diabetes, even if they usually do not prevent insulitis (Babaya et al. 2005). Accordingly, vitamin E may not prevent the autoimmune process which causes the insulitis and initiates β-cell damage, but may protect the β-cells against the cytotoxic effects associated with the autoimmune attack (Beales et al. 1994, Hayward et al. 1992, Hyppönen 2004). The findings on vitamin E in the present and previous work (Knekt et al. 1999) – borderline or significant inverse association of serum concentration with clinical diabetes, borderline inverse association of serum concentration for advancing from preclinical to clinical diabetes, no proven association of serum concentration or maternal intake with pre-type 1 diabetes – fit well into such a scenario.

6.3 Antioxidant intake during pregnancy

There was substantial variation in the intake of antioxidant nutrients among the pregnant women; the difference between the 25th and 75th percentile ranged from almost 50% for selenium to nearly 150% for β-carotene. The supplementary intake of antioxidant nutrients was, on average, modest, as the mean intakes from dietary supplements covered only a small fraction of the total intakes. The most commonly consumed type of dietary supplements containing antioxidant nutrients were multivitamin and mineral supplements, and few pregnant mothers had supplements with large doses of single antioxidant nutrients. Probably the dietary supplements were not consumed specifically to achieve antioxidant protection, but to ensure a sufficient intake of vitamins and minerals in general.

Antioxidant nutrients were supplied by variable dietary sources. β-carotene and vitamin C came from vegetables and fruits, while dietary fats were the main sources of vitamin E. The most important sources of the trace elements selenium, zinc and manganese were cereals and for selenium and zinc also animal products, while retinol was supplied mostly by offal and milk products. In general, foods rich in antioxidant nutrients seem to indicate a healthy diet; they tend also to be rich in other nutrients and dietary fiber, and have a low

content of refined sugar and often a low content of saturated fat as well. Correspondingly, a low dietary intake of antioxidant nutrients may often indicate a refined, energy-dense diet.

All the background factors analyzed had independent effects on the antioxidant intake of pregnant women. Because the results for each background factor are adjusted for all the other background variables, the differences in the intake figures according to any single background factor seem small. For example, the difference in energy-adjusted vitamin E intake between the youngest and oldest age groups, adjusted for all the other background variables, was less than 8%. However, the overall variation in antioxidant intake by the sociodemographic and lifestyle characteristics analyzed was considerable. The variation in the intake of β-carotene was almost threefold, that of manganese and retinol more than twofold, and that of vitamin C almost twofold, while for vitamin E, zinc and selenium the variation was less than 40%. Because of the poor validity of the FFQ for vitamin E, the estimates of variation by background factors may also be misleading.

A wealth of evidence indicates that a higher socioeconomic position is associated with healthier food choices (Prättälä et al. 1992, Roos et al. 1998, De Irala-Estevez et al. 2000, Groth et al. 2001, Laaksonen et al. 2003). During pregnancy, the interest in a healthy diet often increases (Tuffery and Scriven 2005, Grace et al. 2006), and it has been reported that less health-conscious women are more likely to make greater changes towards healthy food choices than more health-conscious women (Olson 2005). However, the socioeconomic gradient in food choices persists during pregnancy. In the present study, young age, low level of education, and smoking during pregnancy were associated with a lower intake of most antioxidant nutrients, and similar findings have been reported in earlier surveys (Wynn et al. 1994, Erkkola et al. 1998, Rogers et al. 1998, Mathews et al. 2000, Freisling et al. 2006). The finding that the intakes of some foods and nutrients among pregnant women are more strongly associated with the partner's rather than own level of education is novel, and may indicate a complex interplay of education level and available income as determinants of food choices.

6.4 Associations of maternal intake of dietary antioxidants during pregnancy and preclinical type 1 diabetes in the offspring

In the present work, no evidence was observed of a protective effect of maternal antioxidant intake during pregnancy against the development of type 1 diabetes in the offspring. Lower ends of the CI for the HR per twofold increase in total intake of each analyzed antioxidant nutrient – which represents a large proportion of the total variation of intake within the study population – varied between 0.70 and 0.90, excluding substantial inverse associations. Although all the sociodemographic and lifestyle variables analyzed were

associated with the maternal diet, there was no confounding as their relationships with the endpoint variable were weak. Among the other baseline characteristics, genetic risk group, diabetes in a first-degree relative and gestational age were associated with pre-type 1 diabetes, but they did not confound the etiologic associations.

The associations were analyzed in a large, prospective, population-based study cohort with a reasonable number of cases. The variation in the intakes of the antioxidant nutrients within the study population was substantial, even if high supplementary intakes were uncommon among the pregnant mothers of the cohort.

Dietary intake during pregnancy was assessed using a detailed FFQ validated specifically for the purposes of the present study (Erkkola et al. 2001). Despite acceptable repeatability and validity of the FFQ, measurement error is still likely to persist in dietary intake figures. For example, the time interval of a few months between the reference period and the completion of the FFQ may lead to memory errors. Most likely, the associations reported are to some degree attenuated towards no association by random measurement error, as is usual in epidemiologic analyses (Willett 1998). A systematic between-person error – overestimation of dietary consumption – was observed in the validation study (Erkkola et al. 2001), but such an error has no effect on the measure of association with the endpoint variable. Systematic within-person errors may result, for example, if food items important for the responder are missing from the food list of the questionnaire, or are misinterpreted. Systematic within-person errors may also exist in the present data, but as long as they are not associated with the outcome variable, they will not bias the results (Willett 1998).

The validity of the FFQ was tested against food records, which in general provide the best available reference method (Willett and Lenart 1998). However, as both methods use the same dietary database to calculate nutrient intakes on the basis of food consumption, a potential error in this phase could lead to overestimation of the validity of the FFQ. For example, the bioavailability of selenium seems to differ according to the source (Finley 2006), which is not taken into account in assessing the nutrient intakes. Therefore both the FFQ and food records could be inaccurate measures of bioavailable selenium, and consequently the association of estimated selenium intake with pre-type 1 diabetes could be attenuated. However, as the differences in bioavailability seem to be modest (Finley 2006), a major attenuation is unlikely.

In trying to assess the effects of potential differences in the bioavailability of nutrients from different food groups, it is useful to analyze the associations of dietary sources of each nutrient with the disease of interest. In the present study, the consumption of none of the main dietary sources of antioxidant nutrients during pregnancy was associated with pre-type 1 diabetes in the child. Among all foods, only low-fat spreads, coffee and berries showed an inverse association after appropiate adjustments, and none of these stands out

as an important source of the antioxidant nutrients studied.

In theory, maternal antioxidant intake during pregnancy could affect the future risk of type 1 diabetes in the offspring by at least two different mechanisms. The most likely explanation is that maternal intake determines the antioxidant status of the newborn, which, in turn, is expected to be associated with his or her risk of developing the disease. Correlations between the blood concentrations of antioxidant nutrients between mother and newborn infant have been observed in several studies (Vobecky et al. 1982, Tsuchiya et al. 1984, Wasowicz et al. 1993, Lee et al. 1995, Dimenstein et al. 1996, Karakilcik et al. 1996, Zapata et al. 1997, Dejmek et al. 2002, Gazala et al. 2003, Takser et al. 2004). However, the baby's own dietary intake is likely to become the most important determinant of his/her antioxidant status soon after birth, or at least as soon as he or she starts to consume other foods than breast milk. Probably type 1 diabetes starts to develop during the fetal or neonatal period in only a minority of cases who will later be affected by pre-type 1 diabetes, and therefore no association with maternal intake is seen. Clinical type 1 diabetes may manifest even several years after the seroconversion to autoantibody positivity (Daneman 2006). As the time span from the fetal time to the endpoint is much longer than for prediabetes, it is not very likely that maternal antioxidant intake is associated with overt disease. In fact, no evidence for such an association was observed in the present study.

An alternative potential mechanism of protection against type 1 diabetes by antioxidants could be fetal programming analogous to what has been proposed for chronic metabolic diseases of adulthood (Barker et al. 1989). According to this hypothesis, low maternal intake of antioxidant nutrients causes permanent metabolic or structural alterations in the fetus, thereby predisposing it to type 1 diabetes later in life. At present, there is no evidence to support this view. As antioxidants are hypothesized to exert their action by protecting the β-cells against free radical action, fetal programming seems a less liss likely mechanism of protection than a direct effect of antioxidants in the islets.

7 SUMMARY AND CONCLUSIONS

7.1 Antioxidant nutrients in early life

The present study covered some aspects of the relatively unexplored field of antioxidant nutrients in the development of type 1 diabetes. The main findings of the present study were possible protection by vitamin E against overt type 1 diabetes, no evidence of a protective role of vitamin E against preclinical type 1 diabetes, and no association between maternal intake of antioxidant nutrients during pregnancy and preclinical type 1 diabetes in the offspring.

Antioxidants have been studied most widely in the context of cardiovascular diseases and cancer, the two major degenerative diseases in adults. The overall conclusion of the research conducted so far can be described as 'the antioxidant paradox' (Halliwell 2000); diets rich in fruits and vegetables are inversely associated with mortality, cardiovascular diseases and certain types of cancer, but antioxidant supplements have not been beneficial in intervention studies (Stanner et al. 2003).

A possible explanation for this paradox is that the protective effects of fruits and vegetables are caused by some other bioactive compounds than antioxidant nutrients. Fruits and vegetables contain a number of other potential anticarcinogens than antioxidants (Potter and Steinmetz 1996), and, conversely, other foods than fruits and vegetables may have a high content of antioxidants (Halvorsen et al. 2006). On the other hand, it is possible that a single or a few antioxidants given in high doses are not beneficial, but a balanced supply of a variety of different antioxidative compounds is needed to achieve protection against free radicals. Different antioxidants may have synergistic effects (Lampe 1999, Stanner et al. 2003, Stahl and Sies 2005). Also, antioxidants locate themselves in different cell compartments according to their hydrophilic or lipophilic characteristics, and a wide variety of antioxidants may be needed for a balanced protective effect (Eastwood 1999). For example, it has been shown that β-cells are best protected not by single antioxidant enzymes, but by a combination of enzymes (Tiedge et al. 1998).

