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ALCOHOL, TOBACCO AND THE RISK OF LADA-LATENT AUTOIMMUNE DIABETES IN ADULTS

Bahareh Rasouli

Karolinska Institutet

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Alcohol, tobacco and the risk of LADA-latent autoimmune diabetes in adults
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By

Bahareh Rasouli

Principal Supervisor:
Associate Professor Sofia Carlsson
Karolinska Institutet
Department of Environmental Medicine
Unit of Epidemiology

Co-supervisors:
Professor Valdemar Grill
NTNU-Trondheim
Norwegian University of Science and Technology
Department of Internal Medicine
Division of Endocrinology

Associate Professor Tiinamaija Tuomi
University of Helsinki
Finnish Institute of Molecular Medicine,
Research Program Unit, Diabetes and Obesity.
Folkhälsan Institute of Genetics,
Biomedicum, Helsinki

Opponent:
Associate Professor Lars Christian Stene
Norwegian Institute of Public Health
Department of Chronic Diseases
Division of Epidemiology

Examination Board:
Associate Professor Marcel den Hoed
Uppsala University
Department of Medical Sciences
Molecular Epidemiology and Science for Life Laboratory

Associate Professor Mats Palmér
Karolinska Institutet
Department of Medicine
Division of Metabolism

Associate Professor Anna Bergström
Karolinska Institutet
Department of Environmental Medicine
Unit of Environmental Epidemiology

To my mother, father, brother, and sisters

تقدیم به مادرم، پدرم، برادرم، و خوهرانم
همراهان همیشگی و پشتوانه های زندگیم
آنها که مانند جان دوستشان میدارم

The knowledge of anything, since all things have causes, is not acquired or complete unless it is known by its causes.

Avicenna

ABSTRACT

Diabetes is a common and increasing public health problem. Knowledge of risk factors is a prerequisite for efficient prevention; such knowledge is extensive for type 2 diabetes but limited for autoimmune forms of diabetes. LADA-latent autoimmune diabetes in adults is an autoimmune form of diabetes that develops in adults and has features of both type 1 and type 2 diabetes. It accounts for relatively large proportion of all diabetes patients, yet risk factors are largely unexplored. The aim of this thesis was to investigate the influence of tobacco exposure and alcohol consumption on the risk of LADA, and also to explore these factors in relation to type 2 diabetes, to compare the etiology of these adult onset forms of diabetes.

Analyses were based on data from two large Scandinavian population-based studies; the Norwegian HUNT-study, a prospective cohort study conducted between 1984 and 2008, and ESTRID, an ongoing Swedish case-control study with incident cases. Information on lifestyle including alcohol consumption and tobacco use was collected by questionnaire. Cases of diabetes were identified by self-report (HUNT) or through the health care system (ESTRID). Patients with LADA had onset ≥35 years and were glutamic acid decarboxylase autoantibody (GADA) positive. Information on lack of insulin treatment (HUNT) or C-peptide levels (ESTRID) were used to indicate a slow onset.

Alcohol consumption was associated with reduced risk of LADA in both HUNT and ESTRID. In ESTRID, stratification by GADA levels indicated that the reduced risk primarily pertained to LADA with low GADA levels (odds ratio [OR] 0.85, 95% confidence interval [CI] 0.76–0.94 for every 5-gram increase in daily alcohol intake). Smoking was associated with a reduced risk of LADA in HUNT which we could not confirm in ESTRID; in contrast we found an increased risk of LADA in heavy smokers (OR; 1.37, 95% CI; 1.02-1.84). With regard to type 2 diabetes, we could confirm that alcohol intake is associated with a reduced risk and smoking with an increased risk. There was no association between moist snuff use and type 2 diabetes or LADA in either the Swedish (type 2 diabetes: OR for >10 box-years; 1.00, 95% CI; 0.47-2.11, and LADA: 1.01, 95% CI; 0.45-2.29) or the Norwegian study.

In conclusion, the results suggest that alcohol consumption reduces the risk of type 2 diabetes and type 2-like LADA. An increased risk of LADA was seen in smokers but results were contradictory and require further exploration. Finally the use of moist snuff was associated neither with type 2 diabetes nor LADA. These findings indicate that the etiology of LADA in part may be shared with type 2 diabetes and involve factors related to insulin sensitivity. Furthermore, they indicate that LADA may to some extent be preventable by lifestyle modification.

SAMMANFATTNING PÅ SVENSKA

Diabetes är en vanligt förekommande sjukdom och ett växande folkhälsoproblem. För effektiv prevention krävs kunskap om riskfaktorer; sådan kunskap finns för typ 2 diabetes men är mycket begränsad för autoimmuna former av sjukdomen. LADA – latent autoimmun diabetes hos vuxna – är en autoimmun form av diabetes som drabbar vuxna och som har drag av både typ 1 och typ 2 diabetes. LADA utgör en förhållandevis stor del av all diabetes, men trots detta är riskfaktorerna i princip okända. Syftet med denna avhandling var att studera hur tobak och alkoholkonsumtion påverkar risken att utveckla LADA, samt att undersöka dessa exponeringar avseende risken för typ 2-diabetes för att därigenom kunna jämföra etiologin för dessa två diabetesformer.

Analyserna är baserade på data från två stora, populationsbaserade studier i Skandinavien; norska HUNT-studien, en prospektiv kohortstudie med data insamlad 1984-2008, och ESTRID, en pågående svensk fall-kontrollstudie med incidenta fall. Information om alkoholvanor, tobaksvanor, och andra livsstilsfaktorer samlades in genom frågeformulär. Fallen identifierades genom självrapporterad diabetes (HUNT) eller via sjukvården (ESTRID). LADA-patienterna var ≥35 år vid diagnos samt positiva för GADA (autoantikroppar mot glutaminsyredekarboxylas). Information om insulinberoende (HUNT) eller C-peptidnivåer (ESTRID) användes som indikation på långsam sjukdomsprogression.

Alkoholkonsumtion var associerat med minskad risk för LADA i både HUNT och ESTRID. Stratifiering på GADA-nivåer indikerade att den minskade risken framför allt var kopplad till LADA med låga nivåer av GADA (oddskvot [OR] 0.85, 95 % konfidensintervall [CI] 0.76–0.94 per 5 grams ökning i daglig konsumtion). Rökning var associerat med minskad risk för LADA i HUNT vilket dock inte kunde konfirmeras i ESTRID; tvärtom fann vi en ökad risk för LADA bland de som rökte mest (OR 1.37, 95 % CI 1.02-1.84). För typ 2 diabetes kunde vi bekräfta att alkoholkonsumtion är associerat med minskad risk medan rökning är kopplat till ökad risk. Vi fann inget samband mellan snus och risken för typ 2 diabetes eller LADA, varken i svenska ESTRID (OR för >10 box-years, typ 2 diabetes: 1.00, 95% CI; 0.47-2.11, LADA: 1.01, 95% CI; 0.45-2.29) eller i norska HUNT.

Sammanfattningsvis tyder resultaten på att alkoholkonsumtion minskar risken för typ 2 diabetes och typ 2 liknande LADA (låg-GADA). En ökad risk för LADA syntes bland rökare, men resultaten var motsägelsefulla och detta behöver utforskas vidare. Slutligen fanns inget samband mellan snus och risk för varken typ 2 diabetes eller LADA. Tillsammans tyder dessa fynd på att etiologin för LADA delvis liknar den för typ 2 diabetes och innefattar faktorer relaterade till insulinkänslighet. Vidare tyder de på att LADA till viss del skulle kunna förebyggas genom livsstilsförändringar.

ABSTRACT IN PERSIAN

چکیده به فارسی

دیابت یکی از شایع ترین بیماریهای مزمن به شمار میرود که پیامدهای جدی برای جامعه، افراد، و سیستم بهداشتی به دنبال دارد. متأسفانه دیابت دارمان ندارد، ولی قابل پیشگیری می‌باشد. شناختن عوامل خطر شرط لازم برای پیشگیری کارآمد است. بسیاری از عوامل خطر دیابت نوع ۲ شناخته شده هستند، در صورتیکه چنین دانشی در مورد نوع خود ایمنی دیابت محدود می‌باشد. بیماری LADA یک فرم خود ایمنی از دیابت است که در بزرگسالی رخ میدهد و دارای ویژگی‌های هر دو نوع دیابت ۱ و ۲ می‌باشد. با اینکه شیوع آن بالا می‌باشد و شامل ٪۹ کلیه بیماران دیابتی در بزرگسالی میشود، ولی هنوز عوامل خطر آن تا حد زیادی ناشناخته می‌باشند. هدف این پایان نامه مطالعه تاثیر مصرف الکل و دخانیات در بروز بیماری LADA می‌باشد. همچنین اثر این عوامل در بروز دیابت نوع ۲ نیز مورد مطالعه قرار گرفت، تا به مقایسه اتیولوژی این دو فرم دیابت در بزرگسالی بپردازد.

این پایان نامه، براساس اطلاعات به کار گرفته شده از۲ مطالعه بزرگ مبتنی بر جمعیت اسکاندیناوی می‌باشد؛ اولین مطالعه، یک مطالعه کوهورت آینده نگر نروژی با ۲۲ سال پیگیری (1984–2008) به نام HUNT و دومی,ESTRID یک مطالعه مورد شاهدی سوئدی می‌باشد. اطلاعات مربوط به شیوه زندگی از جمله مصرف الکل و تنباکو توسط پرسشنامه جمع آوری شد. موارد ابتلا به دیابت توسط خود گزارش (HUNT) و یا از طریق سیستم مراقبت های بهداشتی (ESTRID) مشخص شدند. بیماران دیابتی که بیشتر از ۳۵ سال سن داشتند و علاوه بر این Anti-GAD (گلوتامیک اسید دکربوکسیلاز اتوآنتی بادی) مثبت بودند به عنوان LADA طبقه بندی شدند .اطلاعات مربوط به عدم شروع درمان زودهنگام با انسولین (HUNT) و یا سطح C-peptide (ESTRID) برای جدا کردن بیماران LADA از دیابت نوع ۱ استفاده شد .

مصرف الکل با کاهش خطر ابتلا LADA در هر دو مطالعه HUNT و ESTRID همراه بود. طبقه بندی کردن بیماران LADA با سطح GADA نشان داد که کاهش خطر در درجه اول در بیماران LADA با سطوح پایین GADA مرتبط است، نسبت شانس (Odds Ratio) برای افزایش هر ۵ گرم در مصرف روزانه 0.84 با فاصله اطمینان (Confidence Interval, CI) 0.76–0.94 بود. مشاهدات ما در HUNT نشان داد که سیگار کشیدن با کاهش خطر LADA مرتبط است، گرچه در مطالعه ESTRID این تأیید نشد، و حتی افزایش خطر LADA در افرادی که مصرف سیگار بالا داشتند مشاهده شد (OR; 1.37; CI; 1.02–1.84). مطالعه ما در راستای مطالعات قبلی تأیید کرد که مصرف الکل با کاهش خطرو سیگار کشیدن با افزایش خطر ابتلا به دیابت نوع ۲ همراه است. هیچ ارتباطی بین استفاده moist snuff (انفیه مرطوب سوئدی) و دیابت نوع ۲ یا LADA در مطالعات ما دیده نشد .

بطور خلاصه ، نتایج ما نشان می دهد که مصرف الکل خطر ابتلا به دیابت نوع ۲ و LADA را کاهش می دهد. افزایش خطر ابتلا به LADAدر افراد سیگاری دیده شد اما نتایج متناقض بود و نیاز به مطالعات بیشتر دارد. این یافته ها نشان می دهد که اتیولوژی LADA ممکن است مشابه با اتیولوژی دیابت نوع ۲ و مرتبط با حساسیت به انسولین باشد. به علاوه، نتایج نشان می دهد که LADA ممکن است با اصلاح شیوه زندگی تا حدی قابل پیشگیری باشد.

LIST OF SCIENTIFIC PAPERS

I. **Rasouli B**, Ahlbom A, Andersson T, Grill V, Midthjell K, Olsson L, Carlsson S. Alcohol consumption is associated with reduced risk of Type 2 diabetes and autoimmune diabetes in adults: results from the Nord-Trondelag health study. *Diabet Med 2013;30:56-64.*

II. **Rasouli B**, Andersson T, Carlsson PO, Dorkhan M, Grill V, Groop L, Martinell M, Tuomi T, Carlsson S. Alcohol and the risk for latent autoimmune diabetes in adults: results based on Swedish ESTRID study. *Eur J Endocrinol 2014;171:535-543.*

III. **Rasouli B**, Grill V, Midthjell K, Ahlbom A, Andersson T, Carlsson S. Smoking is associated with reduced risk of autoimmune diabetes in adults contrasting with increased risk in overweight men with type 2 diabetes: a 22-year follow-up of the HUNT study. *Diabetes Care 2013;36:604-610.*

IV. **Rasouli B**, Andersson T, Carlsson PO, Grill V, Groop L, Martinell M, Storm P, Tuomi T, Carlsson S. Smoking and the risk of LADA: results from a Swedish population-based case-control study. *Accepted for publication in Diabetes Care. 2016.*

V. **Rasouli B**, Andersson T, Carlsson P.O, Grill V, Groop L, Martinell M, Midthjell K, Storm P, Tuomi T, Carlsson S. Use of Swedish moist snuff (snus) and the risk of type 2 diabetes and LADA: results based on two Scandinavian studies. *Submitted for publication. 2016.*

This thesis is based on the abovementioned papers which will be referred to in the text by their Roman numerals (I – V).

CONTENTS

LIST OF ABBREVIATIONS

ANDIS	All New Diabetics in Scania
ANDIU	All New Diabetic in Uppsala
BMI	Body mass index
CI	Confidence interval
ELISA	Enzyme-Linked Immunosorbent Assays
ESTRID	Epidemiological Study of Risk factors for LADA and type 2 Diabetes
FPG	Fasting Plasma Plucose
GADA	Glutamic Acid Decarboxylase Autoantibodies
HbA1C	Haemoglobin A1C
HLA	Human Leukocyte Antigen
HR	Hazard ratio
HOMA	Homeostasis model assessment
HUNT	The Nord-Trøndelag Health Study
IAA	Autoimmunity like autoantibodies directed towards insulin
ICA	Autoantibodies to islet cells
Kg	Kilogram
LADA	Latent autoimmune diabetes in adults
OR	Odds ratio
POR	Prevalence odds ratios
WHO	World Health Organization

1 INTRODUCTION

Diabetes is a chronic disease that occurs when the body cannot produce enough insulin and/or use insulin efficiently. It affects more than 415 million individuals globally, and the prevalence is expected to rise 50% by the year 2040. Diabetes contributes considerably to the burden of morbidity and is estimated to be the 7th leading cause of death (1; 2). At present, there is no cure for diabetes, as such prevention and management of the disease are crucial in order to reduce morbidity. Knowledge about risk factors is a necessity for successful preventive action. Such knowledge is extensive for type 2 diabetes but limited for autoimmune forms of diabetes.

Latent autoimmune diabetes in adults (LADA) is described as a hybrid form of diabetes with features of both type 1 and type 2 diabetes (3). Like type 1 diabetes, it is characterized by presence of autoantibodies against pancreatic β-cells (3), but like type 2 diabetes it develops in adults and is afflicted with insulin resistance. Recent findings suggest that LADA accounts for 5% of all diabetes, and is almost as frequent as type 1 diabetes (4). Risk factors are largely unexplored; but considering LADA as a mix of type 1 and type 2 diabetes, one could hypothesize that risk factors may include factors triggering autoimmunity and/or insulin resistance.

The aim of this thesis was to investigate the risk of LADA in relation to alcohol consumption and tobacco use, two modifiable risk factors associated with type 2 diabetes. Analyses were based on data from a Norwegian cohort study and a Swedish case-control study, the largest population-based studies of LADA to date. The overall aim was to contribute to the understanding of the etiology of LADA.

2 BACKGROUND

Diabetes mellitus is a chronic disease characterized by hyperglycemia, but it is a heterogeneous group of disorders with different pathologies: disturbances in insulin secretion, insulin sensitivity, or both (5). Diabetes is one of the world´s fastest increasing diseases; between 2000 and 2015 the number of individuals with diabetes increased from 171 to 415 million worldwide and a further increase to 642 million affected individuals is project by 2040 (1). This rise is projected to be most pronounced in Africa and Middle Eastern countries and the driving forces are aging populations, nutritional transition, and increasing prevalence of obesity and physical inactivity (1; 6). According to a new report from the International Diabetes Federation (IDF), 9.1% of the adult population (59.8 million) in Europe lives with diabetes (1), and in Sweden, 6.8% of adults are affected (7).

Long-term exposure to abnormal high blood glucose can result in damages to blood vessels, heart, kidney and nerves (8); which are associated with premature death, primarily from cardiovascular diseases (1). In many high income countries, diabetes is the primary cause of cardiovascular disease, blindness, nephropathy, and foot/leg amputation (1). Despite improvements in treatment and risk factor control, patient with diabetes still have excess mortality compared to the general population. In a report based on the Swedish National Diabetes Register, type 1 diabetes patients had twice the risk of death from any cause or cardiovascular disease compared to matched controls (9), and patients with type 2 diabetes had 27% higher risk of death from any cause and 33% from cardiovascular diseases, and the risk was substantially higher in patients with poor glycemic control (10)

Diabetes and its complications pose an enormous burden on individuals, families, health systems, and public spending (1). At present diabetes cannot be cured, therefore prevention and control of diabetes is needed. A better understanding of the potential role of lifestyle factors in the etiology of diabetes is important for shaping effective diabetes prevention programs and also contributes to a better understanding of the pathogenesis of the disease.

2.1 DIAGNOSIS OF DIABETES

Hyperglycemia is the hallmark of diabetes. Plasma glucose (fasting and 2-hour plasma glucose) and HbA_{1c} are the basis of diabetes diagnostic criteria (11; 12):

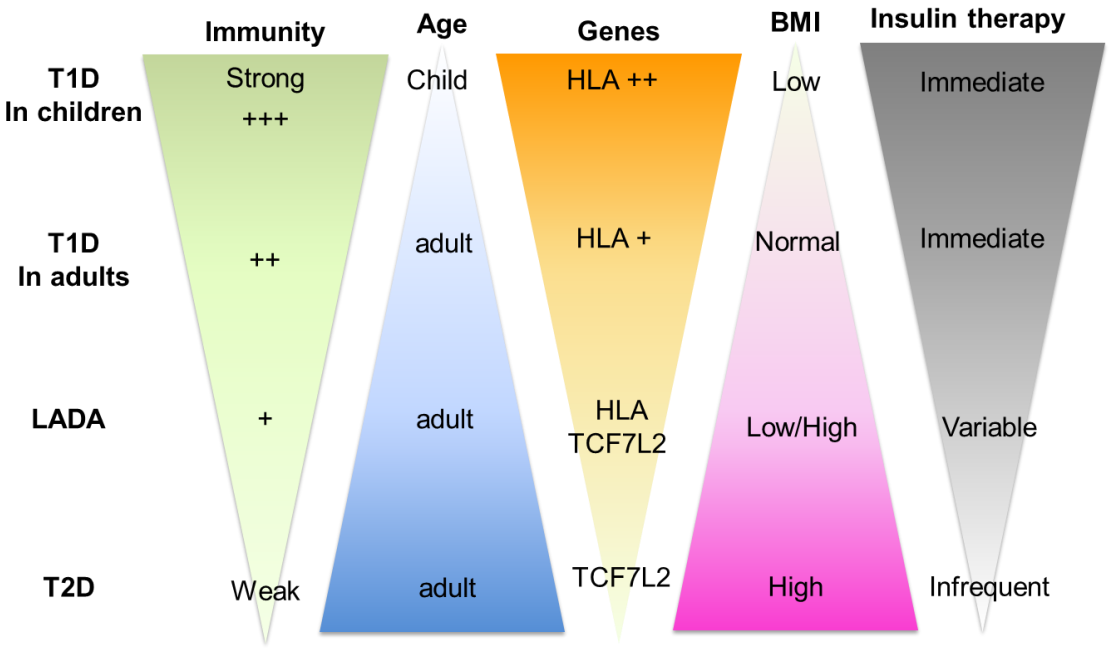
- Fasting Plasma Plucose (FPG); ≥7.0 mmol/L, or
- 2-Hour Plasma Glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT): ≥11.1 mmol/L, or
- Random plasma glucose: ≥11.1 mmol/L, together with classical symptoms of hyperglycemia, or
- HbA1c: ≥6.5%

The latest criterion of HbA1c, is however, not appropriate for diagnosis of diabetes in children and adolescents, some ethnicities, e.g. African Americans, gestational diabetes, and individuals with anemia and hemoglobinopathies (11).