The evidence for the associations of dietary factors with the development of type 1 diabetes is fragmentary, and no protection by fruits and vegetables or by antioxidant nutrients has been confirmed. By contrast, an ecological study among adolescent populations in Europe indicated a positive correlation between combined fruit and vegetable consumption, and the incidence of type 1 diabetes (Thorsdottir and Ramel 2003). The direction of association was similar in the DIPP cohort, in which early age at introduction of fruits and berries into the diet of infants was associated with an increased risk of pre-type 1 diabetes (Virtanen et al. 2006). Certain vegetables and fruits contain nitrate and possibly also other

potentially diabetogenic compounds (reviewed in Thorsdottir and Ramel 2003), and it may be speculated that these could counteract the possible protective effects of vitamin C and β-carotene, the two antioxidants that come mainly from fruits and vegetables.

At present, there is no basis for recommending antioxidants in the form of dietary supplements for children or pregnant women to achieve protection against type 1 diabetes, but dietary consumption of antioxidant nutrients may be encouraged for other reasons. Antioxidant nutrients are supplied by varied sources. The most classical antioxidant nutrients, vitamin E, β-carotene and vitamin C, are mainly provided by vegetarian sources, but animal products are important sources of trace elements which act as cofactors in antioxidant enzymes. A balanced intake of antioxidant nutrients from foods may often serve as an indicator of a balanced and healthy diet rich in other nutrients and dietary fiber as well, with a low consumption of refined foods, added sugar and often also with low supply of saturated fat. This kind of diet corresponds strikingly well to the Finnish dietary recommendations for children and pregnant women (Hasunen et al. 2004).

7.2 Implications for future research

7.2.1 Antioxidants and the risk of type 1 diabetes

The question of a possible protective role of antioxidant nutrients against developing type 1 diabetes remains open, but the findings of the present study may be of use in planning future studies on this issue.

In future research, the main effort should be targeted towards overt type 1 diabetes. The present and earlier observations indicated possible protection by vitamin E against overt disease, and support for this idea can also be found in experimental studies. In light of the present findings, analyzing antioxidant nutrients as protective factors against preclinical type 1 diabetes does not seem to be a promising research strategy.

Maternal intake of antioxidant nutrients during pregnancy did not appear to be associated with the development of type 1 diabetes in the offspring, and attention should next be turned towards the child's own antioxidant intake. As it is possible that antioxidant nutrients do not affect the early, preclinical stage of the disease, it is conceivable that the child's own dietary intake may be a more important predictor of the disease risk than the antioxidant status during fetal or neonatal life.

High doses of single or a few antioxidant nutrients have not been proven to prevent cancer or cardiovascular diseases, in the context of which antioxidants have been most widely studied. Likewise, in studies on the associations of antioxidants with type 1 diabetes, it could be useful to take into account the overall intake of antioxidants instead of analyzing the intake of each antioxidant separately. Efforts have been made to develop

indices of combined antioxidant intake (Wright et al. 2004), but further research on the methodology of such a holistic approach are warranted. Also, assays measuring total antioxidant activity in blood (Benzie and Strain 1996) could be useful tools in future research on the development of type 1 diabetes.

Among the eight chemical forms of vitamin E, α-tocopherol has for a long time received a wealth of scientific attention, probably because of its abundance in human tissues (Hensley et al. 2004) and its superior effect in the traditional test used in measuring vitamin E activity (Jiang et al. 2001). However, other tocopherols and tocotrienols are also worth studying. Recently, γ-tocopherol has been shown to be a more effective antioxidant than α-tocopherol in some circumstances (Jiang et al. 2001, Dietrich et al. 2006). The interaction between serum γ-tocopherol concentration and the age of seroconversion to pre-type 1 diabetes observed in the present work may be interpreted as indicative of short-term protection by γ-tocopherol against pre-type 1 diabetes, which makes γ-tocopherol an interesting new research target in the etiology of the disease.

7.2.2 Methodological implications

In nutritional epidemiology, the main concern is to measure the true long-term intake level of the nutrient of interest for each individual (Willett 1998). In this respect, vitamin E eludes the researcher like the end of the rainbow. No gold standard for measuring vitamin E intake practical enough to be used in large epidemiologic samples has been identified, and each method has problems associated with its application. The validity of blood concentrations as an indicator of dietary vitamin E intake has not been established (Stryker et al. 1988, Sasaki et al. 2000, Kabagambe et al. 2001, McNaughton et al. 2005), especially for children (Byers et al. 1993, Hercberg et al. 1994, Ortega et al. 2005, Drewel et al. 2006, Kim et al. 2006). On the other hand, the lack of validity could result from problems associated with the methods for dietary assessment to which the circulating concentrations are compared (Romieu et al. 1990). There is considerable day-to-day intraindividual variation both in blood concentrations and in dietary intakes. Therefore, repeated blood samples or several days of dietary measurement are needed for a reliable estimate; otherwise the observed associations with the given disease are likely to be attenuated (Tangney et al. 1987). In the FFQ employed in the present study, the validity for vitamin E was poor, which could result from difficulties in identifying the types of fat used in cooking. Further studies are clearly needed to identify the optimal methods for measuring vitamin E intake, and the validity of the instruments used should be tested and reported. In the absence of a gold standard, it may be advisable to employ several different methods in the validation studies (Kabagambe et al. 2001).

The residual method proposed by Willett (1998) has been widely used in nutritional research. In the present work, the use of residuals in etiological analyses caused unexpected changes in the confidence intervals of the measure of association with the disease. The magnitude of the change varied according to the degree of correlation between energy intake and the intake of each nutrient. Therefore, despite the many virtues of the residual method, it may be recommended that the energy-adjusted results are always compared with crude results, and, in case of discrepancy, carefully scrutinized.

The dietary intake during pregnancy was determined by a wide variety of sociodemographic and lifestyle factors. Especially, the partner's education affected the diet of the pregnant mother independently of her own education level. This finding highlights the importance of the family as a socio-economic unit. When studying the social determinants of food choices, or adjusting for socio-economic status in etiological analyses, the social position of other family members and the available family income should accordingly be taken into account.

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Willett W. Nutritional epidemiology. 2nd ed. New York: Oxford University Press; 1998.
Willett W, Lenart E. Reproducibility and validity of food-frequency questionnaires. In: Willett W, editor. Nutritional epidemiology. 2nd ed. New York: Oxford University Press; 1998. p.101-47.
Williams AJK, Bingley PJ, Bonifacio E, Palmer JP, Gale EAM. A novel micro-assay for insulin autoantibodies. J Autoimmun 1997;10:473-8.
Wright ME, Mayne ST, Stolzenberg-Solomon RZ, Li Z, Pietinen P, Taylor PR, et al. Development of a comprehensive dietary antioxidant index and application to lung cancer risk in a cohort of male smokers. Am J Epidemiol 2004;160:68-76.
Wynn S, Wynn A, Doyle W, Crawford M. The association of maternal social class with maternal diet and the dimensions of babies in a population of London women. Nutr Health 1994;9**:**303-15.
Yang J, Cherian MG. Protective effects of metallothionein on streptozotocin-induced diabetes in rats. Life Sci 1994;55:43-51.
Yu MS, Norris JM, Mitchell CM, Butler-Simon N, Groshek M, Follansbee D, et al. Impact on maternal parenting stress of receipt of genetic information regarding risk of diabetes in newborn infants. Am J Med Genet 1999;86: 219-26.
Zapata CL, Melo MR, Donangelo CM. Maternal, placental and cord zinc components in healthy women with different levels of serum zinc. Biol Neonate 1997;72:84-93.
Zhao HX, Mold MD, Stenhouse EA, Bird SC, Wright DE, Demaine AG, et al. Drinking water composition and childhood-onset Type 1 diabetes mellitus in Devon and Cornwall, England. Diabet Med 2001;18:709-17.
Zheng M, Storz G. Redox sensing by prokaryotic transcription factors. Biochem Pharmacol 2000;59:1-6.
Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. JAMA 2003;290:1721-8.
Zunino SJ, Storms DH, Stephensen CB. Diets rich in polyphenols and vitamin A inhibit the development of type I autoimmune diabetes in nonobese diabetic mice. J Nutr 2007;137:1216-21.

APPENDIX 1. SYSTEMATIC LITERATURE SEARCH

1. A PubMed search was conducted on 4th March, 2008, using the following search terms:

#1 Vitamin E	28679
#2 Tocopherols	3851
#3 alpha-Tocopherol	25765
#4 beta-Tocopherol	71
#5 gamma-Tocopherol	838
#6: #1 OR #2 OR #3 OR #4 OR #5	31315
# 7 Diabetes Mellitus, Experimental	23814
#8 Mice, Inbred NOD	4245
#9 Prediabetic State	2380
#10 Diabetes Mellitus, Type 1	47109
#11 Rats, Inbred BB	1171
#12 Autoantibodies	73121
#13 Insulin-Secreting cells	1983
#14 Islets of Langerhans	33633
#15: #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14	168 075
#16 Causality	5289301
#17 Risk	927855
#18 Risk Factors	398068
#19 etiology	5 341 199
#20 prevention and control	714 483
#21 Incidence	1 249 582
# 22 Epidemiology	1 061 680
#23: #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22	6 521 626
#24: #6 AND #15 AND #23	278

The abstracts of the resulting 278 articles were read. The following 9 abstracts fulfilled the inclusion criteria:

Uusitalo L, Knip M, Kenward MG, Alfthan G, Sundvall J, Aro A, et al. Serum alpha-tocopherol concentrations and risk of type 1 diabetes mellitus: a cohort study in siblings of affected children. J Pediatr Endocrinol Metab 2005;18:1409-16.
Not included. Part of the dissertation.

Hyppönen E. Micronutrients and the risk of type 1 diabetes: vitamin D, vitamin E, and nicotinamide. Nutr Rev 2004;62:340-7.
Included

Knekt P, Reunanen A, Marniemi J, Leino A, Aromaa A. Low vitamin E status is a potential risk factor for insulin-dependent diabetes mellitus. J Intern Med 1999;245:99-102.
Included

Leinonen JS, Alho H, Harmoinen A, Lehtimäki T, Knip M. Unaltered antioxidant activity of plasma in subjects at increased risk for IDDM. Free Radic Res 1998;29:159-64.
Included

Ramesh B. Dietary management of pancreatic beta-cell homeostasis and control of diabetes. Med Hypotheses 1996;46:357-61.
Included

Beales PE, Williams AJ, Albertini MC, Pozzilli P. Vitamin E delays diabetes onset in the non-obese diabetic mouse. Horm Metab Res 1994;26:450-2.
Included

Murthy VK, Shipp JC, Hanson C, Shipp DM. Delayed onset and decreased incidence of diabetes in BB rats fed free radical scavengers. Diabetes Res Clin Pract 1992;18:11-6.
Included

Hayward AR, Shriber M, Sokol R. Vitamin E supplementation reduces the incidence of diabetes but not insulitis in NOD mice. J Lab Clin Med 1992;119:503-7.
Included

Behrens WA, Scott FW, Madère R, Trick K, Hanna K. Effect of dietary vitamin E on the vitamin E status in the BB rat during development and after the onset of diabetes.
Ann Nutr Metab 1986;30:157-65.
Included

The following 14 abstracts potentially fulfilled the inclusion criteria, and the full text articles were assessed:

Asayama K, Kooy NW, Burr IM. Effect of vitamin E deficiency and selenium deficiency on insulin secretory reserve and free radical scavenging systems in islets: decrease of islet manganosuperoxide dismutase. J Lab Clin Med 1986;107:459-64.
Included

Hsieh CC, Lin BF. The effects of vitamin E supplementation on autoimmune-prone New Zealand black x New Zealand white F1 mice fed an oxidised oil diet. Br J Nutr 2005;93:655-62.
Not included

Tsujinaka K, Nakamura T, Maegawa H, Fujimiya M, Nishio Y, Kudo M, Kashiwagi A. Diet high in lipid hydroperoxide by vitamin E deficiency induces insulin resistance and impaired insulin secretion in normal rats. Diabetes Res Clin Pract 2005;67:99-109.
Not included

Kauffman LD, Sokol RJ, Jones RH, Awad JA, Rewers MJ, Norris JM. Urinary F2-isoprostanes in young healthy children at risk for type 1 diabetes mellitus. Free Radic Biol Med 2003;35:551-7.
Not included

Mayne ST. Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 2003;133 Suppl 3:933-40.
Not included

Stetinová V, Grossmann V. Effects of known and potential antioxidants on animal models of pathological processes (diabetes, gastric lesions, allergic bronchospasm). Exp Toxicol Pathol 2000;52:473-9.
Included

Torres MD, Canal JR, Pérez C. Oxidative stress in normal and diabetic rats. Physiol Res 1999;48:203-8.
Not included

Tajiri Y, Grill VE. Interactions between vitamin E and glucose on B-cell functions in the rat: an in vivo and in vitro study. Pancreas 1999;18:274-81.
Not included

Pozzilli P, Visalli N, Cavallo MG, Signore A, Baroni MG, Buzzetti R, et al. Vitamin E and nicotinamide have similar effects in maintaining residual beta cell function in recent onset insulin-dependent diabetes (the IMDIAB IV study). Eur J Endocrinol 1997;137:234-9.

Erratum in: Eur J Endocrinol 1997;137:558.
Not included

Papaccio G, Baccari GC, Frascatore S, Sellitti S, Pisanti FA. The vitamin-E derivative U-83836-E in the low-dose streptozocin- treated mouse: effects on diabetes development. Diabetes Res Clin Pract 1995;30:163-71.
Not included

Flechner I, Maruta K, Burkart V, Kawai K, Kolb H, Kiesel U. Effects of radical scavengers on the development of experimental diabetes. Diabetes Res 1990;13:67-73.
Included

el-Hage A, Herman EH, Yang GC, Crouch RK, Ferrans VJ. Mechanism of the protective activity of ICRF-187 against alloxan-induced diabetes in mice.
Res Commun Chem Pathol Pharmacol 1986;52:341-60.
Included

Goodwin JS, Garry PJ. Relationship between megadose vitamin supplementation and immunological function in a healthy elderly population. Clin Exp Immunol 1983;51:647-53.
Not included

Ayres S Jr, Mihan R. Is vitamin E involved in the autoimmune mechanism?
Cutis 1978;21:321-5.
Not included

2. A search in the Web of Science was conducted on 10th March, 2008, using the following search terms:

#1 Vitamin E	37 637
#2 Tocopherols	4167
#3 Alpha-tocopherol OR alphatocopherol	17 157
#4 Beta-tocopherol	1308
#5 Betatocopherol	1
#6 Gamma-tocopherol	1667
#7 Gammatocopherol	4
#8: #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7	45 383
#9 Type 1 diabetes	69 554
#10 Insulin-dependent diabetes	60 452
#11 Juvenile diabetes	1235
#12 Childhood diabetes	3668

#13 Experimental diabetes	21 710
#14 NOD mice	7879
#15 Mice, inbred NOD	4600
#16 Prediabetes	358
#17 BB rats	3584
#18 Rats, inbred BB	1682
#19 Autoantibodies	44 163
#20 Insulin-secreting cells	3080
#21 Islet cells	15 178
#22 Islets of Langerhans	20 996
#23 Beta cells	100 000
#24: #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23	>100 000
#25 Causality	15 092
#26 Risk	>100 000
#26 Risk factors	>100 000
#27 Etiology	>100 000
#28 Prevention	>100 000
#29 Incidence	>100 000
#30 Epidemiology	>100 000
#31 Hazard	46 922
#32: #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31	>100 000
#33: #8 AND #24 AND #32	1408

The titles of the resulting 1408 articles were read. There were 61 studies that potentially met the inclusion criteria. The abstracts of these 61 articles were read.

The following 10 abstracts not yet identified potentially fulfilled the inclusion criteria, and the full text articles were assessed:

Reiter E, Jiang Q, Christen S. Anti-inflammatory properties of alpha- and gamma-tocopherol. Mol Aspects Med 2007;28:668-91.
Not included

Koizumi T, Bando N, Terao J, Yamanishi R. Feeding with both beta-carotene and supplemental alpha-tocopherol enhances type 1 helper T cell activity among splenocytes

isolated from DO11.10 mice. Biosci Biotechnol Biochem 2006;70:3042-5.
Not included

Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: A review. Journal of Biochemical and Molecular Toxicology 2003;17:24-38
Not included

Brigelius-Flohe R, Kelly FJ, Salonen JT, Neuzil J, Zingg JM, Azzi A. The European perspective on vitamin E: current knowledge and futute research. Am J Clin Nutr 2002;76:703-16.
Not included

Feki M, Souissi M, Mebazaa A. Vitamin E deficiency: a risk factor in human disease? Ann Med Interne (Paris) 2001;152:398-406.
Not included

Long AC, Ching S, Quan N, Boileau T, Bray TM. Prevention of type 1 diabetes with dietary antioxidant cocktails in non-obese diabetic (NOD) mice. Free Radic Biol Med 2001;31 Suppl:34.
Not included

Ho E, Bray TM. Antioxidants, NF kappa B activation, and diabetogenesis. Proc Soc Exp Biol Med 1999;222:205-13.
Included

Vendemiale G, Grattagliano I, Altomare E. An update on the role of free radicals and antioxidant defense in human disease. Int J Clin Lab Res 1999;29:49-55.
Not included

Scott, FW. Food-induced type 1 diabetes in the BB rat. Diabetes Metab Rev 1996;12:341-59.
Not included

Bloomgarden ZT. A review of current trends in diabetes. Diabetes Care 1994;17:786-90.
Not included

3. The references of the included articles were inspected for potentially relevant studies not yet found. The following 18 studies were found:

Beales et al. 1994:
Jialal I, Grundy SM. Effect of dietary supplementation with alpha-tocopherol on the oxidative modification of low density lipoprotein. J Lipid Res 1992;33:899-906.
Not included

Behrens et al. 1986:
Slonim AE, Surber ML, Page DL, Sharp RA, Burr IM. Modification of chemically induced diabetes in rats by vitamin E. J Clin Invest 1983;71:1282-8.
Included

Ho and Bray 1999:
Virtanen SM, Aro A. Dietary factors in the aetiology of diabetes. Ann Med 1994;26:469-78.
Included

Dahlquist GG, Blom LG, Persson LA, Sandström AI, Wall SG. Dietary factors and the risk of developing insulin dependent diabetes in childhood. Br Med J 1990;300:1302-6.
Not included

Scott FW, Marliss EB. Conference summary: Diet as an environmental factor in development of insulin-dependent diabetes mellitus. Can J Physiol Pharmacol 1991;69:311-9.
Included

Rocic B, Vucic M, Knezevic-Cuca J, Radica A, Pavlic-Renar I, Profozic V, et al. Total plasma antioxidants in first-degree relatives of patients with insulin-dependent diabetes. Exp Clin Endocrinol Diabetes 1997;105:213-7.
Not included

Nomikos IN, Wang Y, Lafferty KJ. Involvement of O_2 radicals in autoimmune diabetes. Immunol Cell Biol 1989;67:85-7.
Not included

Heller B, Burkart V, Lampeter E, Kolb H. Antioxidant therapy for the prevention of type 1 diabetes. Adv Pharmacol 1997;38:629-38.
Not included

Knekt et al. 1999:
Sumoski W, Baquerizo H, Rabinovitch A. Oxygen free radical scavengers protect rat islet cells from damage by cytokines. Diabetologia 1989;32:792-6.
Not included

Kolb 1989:

Kolb H, Schmidt M, Kiesel U. Immunomodulatory drugs in type 1 diabetes. In: Eisenbarth GS (editor), Immunotherapy of type I diabetes and selected autoimmune disease. Boca Raton: CRC Press; 1989. p. 111-22.

Included

Leinonen et al. 1998:

Knekt P, Alfthan G, Marniemi J, Leino A, Reunanen A, Heliövaara M, Aho K. Vitamin E and selenium in the prevention of diabetes mellitus and rheumatoid arthritis. 1998 World Congress of Oxygen Club of California (Abstract).

Not included

Murthy et al. 1992:

Oberley LW. Free radicals and diabetes. Free Rad Biol Med 1988;5:113-24.

Included

Stetinova and Grossmann 2000:

Stahl W, Sies H. Antioxidant defense: Vitamin E and C and carotenoids. Diabetes 1997;46 Suppl 2:14-7.

Not included

Virtanen and Aro 1994:

Kolb H. Diet and autoimmunity: prospects of prevention of type I diabetes. Editorial conclusions. Diabetes Nutr Metab 1989;2:71-3.

Included

Scott FW. Alterations in single diet constituents and diabetes expression in the BB rat. In: Jaworski MA, Molnar GD, Rajotte RV, Singh B, editors. The immunology of diabetes mellitus. Excerpta Medica International Congress Series 717. Amsterdam: Elsevier Science Publishers; 1986. p. 307-12.

Included

Elliott RB. Dietary prospects of prevention of type I diabetes. Diet and autoimmunity: prospects of prevention of type I prevention. Diabetes Nutr Metab 1989;2:67-71.