2.2 CLASSIFICATION OF DIABETES

In 1985, diabetes was classified by WHO into two groups based on age and insulin dependency; insulin requiring diabetes mellitus (IDDM) and non-insulin requiring diabetes mellitus (NIDDM) (13). This classification was eventually considered inadequate, as not all IDDM patients became insulin deficient at diagnosis nor could all NIDDM patients be treated without insulin therapy (14). Later in 1999, diabetes was classified on basis of etiologies rather than degree of insulin deficiency, and IDDM and NIDDM were replaced by type 1 (β-cell destruction) and type 2 diabetes (insulin resistance/relative insulin deficiency). In clinical practice, general observations of clinical phenotypes such as age at onset, apparent abruptness of onset of hyperglycemia, BMI, presence of ketosis, and immediate need for insulin treatment are the first tools for differentiating between diabetes types (15). It has been argued that the current subdivision of diabetes into type 1 and type 2 is, particularly in adults, an oversimplification of a spectrum of phenotypes spanning different subtypes of diabetes (16) with different degrees of overweight, insulin resistance, autoimmunity, metabolic syndrome and genetic susceptibility to *HLA* haplotypes. In this context, type 1 and type 2 diabetes are considered two extreme ends of the diabetes spectrum (figure 1). LADA-latent autoimmune diabetes in adults, first mentioned in 1993 by Tuomi *et al* (16), is an example of an intermediate diabetes form with clinical and genetic feature of both type 1 and type 2 diabetes, (3; 17-20).

Figure 1. The spectrum of diabetes includes immune changes, age, HLA genetic susceptibility, BMI, and insulin therapy according to type of diabetes. Partly adapted from Leslie D. *et al*. (21)



2.2.1 Type 2 diabetes

Type 2 diabetes is the most prevalent form of diabetes and accounts for approximately 70-90% of all cases of diabetes (2; 4; 11). In type 2 diabetes, hyperglycemia is typically caused by a combination of insulin resistance and impaired insulin secretion. When the β-cells lose the ability to release adequate insulin to compensate for insulin resistance, hyperglycemia becomes manifest. Insulin resistance is closely linked to obesity, and the increase in the prevalence of type 2 diabetes reported since the 1990s coincides with a worldwide rise in prevalence of obesity (22; 23). Progress of type 2 diabetes develops slowly through stages of pre-diabetes. Onset of type 2 diabetes typically occurs in middle-aged people or elderly, but the incidence is increasing in younger age groups (5). Type 2 diabetes is often managed initially with dietary and lifestyle changes, and if hyperglycemia is not sufficiently controlled, by oral lowering glucose medications (Metformin, and Sulfonylurea). Injectable insulin is required in patients who do not achieve glycemic control by oral tablet therapy (1; 24-26).

Genetic risk factors

Type 2 diabetes results from the complex interplay between genetic, epigenetic, and environmental factors (27). It is partly an inherited disease; the risk is two to six times higher in individuals with family history of diabetes (28-30). Twin studies have shown that the concordance rate for identical twins is 35-80% and 15-35% for non-identical twins (31-35); indicating a strong genetic component. Unlike type 1 diabetes for which the genetic risk is mainly related to the *HLA* region, the genetic component of type 2 diabetes appears to be scattered all across genome. Type 2 diabetes is a polygenic disease, and more than 120 genetic variants and >80 loci have been thus far associated with type 2 diabetes (27; 36). However, these variants explain only a small fraction (20%) of the total heritability of type 2 diabetes (27; 36). Most of the genes associated with type 2 diabetes are involved in development, function, or regulation of β-cells including *TCF7L2*, *SLC30A8*, and *KCNQ1* and only a few genes are involved in insulin resistance like *PPARG* gene, and obesity susceptibility locus *FTO*. The strongest genetic risk factor for type 2 diabetes so far is the *TCF7L2* rs7903146 variant (37-39)

Environmental risk factors

Overweight and obesity are major risk factors for type 2 diabetes, by way of promoting insulin resistance (22). Physical inactivity is also recognized as an important risk factor for both obesity and type 2 diabetes. Not only excessive calorie intake but also low quality diet characterized by high glycemic load; trans fatty acids, low fiber content is associated with increased risk of type 2 diabetes (22). Moderate alcohol intake is associated with a reduced risk of type 2 diabetes (40), primarily by way of improving insulin sensitivity and smoking is an established risk factors for type 2 diabetes by way of impairing insulin sensitivity (41). There is also some evidence suggesting that exposure to environmental toxins such as arsenic and persistent organic pollutants might be associated with increased risk of type 2 diabetes (42). Intervention studies show that the risk of type 2 diabetes can be reduced by lifestyle

modification including weight loss and regular physical activity (43). Estimation of population-attributable risks (PAR) in observational studies suggests that the majority (72% to 91%) of type 2 diabetes cases can be prevented by adherence to a healthy lifestyle including maintaining normal weight, being physical active, having a healthy diet, and refraining from smoking (44-46).

2.2.1 Type 1 diabetes

In type 1 diabetes, β-cell destruction leads to an absolute defect in pancreatic function, which results in inability to produce adequate amounts of insulin, and insulin treatment must be provided, since most of β-cells are destroyed (47). Type 1 diabetes accounts for 8-10% of all diabetes patients (4; 47). Europe, particularly the Nordic countries, has the highest incidence of type 1 diabetes in children and adolescents (1). Type 1 diabetes accounts for the vast majority of diabetes in children but type 1 diabetes can develop at any age (48).

Genetic risk factors

Familial aggregation studies support the importance of both genetic and environmental risk factors in the development of type 1 diabetes (49). Among all newly diagnosed type 1 diabetes patients, 10-15% have positive family history of type 1 diabetes (50; 51), and in siblings of affected individuals, the risk of type 1 diabetes is increased 15-fold (51). The concordance rate for identical twins (20-50%) is higher than in non-identical twins (5-10%); suggesting there is a strong genetic component for type 1 diabetes (31; 32; 52-54). About 50% of susceptibility is inherited in *HLA* haplotypes (*DQA1*03:01-DQB*03:02 [DQ8]* and *DQA1*05:01-DQB1*02:01[DQ2]*), and to a lesser extent in non-*HLA* loci including the insulin gene (27; 55). Estimates from candidate-gene and GWAS studies indicated that the identified variants combined could explain around 75% of heritability of type 1 diabetes (56; 57).

Environmental risk factors

Tremendous effort has been put into identifying environmental triggers of autoimmunity through studies like the TEDDY (58), but so far, very few environmental factors have consistently been linked to development of type 1 diabetes. Potential environmental triggers that may initiate autoimmunity or precipitate β-cells destruction included exposure to maternal infection during pregnancy, environmental pollutants, infection, early life exposure to cow milk, socioeconomic factors, and low exposure to environmental microorganisms (the hygiene hypothesis) (59-66).

2.2.2 Latent autoimmune diabetes in adults (LADA)

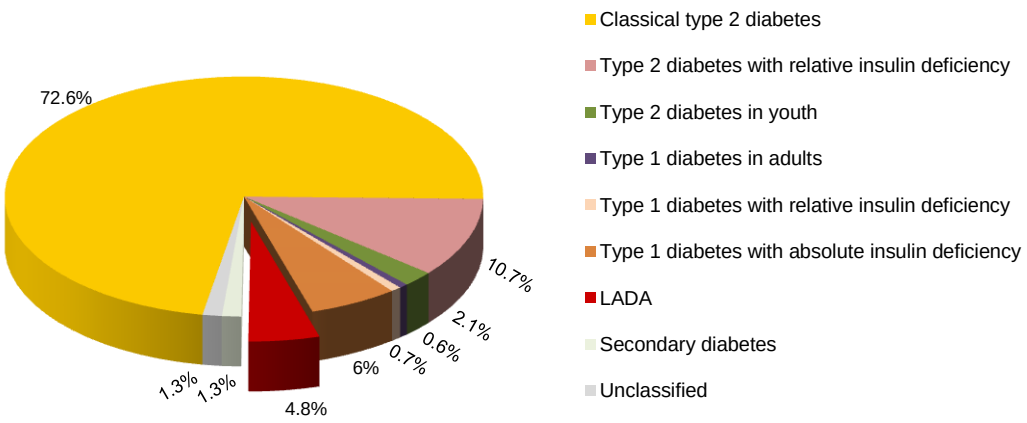
The term LADA was introduced for the first time in 1993 by Tuomi *et al* to define a slowly progressing form of autoimmune diabetes that occurred in adulthood and could be treated initially without insulin (16). Earlier, Irvine *et al* (1977) also identified a group of patients who had originally been diagnosed as type 2 diabetes but who were also positive for islet cell

antibodies (67), and Groop *et al* (1986) described a group of adult patients with a latent form of type 1 diabetes (68). There is no consensus, so far, on the concept of LADA; some continue to consider it type 2 diabetes with positive autoantibodies (69), whereas others including WHO (5; 15; 70) describe it as a slowly progressing form of type 1 diabetes (71; 72). Knowledge on risk factors may help to clarify to what extent LADA shares features of type 1 vs type 2 diabetes and whether it is useful to consider it as a separate form of diabetes.

LADA is considered a hybrid between type 1 and type 2 diabetes, and some have even termed it type 1.5 diabetes (73; 74). LADA patients are insulin resistant and overweight but less pronounced than type 2 diabetes patients and they have better lipid and metabolic profile (19; 75-77). Besides, the insulin requirement in LADA patients is less pronounced and C-peptide levels are higher compared with type 1 diabetes (17; 19; 78). Also, some of the risk factors for LADA so far identified are similar to those of type 2 diabetes (79-83), but not all (84-86). Genetic findings also support the view of LADA as a hybrid, with genetic features of both type 1 and type 2 diabetes (20; 87).

LADA is a common, accounting for about 9% of all patients are initially diagnosed as type 2 diabetes patients (88). However, the prevalence varies by population, ethnicity, and the diagnostic criteria used to define LADA. The estimated prevalence of LADA is around 9% in China (89). In a new Swedish study, ANDIS (All New Diabetics in Scania), all incident cases of diabetes in the county of Scania, are classified according to diabetes type based on clinical and genetic features. Since 2008, 10,226 patients have been included and of those, LADA account for 4.8% of all diabetes patients in children and adults (figure 2).

Figure 2. Prevalence (%) of different types of diabetes; data is from the ANDIS project, adapted and modified from the ANDIS website (4)



Classification of LADA

There is currently no uniform definition of LADA. Three clinical criteria are commonly used: 1) autoantibody positivity, predominantly autoantibodies to glutamic acid decarboxylase (GADA), which differentiates LADA from type 2 diabetes, 2) adult onset (usually 35 years or

older), and 3) insulin independency 6-12 months after diagnosis, to distinguish LADA from type 1 diabetes (27; 90; 91).

All of these criteria have been debated: The age criterion is based on an arbitrary limit, and one could ask; what is the significant difference between a patient with autoimmunity who is diagnosed at age 34 and another who is diagnosed at 36 years of age? (70; 92; 93). GADA has been questioned because there may be other markers of autoimmunity like autoantibodies directed towards insulin (IAA), and autoantibodies to islet cells (ICA). One argument against this is that it has been shown that GADA is the antibody with the highest penetration, being present in 70-80% of LADA patients (94). Finally, the commonly used insulin criterion is a subjective method which depends on the assessment of the individual physician (91). C-peptide is suggested as an alternative, more objective indicator remaining β-cell function (95). Each of these criteria is based on arbitrary cutoffs which makes the diagnosis of LADA variable in different studies and hampers comparisons across studies.

Genetic risk factors

Data from a limited number of studies indicate that the risk of LADA is increased four-fold in individuals with diabetes in the family (82). Knowledge on the genetic predisposition of LADA is not as extensive as for type 1 and type 2 diabetes, but an admixture of genetic characteristics of both type 1 and type 2 diabetes has been suggested (20). Like type 1 diabetes, *HLA*-risk genotype, especially *HLA-DQB1*02/*0302* allele was positively associated with increased risk of LADA (3; 19; 90; 96; 97). The protective *DQB1*0602* allele is higher in LADA than type 1 diabetes (19; 90; 96). Within LADA, the associations of both *HLA*-risk and protective seem to depend on GADA concentrations; LADA patients with high GADA have higher frequency of these risk variants than LADA patients with low GADA (3; 19; 90). Similarly, *PTPN22* was associated with LADA overall or with LADA with high GADA (96; 98). The *CTLA4* gene is also associated with higher risk of LADA (99). Regarding *INS* gene data is inconsistent; some show similar association for LADA as type 1 diabetes (96), whereas others show no association (90).

There are also genetic similarities between LADA and type 2 diabetes. A variant of *TCF7L2*, as the strongest gene for type 2 diabetes, has been associated with LADA in several studies (96; 100; 101). The obesity-associated variant of *FTO* was associated with LADA, particularly LADA with low GADA (90). No association was found between *SLC30A8* and *PPARG* and LADA (90).

This genetic similarity with both classical type 1 and type 2 diabetes supports the view that LADA is a hybrid form of diabetes (20; 90; 96). Moreover, this data suggests that LADA is heterogeneous disease which is mostly related to the variability in GADA levels (20); LADA patients with high GADA titers are found to be genetically more similar to type 1 diabetes, whereas LADA with low GADA levels are more type 2-like (20).

Environmental risk factors

In the pathogenesis of LADA both autoimmunity and insulin resistance seem to be involved (74). It is hence plausible that risk factors for LADA could be the same as those for type 2 diabetes and be mediated primarily by insulin resistance, or they could be the same as for type 1 diabetes, triggering autoimmunity, or they could be a mix. Knowledge on potential risk factors for LADA is at present very limited.

One reason for the lack of studies on risk factors may be that in most observational studies the indicators of autoimmunity which is needed to distinguish LADA from type 2 diabetes patients have not been measured. Exceptions are the Norwegian HUNT-study (102), a longitudinal study conducted in the middle of Norway, and the Swedish ESTRID-study; a population-based case-control study which recruits patients primarily through the ANDIS-registry in Scania, a county in the South of Sweden (4). Data from these studies indicate that risk factors for LADA to some extent are similar to those of type 2 diabetes and include overweight, physical inactivity (81), low birth weight (80), low psychosocial well-being and sleep disturbances (83) In contrast, high education (86), and coffee consumption (85) were associated with an increased risk for LADA, whereas smoking was associated with a reduced risk (84), in line with previous findings in type 1 diabetes (103-105). These findings support the notion that LADA has shared characteristics with both type 1 and type 2 diabetes and that environmental risk factors may be related to autoimmunity as well as insulin resistance. Studies are however few and confirmation and extension of these findings and exploration of additional lifestyle factors are clearly warranted.

2.3 ALCOHOL CONSUMPTION

Moderate alcohol intake is associated with a 30-40% reduced risk of type 2 diabetes (106-109). The beneficial effect of alcohol could be attributed to improvement of insulin sensitivity (110-113), beneficial postprandial effects (alcohol reduces the peak of blood glucose levels after meals (114)), anti-inflammatory effects (115; 116), and elevated circulating adiponectin, which plays an important role in regulating metabolism of glucose and lipids (117-120).

Drinking pattern is also important; frequent drinking is associated with reduced risk of type 2 diabetes compared to episodic or binge drinking (108; 121). The ethanol itself rather than particular components of different alcoholic drinks appears to be carrying beneficial health effects, but some studies have shown that wine is more beneficial than other alcohol containing drinks like beer and liquor (109; 121). Possibly, it is due to presence of other compounds rather than ethanol, such as polyphenols and hydroxylated Stilbenes, which play an anti-oxidative or anti-inflammatory role in the body (122; 123).

Alcohol consumption has previously not been studied in relation to LADA. There are however, some studies indicating that moderate alcohol intake is associated with a reduced risk of other autoimmune disorders such as rheumatoid arthritis (124; 125), and Graves'

hyperthyroidism (126). A possible underlying mechanism could be that alcohol reduces some markers of inflammation and regulates the immune system (115; 116; 127-129).

2.4 SMOKING

Smoking, particularly heavy smoking, is a well-known risk factor for type 2 diabetes (41; 84; 130-137); A systematic review showed that current smoking is associated with a 44% increased risk of type 2 diabetes (41) and the risk increases in a dose-dependent manner (84; 133; 138). Cigarette contains over 4000 chemical substances and more than 200 of them are believed to be toxic, including nicotine, tar, and carbon monoxide. Studies have shown that smoking is more harmful in individuals with high BMI (138), suggesting that overweight may modify the effect of smoking on type 2 diabetes. The association between smoking and type 2 diabetes has primarily been attributed to smoking/nicotine-induced insulin resistance (139; 140), but increased systematic inflammation (141), greater accumulation of abdominal adipose tissues (142), and adverse effects on pancreatic tissue and β-cell function (143) may also contribute to the excess risk.

A small study based on HUNT (follow-up between 1984 and 1997) indicated that smoking may reduce the risk of LADA (84). It was based on only 35 smoking LADA patients and replication of these findings is thus needed. These findings are in line with previous observations in type 1 diabetes, suggesting a reduced risk in the offspring of smoking parents (103-105). A proposed mechanism behind a beneficial effect could be the anti-inflammatory and immune-modulating effect of smoking/exposure to nicotine (144; 145). This potential biologic mechanism is controversial (144-147).

2.5 MOIST SNUFF (SNUS) USE

Swedish moist snuff (Snus) is a smokeless tobacco product that contains ground tobacco, salt, water, aromatic and humidifying substances. The use of moist snuff is popular in Sweden, where 20% of men and 4% of women use it on a daily basis (148). Moist snuff is steadily on the rise in Norway and the United States (149), where the sale of moist snuff is permitted.

The influence of smoking on type 2 diabetes has been attributed, at least in part, to nicotine (139; 140). It has been suggested that moist snuff, with even higher nicotine content and comparable nicotine bioavailability as cigarettes (150), increases the risk of type 2 diabetes (151; 152), but results from the small number of studies are inconsistent [6]. One reason for conflicting results may be insufficient adjustment for smoking, which is common among moist snuff users. Larger studies will allow for restriction of the analyses to never smokers, which is an efficient way of handling this potential confounding.

If smoking reduces the risk of LADA (153) by way of an inhibitory effect of nicotine on autoimmunity/inflammation (144), one could hypothesize that moist snuff, with its higher nicotine content, may have an even stronger beneficial effect. Whether this is the case, remains to be explored.

3 AIM

3.1 OVERALL AIM

To study the influence of alcohol and tobacco use on the risk of LADA, and to address these exposures in relation to type 2 diabetes in order to compare the etiology of LADA and type 2 diabetes.

3.2 SPECIFIC AIMS

Study I and II: To study weather alcohol consumption is associated with the risk of LADA and whether the association is dependent on degree of autoimmunity as assessed by GADA level.

Study III and IV: To assess the association between smoking and the risk of LADA.

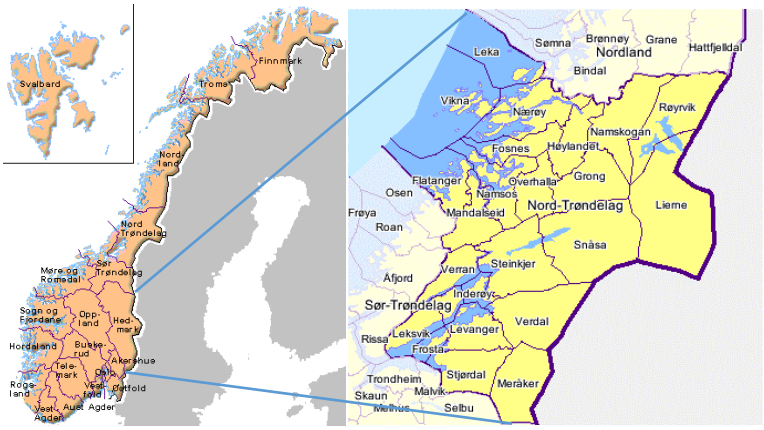
Study V: To study the association between use of moist snuff and the risk of LADA and type 2 diabetes.

4 MATERIAL AND METHODS

4.1 HUNT STUDY (PAPERS I, III, AND V)

The Nord-Trøndelag County is located in the middle of Norway with a total of ~127,500 inhabitants (figure 3). During 1984-2008, all inhabitants aged ≥20 year old living in the county were invited to the HUNT Study, where the health of the population was extensively investigated in three separate surveys (HUNT1, HUNT2, and HUNT3).