Included

Scott and Marliss 1991:

Elliott RB, Reddy SN, Bibby NJ, Kida K. Dietary prevention of diabetes in the non-obese diabetic mouse. Diabetologia 1988;31:62-4.

Not included

Scott FW, Elliott RB, Kolb H. Diet and autoimmunity: prospects of prevention of type 1 diabetes. Diabetes Nutr Metabol 1989;2:61-73.

Included

4. The following articles were found through the search for studies about other antioxidants:

Thorsdottir I, Ramel A. Dietary intake of 10- to 16-year-old children and adolescents in Central and Northern Europe and association with the incidence of type 1 diabetes. Ann Nutr Metab 2003;47:267-75.
Included

5. A PubMed search was conducted on 1st April, 2008, using the following search terms:

#1 Vitamin A	37564
#2 beta Carotene	8413
#3 Carotenoids	52243
#4 Ascorbic acid	35664
#5 Selenium	18523
#6 Zinc	73684
#7 Manganese	26280
#8: #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7	199 877
# 9 Diabetes Mellitus, Experimental	23893
#10 Mice, Inbred NOD	4291
#11 Prediabetic State	2396
#12 Diabetes Mellitus, Type 1	47292
#13 Rats, Inbred BB	1174
#14 Autoantibodies	73348
#15 Insulin-Secreting cells	2070
#16 Islets of Langerhans	33763
#17: #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16	168 682
#18 Causality	5310952
#19 Risk	934 853
#20 Risk Factors	401 018
#21 etiology	5 362 990
#22 prevention and control	718 082
#23 Incidence	1 256 721

#24 Epidemiology	1 068 019
#25: #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24	6 549 603
#26: #8 AND #17 AND #25	650

The titles of the resulting 650 articles were read. There were 50 studies that potentially met the inclusion criteria. The abstracts of these 50 articles were read.

The following 13 abstracts fulfilled the inclusion criteria:

Zunino SJ, Storms DH, Stephensen CB. Diets rich in polyphenols and vitamin A inhibit the development of type I autoimmune diabetes in nonobese diabetic mice. J Nutr 2007;137:1216-21.
Included

Schott-Ohly P, Lgssiar A, Partke HJ, Hassan M, Friesen N, Gleichmann H. Prevention of spontaneous and experimentally induced diabetes in mice with zinc sulfate-enriched drinking water is associated with activation and reduction of NF-kappa B and AP-1 in islets, respectively. Exp Biol Med (Maywood) 2004;229:1177-85.
Included

Moltchanova E, Rytkönen M, Kousa A, Taskinen O, Tuomilehto J, Karvonen M et al. Zinc and nitrate in the ground water and the incidence of Type 1 diabetes in Finland. Diabet Med 2004;21:256-61.
Included

Zhao HX, Mold MD, Stenhouse EA, Bird SC, Wright DE, Demaine AG, et al. Drinking water composition and childhood-onset Type 1 diabetes mellitus in Devon and Cornwall, England. Diabet Med 2001;18:709-17.
*Include*d

Ho E, Quan N, Tsai YH, Lai W, Bray TM. Dietary zinc supplementation inhibits NFkappaB activation and protects against chemically induced diabetes in CD1 mice. Exp Biol Med (Maywood) 2001;226:103-11.
Included

Ohly P, Dohle C, Abel J, Seissler J, Gleichmann H. Zinc sulphate induces metallothionein in pancreatic islets of mice and protects against diabetes induced by multiple low doses of streptozotocin. Diabetologia. 2000;43:1020-30.
Included

Tobia MH, Zdanowicz MM, Wingertzahn MA, McHeffey-Atkinson B, Slonim AE, Wapnir RA. The role of dietary zinc in modifying the onset and severity of spontaneous diabetes in the BB Wistar rat. Mol Genet Metab 1998;63:205-13.
Included

Apostolova MD, Choo KH, Michalska AE, Tohyama C. Analysis of the possible protective role of metallothionein in streptozotocin-induced diabetes using metallothionein-null mice. J Trace Elem Med Biol 1997;11:1-7.
Included

Haglund B, Ryckenberg K, Selinus O, Dahlquist G. Evidence of a relationship between childhood-onset type I diabetes and low groundwater concentration of zinc. Evidence of a relationship between childhood-onset type I diabetes and low groundwater concentration of zinc. Diabetes Care 1996;19:873-5.
Included

Driscoll HK, Chertow BS, Jelic TM, Baltaro RJ, Chandor SB, Walker EM, et al. Vitamin A status affects the development of diabetes and insulitis in BB rats. Metabolism 1996;45:248-53.
Included

Kudriashov BA, Ul'ianov AM, Tarasov IuA. Prophylactic effect of vitamin A, neutralizing the development of experimental insulin-dependent diabetes in animals. Vopr Med Khim 1993; ;39:20-2. [Article in Russian]
Not included

Chertow BS, Webb MD, Leidy JW Jr, Cordle MB. Protective effects of retinyl palmitate on streptozotocin- and alloxan-induced beta cell toxicity and diabetes in the rat. Res Commun Chem Pathol Pharmacol 1989;63:27-44.
Included

Asayama K, Kooy NW, Burr IM. Effect of vitamin E deficiency and selenium deficiency on insulin secretory reserve and free radical scavenging systems in islets: decrease of islet manganosuperoxide dismutase. J Lab Clin Med 1986;107:459-64.
Included

The following 37 abstracts potentially fulfilled the inclusion criteria, and the full text articles were assessed:

Barbosa NB, Rocha JB, Soares JC, Wondracek DC, Gonçalves JF, Schetinger MR, Nogueira CW. Dietary diphenyl diselenide reduces the STZ-induced toxicity. Food Chem Toxicol 2008;46:186-94.
Included

Hwang D, Seo S, Kim Y, Kim C, Shim S, Jee S, et al. Selenium acts as an insulin-like molecule for the down-regulation of diabetic symptoms via endoplasmic reticulum stress and insulin signalling proteins in diabetes-induced non-obese diabetic mice. J Biosci 2007;32:723-35.
Included

Li X, Chen H, Epstein PN. Metallothionein and catalase sensitize to diabetes in nonobese diabetic mice: reactive oxygen species may have a protective role in pancreatic beta-cells. Diabetes 2006;55:1592-604.
Not included

Khaldi MZ, Elouil H, Guiot Y, Henquin JC, Jonas JC. Antioxidants N-acetyl-L-cysteine and manganese(III)tetrakis (4-benzoic acid)porphyrin do not prevent beta-cell dysfunction in rat islets cultured in high glucose for 1 wk. Am J Physiol Endocrinol Metab 2006;29:137-46.
Not included

Satyanarayana S, Sekhar JR, Kumar KE, Shannika LB, Rajanna B, Rajanna S. Influence of selenium (antioxidant) on gliclazide induced hypoglycaemia/anti hyperglycaemia in normal/alloxan-induced diabetic rats. Mol Cell Biochem 2006;283:123-7.
Not included

Quraishi I, Collins S, Pestaner JP, Harris T, Bagasra O. Role of zinc and zinc transporters in the molecular pathogenesis of diabetes mellitus. Med Hypotheses 2005;65:887-92.
Included

Chen H, Li X, Epstein PN. MnSOD and catalase transgenes demonstrate that protection of islets from oxidative stress does not alter cytokine toxicity. Diabetes. 2005;54:1437-46.
Not included

Kawasaki E, Abiru N, Eguchi K. Prevention of type 1 diabetes: from the view point of beta cell damage. Diabetes Res Clin Pract 2004;66 Suppl 1:27-32.
Included

Matthews KA, Rhoten WB, Driscoll HK, Chertow BS. Vitamin A deficiency impairs fetal islet development and causes subsequent glucose intolerance in adult rats. J Nutr 2004;134:1958-63.
Not included

Kang MK, Yoon YE, Yang JY, Kwon KB, Park JW, Jhee EC. Protective effect of retinoic acid on interleukin-1 beta-induced cytotoxicity of pancreatic beta-cells. Mech Ageing Dev 2004;125:483-90.
Included

Virtanen SM, Knip M. Nutritional risk predictors of beta cell autoimmunity and type 1 diabetes at a young age. Am J Clin Nutr 2003;78:1053-67.
Included

Habeck M. Catalytic antioxidants prevent type 1 diabetes. Drug Discov Today 2002 15;7:933-4.
Not included

Stene LC, Hongve D, Magnus P, Rønningen KS, Joner G. Acidic drinking water and risk of childhood-onset type 1 diabetes. Diabetes Care 2002;25:1534-8.
Included

Schulte im Walde S, Dohle C, Schott-Ohly P, Gleichmann H. Molecular target structures in alloxan-induced diabetes in mice. Life Sci 2002;71:1681-94.
Included

Failla ML. Might oral zinc protect pancreatic B-cells against oxidative insults? Exp Biol Med (Maywood). 2002;227:435.
Included

Song MK, Rosenthal MJ, Hong S, Harris DM, Hwang I, Yip I, et al. Synergistic antidiabetic activities of zinc, cyclo (his-pro), and arachidonic acid. Metabolism 2001;50:53-9.
Not included

Kim BJ, Kim YH, Kim S, Kim JW, Koh JY, Oh SH, et al. Zinc as a paracrine effector in pancreatic islet cell death. Diabetes 2000;49:367-72.
Included

Minami T, Shimizu M, Tanaka H, Okazaki Y, Cherian MG. Metallothionein does not protect mouse endocrine cells from damage induced by alloxan injection. Toxicology 1999;132:33-41.
Included

Malizia R, Scorsone A, D'Angelo P, Lo Pinto C, Pitrolo L, Giordano C. Zinc deficiency and cell-mediated and humoral autoimmunity of insulin-dependent diabetes in thalassemic subjects. J Pediatr Endocrinol Metab 1998;11 Suppl 3:981-4.
Not included

Leinonen JS, Alho H, Harmoinen A, Lehtimäki T, Knip M. Unaltered antioxidant activity of plasma in subjects at increased risk for IDDM. Free Radic Res 1998;29:159-64.
Included

al-Zuhair H, Mohamed HE. Vitamin C attenuation of the development of type I diabetes mellitus by interferon-alpha. Pharmacol Res 1998;38:59-64.
Included

Tsuji A, Sakurai H. Generation of nitric oxide from streptozotocin (STZ) in the presence of copper(II) plus ascorbate: implication for the development of STZ-induced diabetes. Biochem Biophys Res Commun 1998;245:11-6.
Not included

Sprietsma JE, Schuitemaker GE. Diabetes can be prevented by reducing insulin production. Med Hypotheses 1994;42:15-23.
Included

Yang J, Cherian MG. Protective effects of metallothionein on streptozotocin-induced diabetes in rats. Life Sci 1994;55:43-51.
Included

Dahlquist GG, Blom LG, Persson LA, Sandström AI, Wall SG. Dietary factors and the risk of developing insulin dependent diabetes in childhood. Br Med J 1990;300:1302-6.
Included

Baly DL, Lee I, Doshi R. Mechanism of decreased insulinogenesis in manganese-deficient rats. Decreased insulin mRNA levels. FEBS Lett 1988;239:55-8.
Not included

Not I, Weis W. Reinvestigation of the diabetogenic effect of dehydroascorbic acid. Int J Vitam Nutr Res 1983;53:51-60.
Not included

Tuvemo T, Gebre-Medhin M. The role of trace elements in juvenile diabetes mellitus. Pediatrician 1983-1985;12:213-9.
Included

Mogre K, Kashalikar SJ, Kendurkar SM. Effect of Mn++ on blood sugar level in rats. Indian J Physiol Pharmacol 1982;26:227-30.
Not included

Tadros WM, Awadallah R, Doss H, Khalifa K. Protective effect of trace elements (Zn, Mn, Cr, Co) on alloxan-induced diabetes. Indian J Exp Biol 1982;20:93-4.
Included

Alexander FW. The role of zinc in childhood diabetes mellitus. Proc Nutr Soc 1979;38:106.
Not included

Mikhail TH, Awadallah R. The effect of ATP and certain trace elements on the induction of experimental diabetes. Z Ernahrungswiss 1977;16:176-83.
Included

Toroptsev IV, Eshchenko VA, Troshkin VG. Zinc content in islet cells of the mammalian pancreas in relation to the functional state of the insular system. Bull Exp Biol Med 1974;77:119-21.
Not included

Shevchuk IA, Sanduliak LI. Relation between the concentration of zinc in the pancreas and the functional activity of the islets of Langerhans. Probl Endokrinol (Mosk) 1971; 17: 113-7. [Article in Russian]
Not included

Merlini D, Caramia F. Effect of dehydroascorbic acid on the islets of Langerhans of the rat pancreas. J Cell Biol 1965;26:245-61.
Not included

Danon G, Pilorge G, Bernard JP, Chauvel A, Lenoir P, Bourel M. Lesions of the islets of Langerhans during chronic poisoning by sodium selenite administered intravenously. Pathol Biol 1965;13:660-4. [Article in French]
Not included

Maske H. Zinc in the islands of Langerhans in pancreas, its physiological behavior and its significance for etiology of various forms of experimental diabetes. Acta Neuroveg (Wien) 1953;8:51-6. [Article in Undetermined Language]
Not included

6. A search in the Web of Science was conducted on 22th April, 2008, using the following search terms:

#1 vitamin A	>100 000
#2 retinol	14068
#3 beta Carotene	16065
#4 beta-carotene	15921
#5 carotene	17250
#6 carotenoids	16064
#7 ascorbic acid	33378
#8 selenium	32838
#9 zinc	>100 000
#10 Manganese	88692
#11: #1 OR #2 OR #3 OR #4 OR #5 OR #6OR #7 OR #8 OR #9 OR #10	>100 000
#12 Type 1 diabetes	70705
#13 Insulin-dependent diabetes	61167
#14 Juvenile diabetes	1245
#15 Childhood diabetes	3733
#16 Experimental diabetes	21 924
#17 NOD mice	8054

#18 Mice, inbred NOD	4673
#19 Prediabetes	368
#20 BB rats	3595
#21 Rats, inbred BB	1685
#22 Autoantibodies	44 610
#23 Insulin-secreting cells	3204
#24 Islet cells	15 337
#25 Islets of Langerhans	21 102
#26 Beta cells	>100 000
#27: #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26	>100 000
#28 Causality	15326
#29 Risk	>100 000
#30 Risk factors	>100 000
#31 Etiology	>100 000
#32 Prevention	>100 000
#33 Incidence	>100 000
#34 Epidemiology	>100 000
#35 Hazard	48 190
#36: #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34	>100 000
#37: #11 AND #27 AND #36	3582

The titles of the resulting 3582 articles were studied. There were 119 studies that potentially met the inclusion criteria. The abstracts of these 119 articles were studied.

The following 38 articles not yet identified potentially fulfilled the inclusion criteria, and the full text articles were assessed:

Fernandes G. Progress in nutritional immunology. Immunol Res 2008;40:244-61.
Not included

Overbeck S, Rink L, Haase H. Modulating the immune response by oral zinc supplementation: a single approach for multiple diseases. Arch Immunol Ther Exp (Warsz) 2008;56:15-30.
Included

Dejkhamron P, Menon RK, Sperling MA. Childhood diabetes mellitus: Recent advances & future prospects. Indian J Med Res 2007;125:231-50.
Not included

Gehrmann W, Elsner M, Lenzen S. The role of reactive oxygen species (ROS) for lipotoxicity in insulin producing cells. Diabetologia 2007; 50 Suppl 1:175.
Not included

Watson PE and MacDonald BW. Seasonal variation of nutrient intake in pregnancy: effects on infant measures and possible influence on diseases related to season of birth. Eur J Clin Nutr 2007;11:1271-80.
Included

Boucher BJ. Dietary risk factors for the emergence of type 1 diabetes-related autoantibodies in 2 ½-year-old Swedish children – Comments by Boucher. Br J Nutr 2006;96:991.
Not included

Elliott RB. Diabetes – A man made disease. Med Hypotheses 2006;67:388-91.
Included

Ludvigsson J. Why diabetes incidence increases- A unifying theory. Ann N Y Acad Sci 2006;1079:374-82.
Included

Morrison EY, Ragoobirsingh D, Peter SA. The unitarian hypothesis for the aetiology of diabetes mellitus. Med Hypotheses 2006; 67:1115-20.
Included

Shepshelovich D, Shoenfeld Y. Prediction and prevention of autoimmune diseases: additional aspects of the mosaic of autoimmunity. Lupus 2006;15:183-90.
Not included

Brady HL, Ross C, Erlich H, Redondo MJ, Rewers M, Norris J. Vitamin D receptor, vitamin intake, and development of islet autoimmunity in children at increased risk for type I diabetes. Diabetes 2005; 54 Suppl 1:30.
Not included

Taylor CG. Zinc, the pancreas, and diabetes: Insights from rodent studies and future directions. Biometals 2005;18:305-12.
Included

Uauy-Dagach RD. Nutrition challenges for the XXI century: from deficiencies to imbalance. FASEB Journal 2005;19 Suppl:1374.
Not included

Olcott AP, Tocco G, Tian J, Zekzer D, Fukuto J, Ignarro L, Kaufman DL. Diabetes 2004;53:2574-80.
Not included

Wang PH. Growing pains in the pursuit of diabetes prevention. Lancet 2004;363:910.
Not included

Stene LC, Joner G, Norwegian Childhood Diabet Study G. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. Am J Clin Nutr 2003;78:1128-34.
Included

Pozzilli P, Adorini L. Prevention of type I diabetes: Where do we start? J Endocrinol Invest 2003;26:292-3.
Not included

Schott-Ohly P, Partke HJ, Lgssiar A, Friesen NTE, Buchau AS, Gleichmann H. Zn2+-enriched drinking water prevented spontaneous diabetes in NOD mice. Diabetologia 2003;46 Suppl 2:181.
Not included

Soltesz G. Diabetes in the young: a paediatric and epidemiological perspective. Diabetologia 2003;46:447-54.
Not included

Stene L, Joner G. Use of cod liver oil in the first year of life is associated with lower risk of childhood onset type 1 diabetes. Diabetes 2003; 52 Suppl 1:230.
Not included

Thorsdottir I, Ramel A. Dietary intake of 10- to 16-year-old children and adolescents in Central and Northern Europe and association with the incidence of type 1 diabetes. Ann Nutr Metab 2003;47:267-75.
Not included

Lu J, Field CJ, Basu TK. The immune responses to diabetes in BB rats supplemented with vitamin A. Journal of Nutritional Biochemistry 2000;11:515-20.
Included

Stene LC, Ulriksen J, Magnus P, Joner G. Use of cod liver oil during pregnancy associated with lower risk of type 1 diabetes in the offspring. Diabetologia 2000;43:1093-8.
Included

Stene LG, Ulriksen J, Magnus P, Joner G. Negative association between use of cod liver oil during pregnancy and risk of type 1 diabetes in the offspring. Diabetologia 2000;43 Suppl 1:94.
Not included

West IC. Radicals and oxidative stress in diabetes. Diabetic Medicine 2000;17:171-80.
Not included

Delattre J, Bonnefont-Rousselot D, Bordas-Fonfrendre M, Jaudon MC. Diabetes mellitus and oxidative stress. Ann Biol Clin (Paris) 1999;57:437-44.
Not included

Ho E, Bray TM. Antioxidants, NF kappa B activation, and diabetogenesis. Proc Soc Exp Biol Med 1999;222:205-13.
Included

Vendemiale G, Grattagliano I, Altomare E. An update on the role of free radicals and antioxidant defense in human disease. Int J Clin Lab Res 1999;29:49-55.
Not included

Chausmer AB. Zinc, insulin and diabetes. J Am Coll Nutr 1998;17:109-15.
Included

Ramesh B. Dietary management of pancreatic beta-cell homeostasis and control of diabetes. Med Hypotheses 1996;46:357-61.
Included

Florence TM. The role of free radicals in disease. Aust N Z J Ophthalmol 1995;23:3-7.
Not included

Bloomgarden ZT. A review of current trends in diabetes. Diabetes Care 1994;17:786-90.
Not included

Ross AC, Ternus ME. Vitamin-A as a hormone – recent advances in understanding the actions of retinol, retinoic acid, and beta-carotene. J Am Diet Assoc 1993;93:1285-90.
Not included

Bendich A. Biological functions of dietary carotenoids. Carotenoids in Human Health 1993;691:61-7.
Not included

Bendich A. Beta-carotene and the immune-response. Proc Nutr Soc 1991;50:263-74.
Not included

Stich HF, Brunnemann KD, Mathew B, Sankaranarayanan R, Nair MK. Chemopreventive trials with vitamin-A and beta-carotene – some unresolved issues. Prev Med 1989;18:732-9.
Not included

Glatthaar C, Whittall DE, Welborn TA, Gibson MJ, Brooks BH, Ryan MMP, et al. Diabetes in Western Australian children – descriptive epidemiology. Med J Aust 1988;148:(3):117-23.
Included