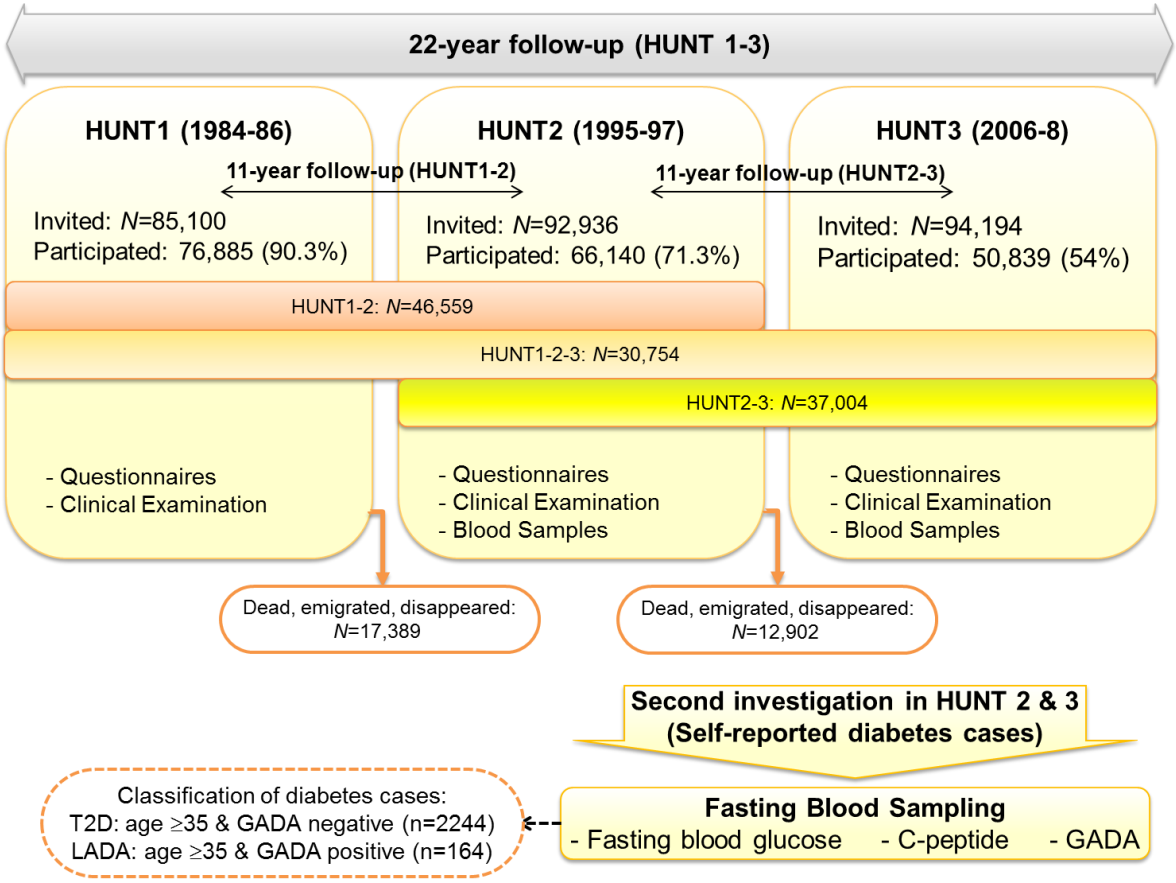
Figure 3. Nord-Trøndelag County (154).



The first survey (HUNT 1) was undertaken in the period of 1984-1986 and the participation rate was 90.3% (n=76,885). HUNT2 was conducted from 1995 to 1997 (102; 155). The participation rate in this follow-up study was 71.3% (n=66,140) and among those still alive, 78% (n=46,559) of participants in HUNT1 re-attended in HUNT2. The third survey (HUNT3) was started in 2006 and completed in 2008, with similar design as the two previous ones, including 50,839 participants (attendance rate=54%), and of those still alive, about 70% (n=37,004) of participants in HUNT2 re-attend in HUNT3 (86; 156-158). An overview of the HUNT Study is given in figure 4.

Participants filled out a self-administrated questionnaire, including detailed information on health, lifestyle, and demographic factors and participated in a clinical examination including anthropometric measurements and blood sampling. Diabetes was identified by self-reporting; a high accuracy is reported between this self-reported information and medical records (96% of the diabetes cases were verified) (159). Individuals who reported having diabetes in HUNT2 or HUNT3 were invited to a separate diabetes investigation, including blood sampling and detailed questions on treatment and age at onset (102; 155; 157; 160; 161).

Figure 4. A chart presentation for the HUNT studies (The Nord-Trøndelag Health Survey); 1984–2008. Adapted from Krokstad *et al* (158)



4.1.1 Study population

Paper I, Alcohol and LADA/type 2 diabetes: For the analysis of frequency of alcohol intake we used data from all three HUNT surveys (HUNT1-3) and formed a cohort consisting of individuals who were free of diabetes at baseline (HUNT1 or HUNT2; depending on when the participant entered the study) who also participated in at least one follow-up survey (HUNT2 or HUNT3). Eligible for the analyses were 90,296 individuals (964,890 person-years) and among those, 1841 incident cases of type 2 diabetes, and 140 cases of LADA were identified during the 22 year follow-up (1984-2008). Information on amount of alcohol consumed was available only at HUNT2 why we formed second cohort including 42,033 participants (444,238 person-years) who were free of diabetes at HUNT2 (baseline) and among those, we identified 940 incident case of type 2 diabetes and 46 cases of LADA during 11-years of follow-up (1995-2008).

Paper III, Smoking and LADA/type 2 diabetes: Information from all three HUNT surveys (HUNT1-3) was used to form a cohort of individuals who could be followed prospectively for incidence of diabetes during 11-22 years. Eligible were individuals who were free of diabetes at baseline (HUNT1 or HUNT2; depending on when the participant entered the study) with complete baseline information on smoking; 1860 incident cases of type 2 diabetes and 140 cases of LADA were identified in 968,641 person-years of follow-up.

Paper V, Moist snuff use and type 2 diabetes: Information on moist snuff use was only available for the latest HUNT investigation (HUNT3), conducted 2006-2008. Hence, moist snuff use was analyzed cross-sectionally in relation to diabetes including 829 prevalent cases of type 2 diabetes and 21,473 individuals without diabetes. LADA patients were not included in the analysis as the numbers were too small (n=10) to allow for meaningful analyses. The analyses were restricted to men due to the low prevalence of moist snuff use in women (~4%).

4.1.2 Biochemical analysis

All individuals with self-reported diabetes at HUNT2 and HUNT3 were invited to a second investigation for fasting blood sampling. Blood samples were analyzed for GADA, C-peptide, and glucose. For the patients who did not attend the supplementary examination, serum samples were available at the HUNT Biobank (86; 90). Among all individuals with diabetes, only 4.5% had missing data on GADA.

The analysis of GADA was performed at Aker University Hospital, Oslo, Norway, by a previously validated method (102). GADA was reported as an antibody index value in relation to standard serum (162). A value of ≥0.08 was considered positive. At this cutoff, the sensitivity and specificity was 0.64 and 1.00, respectively, according to results obtained in through the Diabetes Antibody Standardization Program (163). GADA values were also transformed to World Health Organization units, it was calculated as 0.08=43 WHO units/mL (164). C-peptide was measured via radioimmunoassay (Diagnostic System Laboratories, Webster, TX) (102; 157). Fasting serum levels of glucose were measured by Hemocue at the central laboratory of Levanger Hospital (Levanger, Norway) (13). Homeostasis model assessment for insulin resistance (HOMA2-IR) and β-cell function (HOMA2-% B) were calculated using HOMA2 calculator(165).

4.1.3 Classification of diabetes

GADA and age at onset were used to classify the diabetes patients; Patients with age at onset ≥35 years were classified as having type 2 diabetes if they were GADA negative (<0.08) and as having autoimmune diabetes in adults if they were GADA positive (≥0.08). As a further criterion, we used information on insulin treatment to separate LADA from classical type 1 diabetes. Individuals were classified as having LADA if they were GADA positive and did not use insulin during the first year after diagnosis and as having classical type 1 diabetes if insulin treatment was started less than one year after onset. Unfortunately information on treatment was only available for 79.5% of patients, and thus the distinction between LADA and type 1 diabetes could not be made for all autoimmune patients with adult onset.

Since the majority of autoimmune diabetes patients in HUNT had LADA (85.7% of those with information on treatment belonged to this group), and because of the limited number of cases, we chose to combine LADA and type 1 diabetes in the main analysis of paper I and III. Throughout the thesis, for practical reasons and in order to avoid introducing a new term

(AIDA-autoimmune diabetes in adults), I will refer to this patient group as LADA. Importantly, sensitivity analyses were run with LADA as outcome in all papers based on HUNT data and the results were similar to those based on the whole group of autoimmune patients with adult onset (paper I and III).

4.1.4 Questionnaire data

Information on health and lifestyle factors, including tobacco use and alcohol consumption was collected by questionnaire. The questionnaires used in the HUNT surveys can be found at http://www.ntnu.edu/hunt/data/que.

4.1.4.1 Alcohol consumption (Paper I)

In the HUNT1 questionnaire information on frequency of alcohol intake was available, through a question on how often, on average over the last 14 days, participants consumed alcoholic drinks; and the five response options ranged from: non-consumers to >10 times. No information on the amount consumed was collected.

The HUNT2 questionnaires contained detailed questions about alcohol consumption. The following question were used to determine the quantity and also type of consumed alcoholic drinks: "How many glasses of beer, wine or spirits do you usually drink in the course of two weeks?". To calculate the total average grams of alcohol consumption per day we multiplied the reported amount by the estimated alcohol content for each alcoholic beverage, and then we summed them up to get the total daily alcohol consumption. The estimated alcohol contents were 16 gram for one can/bottle/glass of beer, 12 gram for one glass of wine and 12 gram for one standard drink of spirit (166). Participants were categorized into different consumption groups according to this information, varying from 0.01 to ≥15 gram/day (reference group: 0.01-5 gram/day). For LADA analysis due to few numbers in each category, alcohol consumption was reclassified based on quartiles. The frequency of alcohol consumption was also derived from this question: "How many times a month do you usually drink alcohol?".

4.1.4.2 Smoking (Paper III)

Detailed information on smoking history was collected in both HUNT1 and HUNT2. Based on information on smoking habits participants were categorized into three groups: never smokers, former smokers and current smokers. Current and former smokers were asked; "How old were you when you started smoking?", "How many years in total have you smoked daily?", and "How many cigarettes do you or did you usually smoke daily?". The intensity of smoking among current and former smokers was assessed in two categories; light smokers (<20 cigarettes/day) and heavy smokers (≥20 cigarettes/day). One pack year corresponds to smoking 20 cigarettes per day for a year. Pack-year was assessed in three categories; <6, 6-12 and ≥13.