Pipeleers D, Vandewinkel M. Pancreatic B-cells possess defense-mechanisms against cell-specific toxicity. Proc Natl Acad Sci U S A 1986;83:5267-71.
Not included

7. The references of the included articles were inspected for potentially relevant studies not yet found. The following 44 studies were found:

Al-Zuhair and Mohamed 1998:

Sumoski W, Baquerizo H, Rabinovitch A. Oxygen free radical scavengers protect rat islet cells from damage by cytokines. Diabetologia 1989;32:792-6.
Not included

Barbosa et al. 2008:

Barbosa NBV, Rocha JBT, Wondracek DC, Perottoni J, Zeni G, Nogueira CW. Diphenyl diselenide reduces temporarily hyperglycemia: possible relationship with oxidative stress. Chem Biol Interact 2006;163:230-38.
Not included

Berg EA, Wu JY, Campbell L, Kagey M, Stapleton SR. Insulin-like effect of vanadate and selenate on the expression of glucose-6-phosphate dehydrogenase and fatty acid synthase in diabetic rats. Biochemistry 1995;77:919-24.
Not included

Blasiak J, Sikora A, Wozniak K, Drzewoski J. Genotoxicity of streptozotocin in normal and cancer cells and its modulation by free radical. Cell Biol Toxicol 2004;20:83-96.
Not included

El-Dermerdasch FM, Yousef MI, Abou El-Naga NI. Biochemical study on the hypoglycaemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol 2005;43:57-63.
Not included

Ezaki O. The insulin-like effects of selenate in rat adipocytes. J Biol Chem 1990;265:1124-30.
Not included

Liu CT, Hse H, Lii CK, Chen PS, Sheen LY. Effects of garlic oil and diallyl trisulfide on glycemic control in diabetic rats. Eur J Pharmacol 2005;516:165-73.
Not included

Liu CT, Wong PL, Lii CK, Hse H, Sheen LY. Antidiabetic effect of garlic oil but not diallyl disulfide in rats with streptozotocin-induced diabetes. Food Chem Toxicol 2006;44:1377-84.
Not included

McNeill JH, Delgatty HLM, Battell ML. Insulin-like effects of sodium selenate in streptozotocin-induced diabetic rats. Diabetes 1991;40:1675-8.
Not included

Mukherjee B, Anbazhagan S, Roy A, Ghosh R, Chatterjee M. Novel implications of the potential role of selenium on antioxidant status in streptozotocin-induced diabetic mice. Biomed Pharmacother 1998;52:89-95.
Included

Navarro-Alarcon M, Lopes-Martinez MC. Essentiality of selenium in the human body: relationship with different diseases. Sci Total Environ 2000;249:347-71.
Not included

Naziroglu M, Cay M. Protective role of intraperitoneally administered vitamin E and selenium on the antioxidative defense mechanisms in rats with diabetes induced by streptozotocin. Biol Trace Elem Res 2001;79:149-59.
Not included

Sheng XQ, Huang KX, Xu HB. New experimental observation on the relationship of selenium and diabetes mellitus. Biol Trace Elem Res 2004;99:241-53.
Not included

Sheng XQ, Huang KX, Xu HB. Influence of alloxan-induced diabetes and selenite treatment on blood glucose and glutathione levels in mice. J Trace Elem Res Med Biol 2005;18:261-7.
Not included

Stapleton SR. Selenium: an insulin-mimetic. Cell Mol Life Sci 2000;57:1874-9.
Not included

Stapleton SR, Garlock GL., Foellmi-Adams L, Kletzien RF. Selenium: potent stimulator of tyrosyl phosphirylation and activator of MAP kinase. Biochim Biophys Acta 1997;1355:259-69.
Not included

Glatthaar et al. 1988:
Rerup CC. Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev 1970;22:485-518.
Included

Haglund et al. 1996:
Okamoto K. Experimental studies on the pathogenesis of diabetes mellitus. Acta Sch Med Univ Kyoto 1949;27:43-65.
Not included

Boquist L, Lernmark Å. Effects on the endocrine pancreas in Chinese hamsters fed zinc deficient diets. Acta Pathol Microbiol Scand 1969;76:215-28.
Included

Wilson GL, Patton NJ, McCord JM, Mullins DW, Mossman BT. Mechanisms of streptozotocin and alloxan-induced damage in rat b-cell. Diabetologia 1984;27:587-91.
Not included

Chertow et al. 1989:
Chertow BS, Baker GR. The effects of vitamin A on insulin release and glucose oxidation in isolated rat islets. Endocrinology 1978;103:1562-72.
Not included

Chertow BS, Baranetsky NG, Sivitz WI, Meda P, Webb MD, Shih JC. Cellular mechanisms of insulin release. Effects of retinoids on rat islet cell-to-cell adhesion, reaggregation, and insulin release. Diabetes 1983;32:568-74.
Not included

Chertow BS, Buschmann RJ, Kaplan RL. Cellular mechanisms of insulin release: Effects of retinol on insulin release and islet ultrastructure. Diabetes 1979;28:754-61.
Not included

Ho et al. 2001:
Kubisch HM, Wang J, Luche R, Carlson E, Bray TM, Epstein CJ, et al. Transgenic copper/zinc superoxide dismutase modulates susceptibility to type 1 diabetes. Proc Natl Acad Sci U S A 1994;91:9956-9.
Not included

Kubisch HM, Wang J, Bray TM, Phillips JP. Targeted overexpression of Cu/Zn superoxide dismutase protects pancreatic β-cells against oxidative stress. Diabetes 1997;46:1563-6.
Not included

Schulte im Walde et al. 2002:
Mathews CE, Leiter E. Resistance of ALR/Lt islets to free radical-mediated diabetogenic stress is inherited as a dominant trait. Diabetes 1999;48:2189-96.
Not included

Mathews CE, Leiter EH. Constitutive differences in antioxidant defense status distinguish alloxan-resistant and alloxan-susceptible mice. Free Radic Biol Med 1999;27:449-55.
Not included

Kawasaki et al. 2004:
Hohmeier HE, Thigpen A, Tran VV, Davis R, Newgard CB. Stable expression of manganese superoxide dismutase (MnSOD) in insulinoma cells prevents IL-1β-induced cytotoxicity and reduces nitric oxide production. J Clin Invest 1998;101:1811-20.
Not included

Lortz S, Tiedge M, Nachtwey T, Karlsen AE, Nerup J, Lenzen S. Protection of insulin-producing RINm5F cells against cytokine-mediated toxicity through overexpression of antioxidant enzymes. Diabetes 2000;49:1123-30.
Not included

Ohly et al. 2000:
Tiedge M, Lortz S, Munday R, Lenzen S. Complementary action of antioxidant enzymes in the protection of bioengineered insulin-producing RINm5F cells against the toxicity of reactive oxygen species. Diabetes 1998;47:1578-85.
Not included

Ohly P, Wang Z, Abel J, Gleichmann H. Zincsulphate induced metallothionein in pancreatic islets and protected against the diabetogenic toxin streptozotocin. Talanta 1998;46:355-9.
Included

Quraishi et al. 2005:
Liuzzi JP, Cousins RJ. Mammalian zinc transporters. Ann Rev Nutr 2004;24:151-72.
Not included

Collier G, Walder K, De Silva A et al. New approaches to gene discovery with animal models of obesity and diabetes. Ann N Y Acad Sci 2002;967:403-13.
Not included

Schott-Ohly et al. 2004:
Xu B, Moritz JT, Epstein PN. Overexpression of catalase provides partial protection to transgenic mouse beta cells. Free Radic Biol Med 1999;27:830-7.
Not included

Tuvemo and Gebre-Medhin 1983-1985:
Roth HP, Kirchgessner M. Insulingehalte in serum bzw. Plasma von zinkmangelratten vor und nach glucosestimulierung. Int J Vitam Nutr Res 1975;45:202-8.
Not included

Macapinlac MP, Pearson WN, Darby WJ. Some characteristics of zinc deficiency in the albius rat. In Prasad, editor.Zinc metabolism. Springfield: Charles Thomas; 1966. p. 142-68.
Included

Quarterman J, Florence, E. Observations on glucose tolerance and plasma levels of free fatty acids and insulin in the zinc-deficient rat. Br J Nutr 1972;29:75-9.
Not included

Awadallah R, Tahani HM, El-Dessonkey EA. Serum mineral changes due to exogenous ATP and certain trace elements in experimental diabetes. Z Ernähr Wiss 1979;18:1-7.
Included

Zhao et al. 2001:
Chandra S, Chandra RK. Nutrition, immune response, and outcome. Proc Food Nutr Sci 1986;1-65.
Not included

Morrison et al. 2006:
Haskins K, Kench J, Powers K et al. Role for oxidative stress in the regeneration of islet beta cells. J Invest Med 2004;52:45-9.
Not included

Taylor 2005:
Chen H, Carlson EC, Pellet L, Moritz JT, Epstein PN. Overexpression of metallothionein in pancreatic β-cells reduces streptozotocin-induced DNA-damage and diabetes. Diabetes 2001;50:2040-6.
Not included

Chausmer 1998:
Quarterman J, Mills C, Humphries W. The reduced secretion of and sensitivity to insulin in Zn deficient rats. Biochem Biophys Res Commun 1966;25:354-8.
Not included

Roza A, Pieper G, Johnson C, Adams M. Pancreatic antioxidant enzyme activity in normoglycemic diabetic prone BB rats. Pancreas 1995;10:53-8.
Not included

Boquist and Lernmark 1969:
Hove E, Elvehjem CA, Hart EB. The physiology of zinc in nutrition of the rat. Am J Physiol 1937;119:768-75.
Included

8. The following articles were found through the search for studies about vitamin E:

Knekt P, Reunanen A, Marniemi J, Leino A, Aromaa A. Low vitamin E status is a potential risk factor for insulin-dependent diabetes mellitus. J Intern Med 1999;245:99-102.
Included

APPENDIX 2. INCLUSION AND EXCLUSION CRITERIA OF STUDIES FOR THE SYSTEMATIC REVIEW

The criteria for inclusion and exclusion of the studies determined prior to the search procedure were as follows:

Inclusion criteria:

- explanatory variable: intake and/or biomarker of vitamin E, vitamin A, β-carotene, vitamin C, selenium, zinc or manganese
- response variable: type 1 diabetes, its animal model, or a variable indicating preclinical type 1 diabetes

Exclusion criteria:

- cross-sectional studies comparing the intakes and/or biomarkers of diabetic and non-diabetic study subjects
- studies analyzing the associations of nutrients with complications or management of diabetes

During the inspection of the studies identified for the review, the criteria were further refined as follows:

- conference abstracts were excluded
- studies published in languages other than English, Finnish or Swedish were excluded. In practice, all the included articles were in English.
- studies dealing with synthetic derivatives of nutrients were excluded
- studies dealing with dehydroascorbic acid, an oxidized derivative of vitamin C, were excluded
- studies dealing with an enzyme with a nutrient as a cofactor instead of analysing the nutrient as such, were excluded
- all models of drug-induced or spontaneous diabetes in animal models were included, unless the authors defined the experiment as a model of type 2 diabetes or the study dealt with insulin resistance. The type of model employed is explicitly stated in the text.
- in animal studies, the response variable must be diabetes, hyperglycemia, blood glucose concentration, glucose tolerance test, insulitis or β-cell destruction
- in *in vitro* -studies, the response variable must be β-cell destruction or viability

APPENDIX 3. ARTICLES INCLUDED IN THE SYSTEMATIC REVIEW – VITAMIN E[1]

Reference	Design	Defined as a model of T1D	Subjects	n	Outcome	Explanatory variable	Association
Knekt et al. 1999	Nested case-control		Finnish men >=20 y	19 cases, 57 controls	T1D	Serum α-tocopherol concentrarion (also adjusted for cholesterol)	–, OR 3rd vs. 1st tertile 0.15 (0.03–0.79)
Leinonen et al. 1998	Cross-sectional		Finnish children and adolescents	51	Diabetes-associated autoantibodies	Plasma α-tocopherol concentrarion (not adjusted for cholesterol)	NS
Thorsdottir and Ramel 2003	Ecological	Yes	10–16 year-old adolescents from 11 European countries	90–1705 from each country	Incidence of T1D	Vitamin E intake	NS
Asayama et al. 1986	Experimental	No	Sprague-Dawley rats, male	7 in each group	Intraperitoneal glucose tolerance test	Vitamin E deficiency vs supplementation with 90 IU vitamin E/ L water	NS
						Vitamin E and selenium deficiency vs supplementation with 90 IU vitamin E and 0.15 mg Se /L water	–, p<0.02
Beales et al. 1994	Experimental	Yes	NOD mice, female	24 in each group	Diabetes	Vitamin E supplementation 1 g/kg diet = ca. 150 mg/kg body weight/d vs standard diet	NS, but vit E delayed the onset of diabetes
					Insulitis		NS
Behrens et al. 1986	Experimental	Yes	BB rats, male	23 in each group	Diabetes	High (1 g/kg diet) vs. low (<0.02 g/kg diet) vitamin E diet	–, p<0.09
			BB rats, female	22 in each group	Diabetes	High (1 g/kg diet) vs. low (<0.02 g/kg diet) vitamin E diet	NS
			BB rats, both sexes	45 in each group	Insulitis	High (1 g/kg diet) vs. low (<0.02 g/kg diet) vitamin E diet	–, p<0.05
El-Hage et al. 1986	Experimental	No	Charles River ICR Mice, male	6–10 in each group	Blood glucose concentration	345 mg/kg vitamin E 4x vs no vit E supplementation prior to 75 mg/kg ALX iv	–, p<0.05
Elliott 1989	Experimental	Yes	NOD mice, female	27 in each group	Diabetes	1 mg/ml vit E + 1 µg/ml selenium in drinking water from weaning vs controls	NS
Flechner et al. 1990	Experimental	Yes	BB rats, both sexes	26–28 in each group	Diabetes	2*200 IU, 50–150 days along with ebselen and Max EPA vs standard diet	–, p<0.05
						2*200 IU, 50–150 days along with nicotinamide and Max EPA vs standard diet	NS
Hayward et al. 1992	Experimental	Yes	NOD mice, female	6–8 in each group	Diabetes	High vitamin E (1000 IU/kg diet) vs. control (50 IU/kg diet) diet	–, p<0.02
					Diabetes	Low vitamin E (< 10 IU/kg diet) vs. control (50 IU/kg diet) diet	–, p<0.02
					Insulitis	High vitamin E vs. control diet	NS

Reference	Design	Defined as a model of T1D	Subjects	n	Outcome	Explanatory variable	Association
					Insulitis	Low vitamin E vs. control diet	NS
Kolb et al. 1989	Experimental	Yes	BB rats, both sexes	30–31 in each group	Diabetes	400 IU/kg diet + niacin	NS
Murthy et al. 1992	Experimental	Yes	BB rats, both sexes	29 in experimental group, 30+30 in control groups	Diabetes	A mixture of vitamin E (1.25 g/kg diet) + 3 other free radical scavengers vs. standard diet ad libitum or pair-fed	–, p=0.03–0.04
Scott 1986	Experimental	Yes	BB rats	45 in each group	Diabetes	High (1.0 g/kg diet) vs low (<0.02 g/kg) alpha-tocopherol diet	–, p<0.01
Slonim et al. 1983	Experimental	No	Wistar rats, male	12 in each group	Glucose response to IVGTT	Vitamin E supplementation 3x ip (200 U + 100 U + 100 U) vs no vit E supplementation prior to 45 mg/kg STZ iv	–, p<0.01
					Glucose response to IVGTT	Vitamin E supplementation 2x ip (200 U + 100 U) vs no vit E supplementation prior to 45 mg/kg STZ iv	–, p<0.01
					Glucose response to IVGTT	Vitamin E supplementation 1x ip (100 U) vs no vit E supplementation prior to 45 mg/kg STZ iv	–, p<0.01
					Islet cell damage		–
					Glucose response to IVGTT	Vitamin E supplementation 3x ip (200 U + 100 U + 100 U) vs no vit E supplementation prior to 90 mg/kg ALX iv	–, p<0.01
					Islet cell damage		–
					Glucose response to IVGTT	Diet deficient in vitamin E + selenium vs. normal diet prior to 25 mg/kg STZ	+, p<0.01
Stetinova and Grossman 2000	Experimental	No	H strain mice, female	6–10 in each group	Hyperglycaemia	10 mg/kg vitamin E intraperitoneally vs no supplementation prior to 120 mg/kg ALX	NS

[1] Articles are listed according to the study design (epidemiologic studies, descriptive studies, ecological studies, experimental (animal) studies, *in vitro* studies). Within each study design, articles are listed in alphabetical order according to the first author.

APPENDIX 4. ARTICLES INCLUDED IN THE SYSTEMATIC REVIEW – OTHER ANTIOXIDANT NUTRIENTS[1]

Reference	Design	Defined as T1D	Subjects	n	Outcome	Explanatory variable	Association
Knekt et al. 1999	Nested case-control		Finnish men >=20 y	19 cases, 57 controls	T1D	Serum retinol concentration	NS
						Serum selenium concentration	NS
Dahlquist et al. 1990	Case-control		Swedish children and adolescents	339 cases, 528 controls	T1D	Frequency of consumption of foods rich in vitamin C	+, *p=0.02* crude association NS stratified for nitrates and nitrites
Glatthaar et al. 1988	Case-control		Children and adolescents in Western Australia	194 cases, 753 controls	T1D	The use of vitamin C supplements for more than 1 mo, prior to diagnosis of the index case	–, OR 0.46 (0.30–0.70), *p*<0.001
Haglund et al. 1996	Case-control /semi-ecological		Incident cases with T1D, 3–14 yrs, matched controls	2,957 cases, 7,165 controls	T1D	Groundwater concentration of zinc at the place of residence 3 yrs before diagnosis	–, OR per 10–fold increase 0.8 (0.7–0.9) for total population, 0.6 (0.4–0.9) for rural areas)
						Groundwater concentration of selenium at the residence 3 yrs before diagnosis	0
Stene et al. 2000	Case-control		Children and adolescents in Vest-Agder county of Norway	85 cases, 1,071 controls	T1D	Maternal use of cod liver oil during pregnancy	–, OR 0.30 (0.12–0.75)
						Use of cod liver oil during the first year of life	–, NS
Stene et al. 2002	Case-control		Children and adolescents in Vest-Agder county of Norway	64 cases, 250 controls	T1D	Tap water concentration of zinc	–, highest vs lowest quarter, adjusted for acidity, use of well water and sociodemographic factors
						Tap water concentration of manganese	NS
Stene et al. 2003	Case-control		Norwegian children and adolescents	545 cases, 1,668 controls	T1D	Maternal use of cod liver oil during pregnancy	NS
						Use of cod liver oil during the first year of life, times/wk	–, *p*=<0.001–0.04
						Use of cod liver oil during the first year of life, starting age	–, *p*=<0.001–0.06
Leinonen et al. 1998	Cross-sectional		Finnish children and adolescents	51	Diabetes-associated autoantibodies	Plasma vitamin C concentration	NS
Moltchanova et al. 2004	Ecological		Finnish children and adolescents	3564	T1D	Groundwater concentration of zinc	NS

Reference	Design	Defined as T1D	Subjects	n	Outcome	Explanatory variable	Association
Zhao et al. 2001	Ecological		Children and adolescents in southwestern England	517 cases	T1D	Zn concentration in domestic drinking water, middle vs lowest third	–, $p=0.046$
Al-Zuhair and Mohamed 1998	Experimental	Yes	Albino rats, male	8 in each group	Plasma glucose concentration	Vitamin C (90 mg/kg/day) along with IFN-α vs neither of them	– (indirect comparison; IFN-α alone and vit C+ IFN-α tested against controls)
Apostolova et al. 1997	Experimental	Yes	MT--/- mice, female	4 in each group	Serum glucose concentration	Intraperitoneal zinc injection 1 mg/kg vs standard diet prior to STZ (85 mg/kg, ip)	–, $p<0.05$
						Intraperitoneal zinc injection 5mg/kg vs standard diet prior to STZ (85 mg/kg, ip)	–, $p<0.05$
						Intraperitoneal zinc injection 10 mg/kg vs standard diet prior to STZ (85 mg/kg, ip)	–, $p<0.05$
			MT+/+ mice, female			Intraperitoneal zinc injection 1 mg/kg, vs standard diet prior to STZ (85 mg/kg, ip)	0
						Intraperitoneal zinc injection 5mg/kg vs standard diet prior to STZ (85 mg/kg, ip)	–, $p<0.05$
						Intraperitoneal zinc injection 10 mg/kg vs standard diet prior to STZ (85 mg/kg, ip)	–, $p<0.05$
Asayama et al. 1986	Experimental	No	Sprague-Dawley rats, male	7 in each group	Intravenous glucose tolerance test	Selenium deficiency vs supplementation with 0.15 mg Se /L water	NS
						Vitamin E and selenium deficiency vs supplementation with 90 IU vitamin E and 0.15 mg Se /L water	–, $p<0.02$
Awadallah et al. 1979	Experimental	No	Sprague-Dawley rats	10–15 in each group	Hyperglycaemia	Iv injection of 1 mg/kg zinc 3x vs none with ALX 150 mg/kg ip	–, $p=0.005$
						Iv injection of 1 mg/kg Mn 3x vs none with ALX 150 mg/kg ip	–, $p=0.005$
Barbosa et al. 2008	Experimental	No	Wistar rats, male	10–27 in each group	Blood glucose concentration	Diet enriched with diphenyl diselenide (10 ppm) vs standard (0.4 ppm) diet prior to STZ (45 mg/kg ip)	NS (indirect comparison; STZ and STZ/Se groups tested against non-treated controls)
Boquist and Lernmark 1969	Experimental	No	Chinese hamsters		Glucosuria	Zinc deficient diet vs standard or Zn-supplemented diet	0, not tested
					Hyperglycaemia		0, not tested
				10–28 in each group	Glucose concentration in IPGTT		+, not tested
				7–13 in each group	Glucose concentration in IVGTT	Zinc deficient diet vs standard diet	+, not tested