4.1.4.3 Moist snuff use (Paper V)

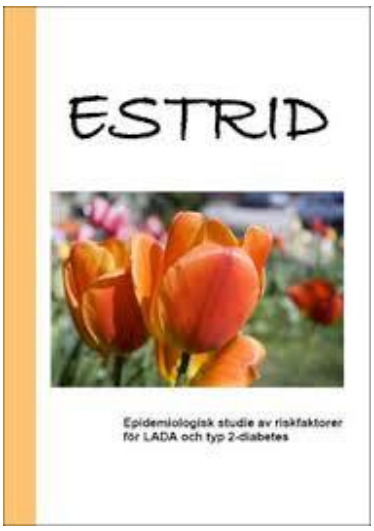
Information on moist snuff use was available from HUNT3. Participants were asked whether they had ever used moist snuff. Individuals were considered as current users if they used moist snuff on a daily basis or occasionally. Ever moist snuff users were asked about the number of boxes consumed per month, and they were classified into two groups (<3 and ≥3 boxes/week).

4.1.4.4 Confounding factors

In HUNT height and weight was measured at the clinical investigation. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Family history of diabetes was considered positive if any first-degree relative had any type of diabetes (yes/no). Educational level categorized into three groups: primary school, upper secondary school or university. Smoking was analyzed continuous (pack-year; in moist snuff analysis) or categorized as: never, former, and current smokers. Questions about leisure time physical activity was used to categorize participants as having high activity, moderate activity or as being inactive; categorization details are described elsewhere (167).

4.2 ESTRID STUDY (PAPERS II, IV, AND V)

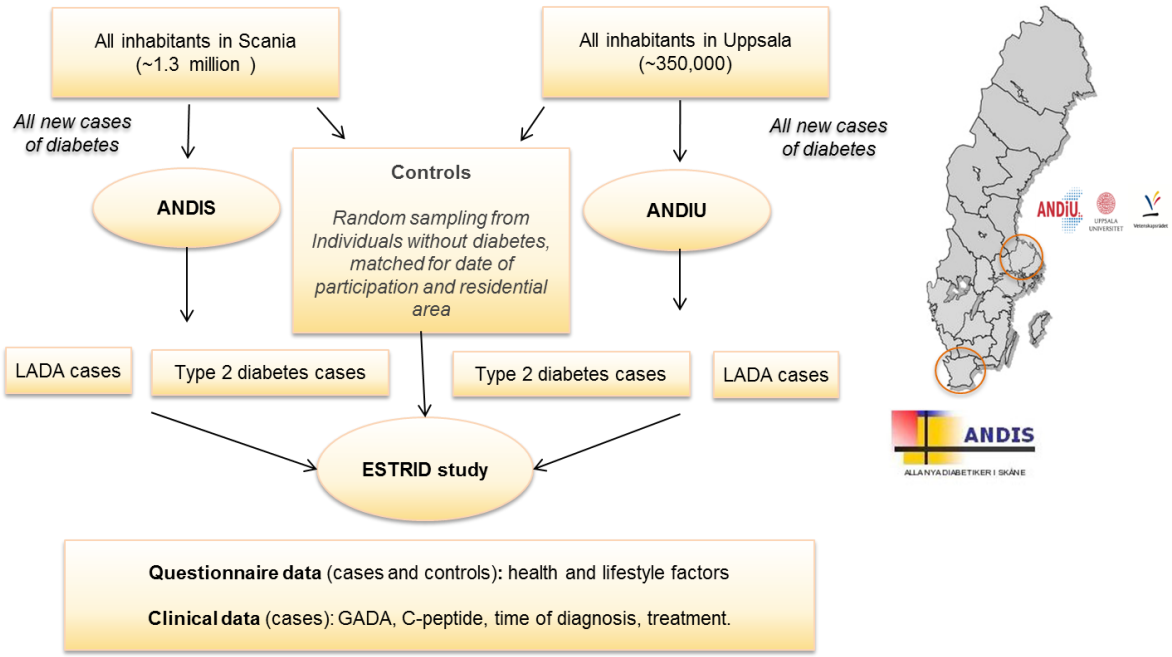
ESTRID (Epidemiological Study of Risk factors for LADA and type 2 Diabetes; http://ki.se/imm/estrid) is an ongoing population-based case-control study conducted in Sweden. Patients are recruited primarily through the before mentioned ANDIS-study (http://andis.ludc.med.lu.se).



ANDIS is an ongoing large scale study attempting to classify and monitor all new diabetes cases in Scania, a county in southern part of Sweden with ~1,300,000 inhabitants (figure 5). The aim of ANDIS is to describe the diabetes spectrum on the basis of clinical features and genetic factors.

To ESTRID we invite all incident cases of LADA identified in ANDIS since 2010, together with a random sample of type 2 diabetes cases (4 per LADA case). Controls ≥35 years old without diabetes are randomly selected from the population registry and matched to the case by date of participation and residential area (incidence density sampling (168)). Six controls are selected per LADA case, corresponding to 1 control per case of diabetes (LADA or type 2). In 2012, a sister-study to ANDIS was launched in Uppsala County (~350 000 inhabitants), the ANDIU-study (All New Diabetic in Uppsala; http://www.andiu.se/). Since 2012, ESTRID recruits cases and controls also in Uppsala. To date (February 2016), 414 LADA and 1380 type 2 diabetes cases and 1793 controls have been included in ESTRID with a participation rate of 81% among cases and 66% among controls. Of the participants, 97% come from Scania and 3% from Uppsala.

Figure 5. A chart presentation of the ESTRID Study, 2010–2016



4.2.1 Study population

ESTRID is an ongoing study and the data collection is continuing. The ESTRID dataset has been updated annually.

Paper II: For this paper we used information from the dataset updated in August 2013; and included 250 cases of LADA, 764 cases of type 2 diabetes, and 1012 controls with complete information on alcohol consumption and covariates.

The newest update was done in July 2015 and analyses of papers IV and V were based on that dataset.

Paper IV: It included cases and controls with available information on smoking and other covariates of interest, consisting of 377 LADA cases, 1188 type 2 diabetes cases, and 1472 controls.

Paper V: It was restricted to men, due to low prevalence of moist snuff use among women (~4%). All men with complete data on moist snuff use and covariates of interest were included in paper V, comprising 200 LADA cases, 724 type 2 diabetes cases, and 699 controls.

4.2.2 Biochemical analysis

The clinical measurements were conducted only in diabetes patients. All blood samples were collected from patients at time of diagnosis and analyzed at Lund university laboratory, Malmö, Sweden. GADA was analyzed with ELISA assay (Enzyme-linked immunosorbent assay; ELISA, RSR Ltd.). GADA was regarded as positive if the serum antibody level >10 IU/mL. ELISA assay showed 84% sensitivity and 98% specificity at a cutoff of 10.7 IU/mL

(14). C-peptide concentrations were determined by using the IMMULITE 2000 (Siemens Healthcare Diagnostics Product Ltd., Llanberis, UK) or by Cobas e 601 analyzer (Roche Diagnostics, Mannheim, Germany) (169). Fasting plasma glucose and C-peptide were used to calculate Homeostasis model assessment (HOMA)-IR to estimate insulin resistance and HOMA-%B to assess β-cell function (165).

4.2.3 Classification of diabetes

Patients were diagnosed in the primary health care of Scania and Uppsala counties. Based on age at onset and GADA and C-peptide measurements, cases with age-at-onset ≥35 years were classified as LADA if they were GADA positive (≥10 IU/mL) with C-peptide ≥0.2 nmol/l (IMMULITE)/or ≥0.3 nmol/l (Cobas e 601). C-peptide was used here to indicate remaining β-cell function and separate LADA from type 1 diabetes, as previously suggested (14; 170). Patients were classified as having type 2 diabetes if they had onset ≥35 years, were GADA negative (<10) and had C-peptide >0.6 nmol/l (IMMULITE) /or ≥0.72 with Cobas e 601. Cases of LADA were further stratified into subgroups by median GADA levels; LADA with high GADA, and LADA with low GADA (Papers II and IV).

4.2.4 Questionnaire data

ESTRID participants filled out questionnaires containing information on health, anthropometrics, and lifestyle factors, including tobacco use and alcohol consumption. The questionnaires used in the ESTRID are available at http://ki.se/imm/estrid. Patients received questionnaires in proximity to diagnosis with careful instructions to report their lifestyle as it was prior to diagnosis.

4.2.4.1 Alcohol consumption (Paper II)

The ESTRID questionnaires contained a previously validated food frequency questionnaire for alcohol intake (171). Participants were asked how often they consumed alcoholic drinks. Among current drinkers, data on frequency of consumption for different alcoholic drinks were collected. Information on the amount of each alcoholic beverage consumed on each occasion was also collected with an open ended question (given in centiliters [cl] for each unit of can, bottle, or glass). Total average intake of alcohol in grams per day was computed by combining information of frequency and amount of drinking. The content of alcohol per cl alcoholic beverage was estimated as follows: 0.25 g for light beer, 0.40 g for strong beer, 0.40 g for cider, 0.85 g for wine, 1.5 g for liquor, and 3 g for hard liquor. The variable was analyzed both categorized and continuous. Frequency of alcohol consumption was also classified into five groups ranging from ≤1time/month (as reference group), to ≥1 time/day.

4.2.4.2 Smoking (Paper IV)

There were detailed questions on lifetime smoking habits, including smoking status (current, former, and never), time-points for start and stop of smoking, and number of cigarettes smoked per day. Participants were categorized by smoking intensity into light and heavy

smokers (<20 or ≥20 cigarettes/day). Cumulative dose of smoking (pack-year) was regarded as equivalent to 20 cigarettes smoked daily for one year, and assessed in three categories (never, 0–15, and ≥15). The exposure to smoking was assessed for the period of time before index year (for cases the year of diagnosis, and for controls, participation year).

4.2.4.3 Moist snuff use (Paper V)

Lifetime history of moist snuff use was assessed, including status of snuffing (current, former, never), number of boxes consumed weekly, and time-points for start and stop of snuffing. Moist snuff use was categorized according to intensity of consumption (<5, and ≥5 boxes/week), and cumulative dose of snuffing (<10, and ≥10 box-year). One box-year was defined as consumption of one box of moist snuff per day for one year. An index year was defined as year of diagnosis for cases and year of participation for controls. The exposure to moist snuff use was assessed prior to the index year.

4.2.4.4 Confounding factors

Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Any family history of diabetes was considered as positive (yes/no). Educational level classified into: primary school, upper secondary school or university. Participants were categorized according to their smoking habits into: never, former, and current smokers; pack-year of smoking (continuous) was also available. Alcohol consumption was categorized as (0.1–4.9 g/day, 5–14.9 g/day, and ≥15 g/day). Educational level was classified into three categories; primary, secondary, and university. Physical activity categorized as physically active, or inactive.

4.3 STATISTICAL ANALYSIS

HUNT Study:

For the prospective studies (Papers I and III), hazards ratios (HR) and confidence intervals (CI) were estimated by Cox proportional hazards regression models. We used age as underlying timescale in the cox models. Person-years of follow-up were calculated from the age that the participant entered the study (HUNT1 or HUNT2) until age at onset of diabetes, death or age at end of the follow-up period at HUNT2 (1997) or HUNT3 (2008), whichever came first.

In the cross-sectional analysis of paper V we used logistic regression to estimate prevalence odds ratios (POR) for type 2 diabetes in relation to moist snuff use.

ESTRID Study:

In the case-control analyses of papers II, IV, and V based on the ESTRID study, we used logistic regression to estimate odds ratios (OR) and CIs according to case-control methodology. The incidence density sampling scheme of the controls (matching for date of participation and residential area) allowed us to interpret ORs as incidence rate ratios (168). We performed conditional logistic regression, matched for residential area and time of

participation in papers IV and V. For paper II both conditional and unconditional analyses were conducted. The ORs of unmatched analyses were in close agreement with those from matched analyses; we preferred to present the results of unconditional analyses as they made more efficient use of our limited data.

Restricted cubic spline regression was used in paper II to model potential dose-response relationship between total grams of alcohol consumption per day and risk of LADA and type 2 diabetes. This model is a common tool for testing non-linearity function of a continuous covariate relaxing the assumption of linearity, it also has the advantage compared to using categorical data that we do not lose information (172).

Linear least squares regression was used to assess the association between alcohol consumption (papers I and II), smoking (paper IV), and HOMA indices. The association with GADA was assessed using the Tobit regression model to account for the fact that GADA (outcome variable) was truncated at 250 (173).

All analyses were carried out using the Statistical Analysis System (SAS 9.2, 9.3, and 9.4; SAS Institute Inc, Cary, NC). We used Stata for restricted cubic spline regression (Stata version SE13.0, College Station, TX, USA).

5 RESULTS

5.1 POPULATION CHARACTERISTICS; COMPARISON OF HUNT AND ESTRID

The HUNT and ESTRID participants were similar with regard to many characteristics (table 1), however, compared to ESTRID participants, those in HUNT were slightly leaner, and had lower prevalence of heavy smoking and family history of diabetes, and higher proportion of low education and physical inactivity.

LADA patients at HUNT compared to those in ESTRID were heavier, and had higher prevalence of family history of diabetes, and lower proportion of ever/or heavy smoking. They had lower levels of HOMA-IR and higher HOMA-β. The median levels of GADA were higher in the LADA patients of ESTRID (177 WHO unites/ml) compared to those of HUNT (140 WHO unites/ml).

5.2 PAPER I, ALCOHOL AND LADA/TYPE 2 DIABETES IN HUNT

Results based on 22 years follow-up suggested that frequent alcohol intake was associated with a reduced risk of LADA (HR for 1-4 times over the last 14 days; 0.70, 95% CI; 0.45-1.08) and type 2 diabetes (HR; 0.68, 95% CI; 0.49–0.93) (table 2). Results of 11 years follow-up, with information on amount of alcohol consumed indicated a 60% reduced risk of LADA (HR, 0.38, 95 % CI=0.15-0.98) in those consuming 2-7 gram/day. Similar findings were seen for type 2 diabetes (HR for 10-15 gram alcohol /day; 0.48, 95% CI; 0.28-0.77).

5.3 PAPER II, ALCOHOL AND LADA/TYPE 2 DIABETES IN ESTRID

The findings suggested that alcohol consumption was dose-dependently (tested with restricted cubic spline models) and inversely associated with the risk of LADA (OR for every 5-g increment; 0.94; 95% CI; 0.89-0.99). The lowest risk was seen in those consuming 5–15 g/day (OR; 0.56, 95% CI; 0.41–0.77) (table 3). Stratification of LADA cases by median GADA (≤median; 152 IU/ml) revealed that the risk reduction was restricted to more type 2-like LADA (LADA with low GADA) (OR for every 5-g increment 0.85; 95% CI; 0.76-0.94) whereas no reduced risk was seen for more autoimmune, high-GADA LADA. In line with this, every 5-g increment of daily alcohol intake was associated with 10% reduction in HOMA-IR (p=0.0418) in LADA patients, and an 8% reduction in patients with type 2 diabetes (p=0.0345). Wine consumption (OR 0.95 per 5-g wine, 95% CI; 0.89-1.02 [LADA]; OR; 0.94, 95% CI; 0.90–0.99 [type 2 diabetes]) rather than beer and liquor intake was inversely related to diabetes risk.

We reanalyzed the HUNT data stratified by median GADA levels (paper II). The results indicated that the possible benefit of alcohol intake on LADA was restricted to LADA patients with low GADA (HR; 0.54, 95% CI; 0.29–0.99) but not LADA with high GADA (0.93, 95% CI 0.48–1.80). Of note, the numbers of cases were very low in these analyses.

Table1. Characteristics of participants in in HUNT (1984-2008) and in ESTRID (2010-2015).

	HUNT study [a]				**ESTRID study** [a]		
	Individuals without diabetes	LADA [β]	Type 2 diabetes		Controls	LADA	Type 2 diabetes
No. of individuals (N)	114549	164	2244		1495	378	1204
Age at onset of diabetes, mean, y (SD)	-	59 (11)	61 (11)		-	59 (12)	63 (10)
Men, N (%)	54205 (47)	80 (49)	1170 (52)		705 (47)	201 (53)	726 (60)
Low education, N (%)	45086 (51)	81 (59)	1140 (61)		361 (24)	113 (30)	450 (37)
BMI, mean, kg/m2 (SD)	25.5 (3.8)	29.1 (5.0)	29.8 (4.5)		25.9 (4.1)	28·1 (5.3)	31.1 (5.4)
Obese (BMI≥30), N (%)	1152 (12)	59 (42)	852 (43)		218 (15)	118 (31)	613 (51)
Physical inactive, N (%)	28650 (33)	58 (42)	688 (38)		221 (15)	63 (17)	271 (23)
Alcohol abstainers, N (%)	10503 (12)	20 (14)	262 (14)		142 (10)	43 (11)	165 (14)
With Family History of Diabetes, N (%)	37449 (33)	90 (55)	1436 (64)		354 (24)	167 (44)	595 (49)
Ever smokers, N (%)	48084 (54)	65 (46)	1027 (55)		767 (51)	207 (55)	756 (63)
Heavy smokers, N (%)	6203 (7)	7 (5)	213 (12)		168 (12)	67 (18)	238 (21)
C-peptide, mean (SD), nmol/l	-	0.66 (0.56)	0.93 (0.53)		-	0.81 (0.53)	1.33 (0.58)
Fasting plasma glucose, mean (SD), mmol/L	-	8.8 (3.2)	8.3 (2.4)		-	15.5 (8.3)	11.3 (6.2)
HOMA_IR, mean (SD)	-	2.3 (1.4)	2.5 (1.3)		-	5.2 (13.0)	5.8 (13.4)
HOMA_B, mean (SD)	-	70 (47)	71 (41)		-	45 (35)	69 (36)
GADA, median (interquartile range), WHO unites/ml	-	140 (59-572)	-		-	177 (25-250)	-

[a] The total numbers in ESTRID and HUNT studies. The numbers might differ with the ones presented in the studies due to missing values of covariates included in the models.

[β] Adults with autoimmune diabetes, including LADA (85.7%) and type 1 diabetes patients in adult

Table 2. HRs of LADA and type 2 diabetes in relation to frequency of alcohol consumption, HUNT Study, 1984-2008

		LADA			Type 2 diabetes	
	Person-year	No. cases	HR (95% CI) [α]		No. cases	HR (95% CI) [α]
Frequency of alcohol intake during the last 14 days						
Abstainers	104,870	20	0.77 (0.42-1.40)		262	0.94 (0.79-1.10)
< 1 time	358,980	70	Reference		765	Reference
1-4 times	456,271	43	0.70 (0.45-1.08)		736	0.99 (0.88-1.11)
5-10 times	27,782	7 [β]	0.72 (0.28-1.83)		40	0.71 (0.51-0.99)
>10 times	16,987	-	-		38	0.88 (0.61-1.26)

[α] HRs are adjusted for age, sex, BMI, smoking, family history of diabetes, education, and physical activity
[β] Two high frequent alcohol intake categories were combined because of small numbers.

Table 3. ORs of LADA and type 2 diabetes in relation to alcohol consumption, ESTRID Study, 2010-2013

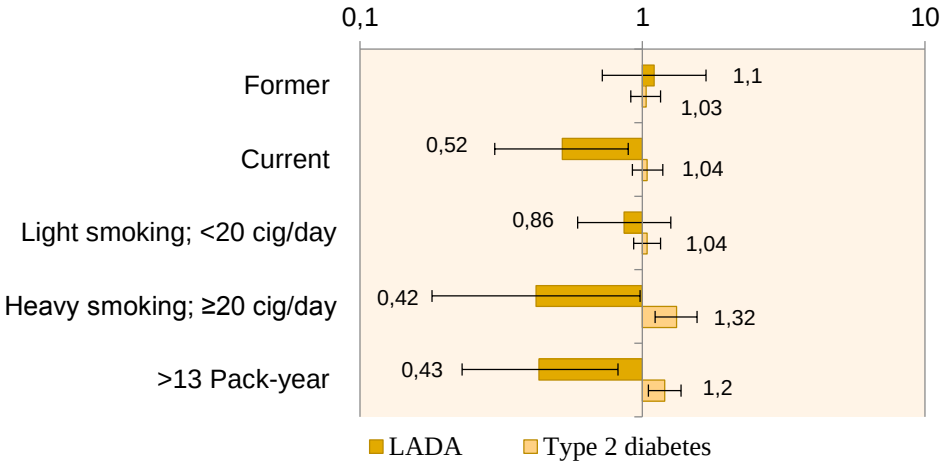
	Type 2 diabetes		LADA Low GADA (≤median;152 IU/mL)		LADA High GADA (>median;152 IU/mL)	
	Cases/ controls	OR (95% CI) [α]	Cases/ controls	OR (95% CI) [α]	Cases/ controls	OR (95% CI) [α]
Alcohol intake (g/day)						
Non-drinkers [β]	106/94	1.34 (0.89-2.00)	16/94	0.97 (0.51–1.84)	9/94	0.92 (0.42-2.01)
0·01-4.9	305/343	Reference	54/343	Reference	39/343	Reference
5-14·9	177/340	0.56 (0.41-0.77)	36/340	0.60 (0.37–0.97)	38/340	0.95 (0.58-1.56)
15-24·9	83/130	0.59 (0.39-0.87)	11/130	0.42 (0.21–0.86)	23/130	1.33 (0.74-2.38)
≥25	93/105	0.58 (0.38-0.88)	6/105	0.23 (0.10–0.57)	14/105	1.01 (0.50-2.02)
Alcohol (per 5g/day)		0.95 (0.92-0.99)		0.85 (0.76-0.94)		1.00 (0.94-1.06)

[α] Adjusted for age, sex, BMI, family history of diabetes, smoking, and education
[β] Non-drinkers: including abstainers and former drinkers
[γ] Analyses were run only in alcohol drinkers; additional adjustment for total alcohol intake

5.4 PAPER III, SMOKING AND LADA/TYPE 2 DIABETES IN HUNT

Current smoking was associated with a 48% reduced risk of LADA (HR; 0.52, 95% CI; 0.30–0.89), and heavy smoking with 58% reduced risk (HR; 0.42, 95% CI; .18–0.98) (figure 6). Compared to never smoking, heavy smoking was associated with lower levels of GADA (0.009 vs.0.056; *p*=0.001). In contrast, heavy smoking was associated with an increased risk of type 2 diabetes (HR; 1.32, 95% CI=1.11-1.56), which was most evident in overweight men (HR; 1.70, 95% CI=1.38-2.10).