Appendix 4. continued

Reference	Design	Defined as T1D	Subjects	n	Outcome	Explanatory variable	Association
					Islet infiltration	Zinc deficient vs standard diet	0
						Zinc deficient diet vs 100 µg zinc in water	0
Chertow 1989	Experimental	No	Sprague-Dawley rats, male	5 in each group	Blood glucose concentration	2.7 mg/kg retinyl palmitate ip with 60 mg/kg STZ ip vs STZ alone	–, p<0.01
				7 in each group		2.4 mg/kg retinyl palmitate ip with 100 mg/kg ALX ip vs ALX alone	–, *p*<0.05
				12 in each group		2.5 mg/kg retinyl palmitate iv with 60 mg/kg STZ iv vs STZ alone	0 overall –, p<0.05 weeks 3 and 4
				5–6 in each group		16 mg/kg retinyl palmitate iv with 60 mg/kg STZ iv vs STZ alone	–, *p*<0.05
				5–6 in each group		10 mg/kg retinyl palmitate iv with 60 mg/kg STZ iv vs STZ alone	–, *p*<0.05
Driscoll et al. 1996	Experimental	Yes	BB-DP rats, both sexes	27–31 in each group	Diabetes	Vitamin A deficient diet vs diet with 4 µg retinyl palmitate/g	–, *p*<0.02
						Vitamin A deficient diet with retinoic acid vs diet with 4 µg retinyl palmitate/g	–, *p*<0.02
					Insulitis	Vitamin A deficient diet vs diet with 4 µg retinyl palmitate/g	–, *p*<0.05
			BB-DR rats, both sexes		Insulitis	Vitamin A deficient diet vs diet with 4 µg retinyl palmitate/g	NS
Elliott 1989	Experimental	Yes	NOD mice, female	27 in each group	Diabetes	1 mg/ml vit E + 1 µg/ml selenium in drinking water from weaning vs controls	0
Ho et al. 2001	Experimental	Yes	CD-1 mice, male	5 in each group	Blood glucose concentration	500 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 50 mg/kg ALX iv	NS
						1000 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 50 mg/kg ALX iv	–, *p*<0.05
						500 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 40 mg/kg STZ ip for 5 d	NS
						1000 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 40 mg/kg STZ ip for 5 d	–, *p*<0.05
					Necrosis in islets	500 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 50 mg/kg ALX iv	–, not tested
						1000 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 50 mg/kg ALX iv	-, not tested
						500 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 40 mg/kg STZ ip for 5 d	–, not tested
						1000 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 40 mg/kg STZ ip for 5 d	–, not tested
					Infiltration of lymphocytes	500 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 40 mg/kg STZ ip for 5 d	–, not tested
						1000 ppm Zn diet vs 50 ppm Zn diet 2 wk prior to 40 mg/kg STZ ip for 5 d	–, not tested

Reference	Design	Defined as T1D	Subjects	n	Outcome	Explanatory variable	Association
Hove et al. 1937	Experimental	No	Rats		Oral glucose tolerance test	22 vs. 40 µg/d	Some differences,not tested
					Blood glucose concentration		0, not tested
					Urinary glucose concentration		0, not tested
Hwang et al. 2007	Experimental	No	NOD mice, non-diabetic at 25–27 wk of age	?	Blood glucose	5 µmol/kg/d sodium selenite ip for 3 wk vs placebo	0
Kim et al. 2000	Experimental	No	Sprague-Dawley rats	10 in each group	Diabetes	ZnEDTA vs CaEDTA infusion started 30 min prior to 65 mg/kg STZ ip	+, p<0.05
						ZnEDTA infusion started 30 min prior to 65 mg/kg STZ ip vs STZ alone	0
Lu et al. 2000	Experimental	Yes	BB rats	12 in each group	Diabetes	Diet supplemented with retinyl palmitate 60.5 IU/g diet vs standard diet	NS
						Diet supplemented with retinyl palmitate 60.5 IU/g diet and zinc 180 µg/g diet vs standard diet	+, NS
Macapinlac et al. 1966	Experimental	No	Sprague-Dawley rats, female	2–3 in each group	Intraperitoneal glucose tolerance test	Zn-deficient vs restricted-fed controls supplemented with 120 µg zinc	0, not tested
						Zn-deficient vs ad libitum fed controls supplemented with 120 µg zinc	0, not tested
Mikhail and Awadallah 1977	Experimental	No	Sprague-Dawley rats	20 in each group	Hyperglycaemia	Iv injection of 1 mg/kg zinc 2x with ALX (150 mg/kg ip) or dithizone 200 mg/kg iv) vs ALX or dithizone alone	–, not tested
					Islet destruction		–, not tested
					Hyperglycaemia	Iv injection of 1 mg/kg Mn 2x with ALX (150 mg/kg ip) or dithizone 200 mg/kg iv) vs ALX or dithizone alone	–, not tested
					Islet destruction		0
Minami et al. 1999	Experimental	No	ddY mice, male	6 in each group	Blood glucose	0.05% vs 0 zinc in drinking water 2 wk prior to 60 mg/kg ALX iv	NS
						0.1% vs 0 zinc in drinking water 2 wk prior to 60 mg/kg ALX iv	NS
						0.5% vs 0 zinc in drinking water 2 wk prior to 60 mg/kg ALX iv	NS
				2 in each group	Atrophy of islet cells	0.5% vs 0 zinc in drinking water 2 wk prior to 60 mg/kg ALX iv	0, not tested
Mukherjee et al. 1998	Experimental	No	Swiss albino mice, male	20 in each group	Blood glucose	0.5 µg sodium selenite/d and 55 mg/kg STZ ip vs 55 mg/kg STZ ip	–, p<0.05 (?)
Ohly et al. 1998	Experimental	Yes	C57BL/6 mice	10 in each group	Diabetes	Pretreatment with 10 mg ZnSO4/kg before 40 mg STZ on 5 consecutive days vs STZ alone	–, p<0.05
Ohly et al. 2000	Experimental	Yes	C57BL/6 mice, male	10–15 in each group	Hyperglycaemia	25 mmol/l zinc in drinking water started 1 week prior to STZ 5 * 40 mg/kg ip vs STZ alone	–, p<0.01
			B6SJL/F_1 mice, male				–, p<0.01

Appendix 4. continued

Reference	Design	Defined as T1D	Subjects	n	Outcome	Explanatory variable	Association
			C57BL/6 mice, male	15 in each group	Oral glucose tolerance		+, $p<0.001$
			B6SJL/F_1 mice, male				+, $p<0.01$
			C57BL/6 mice, male		Hyperglycaemia	25 mmol/l zinc in drinking water started 1 week prior to STZ 5 * 40 mg/kg ip and discontinued 1 d after last STZ dose vs STZ alone	NS
Schott-Ohly et al. 2004	Experimental	Yes	NOD mice, female	30–44 in each group	Diabetes	Continuous treatment of parents and offspring with Zn vs tap water	–, $p<0.05$
						Zn only to either the breeding pair or to their offspring vs tap water	0
					Insulitis	Continuous treatment of parents and offspring with Zn vs tap water	–, $p=0.008$
Schulte im Walde et al. 2002	Experimental	Yes	C57BL/6 mice, male	10 in each group	Blood glucose	25 mmol/l Zn in drinking water started 1 wk prior to 50 mg ALX /kg iv vs ALX alone	–, $p<0.001$
Tadros et al. 1982	Experimental	No	Albino rats	10 in each group	Diabetes	Zn injection 1 mg/kg before and after ALX 50 mg/kg vs ALX alone	–, not tested
					Islet cell degeneration		–, not tested
					Diabetes	Mn injection 1 mg/kg before and after ALX 50 mg/kg vs ALX alone	–
					Islet cell degeneration		0
Tobia et al. 1998	Experimental	Yes	BB rats, male	15–16 in each group	Diabetes	1000 vs 50 ppm zinc starting at 30 d of age	–, $p=0.014$
						1 vs 50 ppm zinc starting at 30 d of age	NS
					Islet inflammation	1000 vs 50 ppm zinc starting at 30 d of age	–, not tested
						1 vs 50 ppm zinc starting at 30 d of age	0
Yang and Cherian 1994	Experimental	No	Sprague-Dawley rats, male	9 in each group	Plasma glucose	10 mg/kg Zn injected 12 h prior to 60 mg STZ ip vs STZ alone	–, p=0.01
Zunino et al. 2007	Experimental	Yes	NOD/Lt mice, female	13 in each group	Diabetes	Diet supplemented with 262 µmol vit A/kg food vs standard diet	–, $p<0.05$
					Insulitis		–, $p<0.05$
Kang et al. 2004	In vitro	Yes	Rat insulinoma cells		Cell death	Incubation of cells with cytokines in the presence vs absence of RA	–, $p<0.05$
Kim et al. 2000	In vitro	No	Insulinoma cells		Cell death	Zn	+
			Human islet cells		Cell death	Zn	+

[1] Articles are listed according to the study design (epidemiologic studies, descriptive studies, experimental (animal) studies, *in vitro* studies). Within each study design, articles are listed in alphabetical order according to the first author.