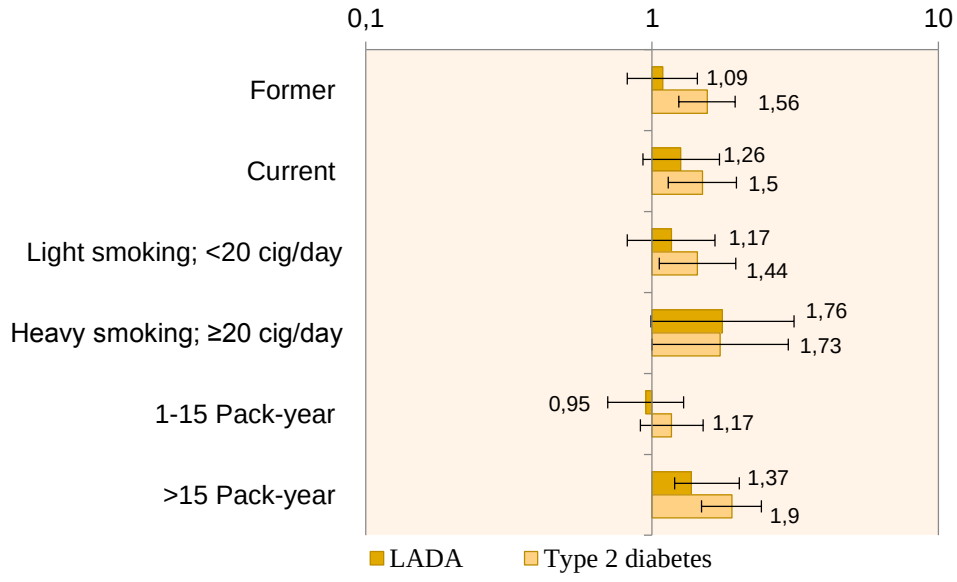
Figure 6. HR of LADA and type 2 diabetes in relation to smoking, results from HUNT, 1984-2008.



5.5 PAPER IV, SMOKING AND LADA/TYPE 2 DIABETES IN ESTRID

In this case-control study, we did not find any indication of reduced risk of LADA in smokers (OR in current smokers; 1.26, 95% CI; 0.93-1.72). These findings were consistent when analyses were stratified by family history of diabetes (yes, no), BMI (<25, ≥25 kg/m2), age (<55, ≥55 year), and median level of GADA (<178, ≥178 IU/mL). Instead, heavy smoking (≥15 pack-year) was associated with an increased risk of LADA (OR; 1.37, 95% CI; 1.02-1.84) (figure 7). Comparing heavy and never smoking LADA patients indicated higher mean levels of HOMA-IR (9.89 vs. 4.38, *p*=0.0479) and HOMA-B (55.7 vs. 42.5, *p*=0.0204) in smokers. Also, heavy smokers had lower median levels of GADA (75 vs. 250, *p*=0.0445), compared with never smokers. Smokers also had an increased risk of type 2 diabetes (OR for ≥20 cigarettes/day; 1.73, 95% CI; 1.00-2.99) (figure 7).

Figure 7: OR of LADA and type 2 diabetes in relation to smoking, results from ESTRID, 2010-2015.



5.6 PAPER V, SNUFFING AND LADA/TYPE 2 DIABETES IN ESTRID AND HUNT

In ESTRID there was no indication of an excess risk of LADA in snuff users; in never smokers, OR was estimated at 1.01 (95% CI; 0.45-2.29) for ≥10 box-years (table 4). For type 2 diabetes, results were similar, high snuff consumption was associated an OR of 1.01 (95% CI; 0.42-2.41) in ESTRID and a POR of 0.89 (95% CI; 0.21-3.78) in HUNT.

Combining exposure from moist snuff and cigarettes indicated that exclusive smokers (ever) have an increased risk of type 2 diabetes (OR; 1.59, 95% CI; 1.16-2.18) which was even more pronounced in heavy smokers (OR; 2.20, 95% CI; 1.40-3.45). No increased risk was seen in exclusive moist snuff users, nor in subjects who combine snuffing and smoking. Similar findings were seen in HUNT (table 5).

Table 4. OR of LADA and type 2 diabetes in relation to moist snuff use in men, ESTRID (2010-2015), and HUNT3 (2006-2008)

	Ever smokers		**Never smokers**	
	Cases/ subjects without diabetes	OR (95% CI) [α β]	Cases/ subjects without diabetes	OR (95% CI) [α β]
ESTRID				
Type 2 diabetes				
Never	310/223	reference	205/254	reference
Current	92/92	0.91 (0.39-1.01)	27/36	1.17 (0.58-2.37)
Light snuff users (<5 box/week)	121/115	0.78 (0.53-1.14)	22/46	0.83 (0.41-1.71)
Heavy snuff users (≥5 box/week)	46/29	0.92 (0.49-1.72)	16/26	1.01 (0.42-2.41)
<10 box -year	105/104	0.77 (0.52-1.15)	13/39	0.74 (0.31-1.77)
≥10 box -year	60/36	1.00 (0.57-1.74)	22/32	1.00 (0.47-2.11)
LADA				
Never	66/223	Reference	70/254	Reference
Current	32/92	1.08 (0.64-1.80)	13/41	0.98 (0.45-2.11)
Light snuff users (<5 box/week)	29/115	0.76 (0.46-1.27)	10/46	0.75 (0.34-1.67)
Heavy snuff users (≥5 box/week)	16/29	1.64 (0.80-3.35)	6/26	0.67 (0.24-1.86)
<10 box -year	23/104	0.67 (0.39-1.16)	5/39	0.46 (0.16-1.31)
≥10 box -year	22/36	1.82 (0.96-3.46)	11/32	1.01 (0.45-2.29)
HUNT				
Type 2 diabetes				
Never	488/7807	Reference	184/7019	Reference
Ever	130/3998	0.86 (0.70-1.07)	27/1779	1.12 (0.72-1.72)
Light snuff users (<3 box/week)	107/3521	0.82 (0.65-1.03)	23/1552	1.15 (0.72-1.82)
Heavy snuff users (≥3 box/week)	7/262	0.90 (0.41-2.00)	2/176	0.89 (0.21-3.78)

[α] Adjusted for age, BMI, and family history of diabetes; and also for pack-year of smoking in ever-smokers

[β] OR for analysis based on ESTRID; and POR for analyses based on HUNT

Table 5. OR of LADA and type 2 diabetes for different combinations of smoking and moist snuff use, results from ESTRID, 2010-2015.

Tobacco use		Type 2 diabetes		LADA	
	No. controls	No. cases	OR (95% CI) [α]	No. cases	OR (95% CI) [α]
None	254	205	Reference	70	Reference
Ever smoking (never snuff)	223	310	1.59 (1.16-2.18)	66	1.06 (0.71-1.57)
Ever snuff (never smoking)	72	38	0.95 (0.55-1.66)	17	0.74 (0.40-1.39)
Ever smoking and snuff use	150	171	1.16 (0.81-1.66)	47	0.97 (0.62-1.52)
Heavy smoking, ≥20 cigarette/day (never snuff use)	54	119	2.20 (1.40-3.45)	27	1.48 (0.84-2.62)
Heavy smoking and snuff use	46	66	1.45 (0.83-2.51)	21	1.44 (0.78-2.66)

[α] Adjusted for age, BMI, and family history of diabetes

6 DISCUSSION

6.1 MAIN FINDINGS

This doctoral thesis aimed to contribute to a better understanding of the association between tobacco use and alcohol consumption and the risk of LADA and type 2 diabetes. Identifying risk factors for LADA does not only contribute with information that may eventually be useful for prevention, but also contributes to understanding of the pathogenesis of LADA.

The findings indicate that alcohol consumption is associated with reduced risk of more type 2-like LADA. The possible beneficial effect appears to be mediated through improved insulin sensitivity rather than an immune-modulating effect as there was no indication of a beneficial effect of alcohol on autoimmunity or LADA with high GADA-levels. With regard to smoking, this thesis provided inconsistent but somewhat interesting, results; A reduced risk of LADA was seen in smokers in the Norwegian study, whereas in the Swedish study, there was no indication of a reduced risk. In contrast, heavy smokers had an increased risk of LADA. Given the hybrid pathogenesis of LADA, including both autoimmunity and insulin resistance, it is plausible that smoking has both positive (by way of reducing autoimmunity) and negative (promoting insulin resistance) effects on development of LADA. Which of these effects that dominates may depend on phenotypic or genetic characteristics of the individual. Finally, the results indicated that moist snuff, a smokeless tobacco product with high nicotine content, is unrelated to the risk of type 2 diabetes and LADA. This suggests that the increased risk of type 2 diabetes (and LADA) seen in smokers is not attributed primarily to nicotine exposure but to other components of tobacco smoke.

6.1.1 Alcohol

The findings from the HUNT study, although based on few patients, suggested that moderate intake of alcohol is associated with a reduced risk of LADA. We could confirm and extend these findings with data from ESTRID, where we also found that this association was limited to the risk of LADA with low GADA (more type 2-like diabetes). With regard to type 2 diabetes, our findings confirm a similar risk reduction associated with moderate drinking as seen in other studies (40; 174; 175).

The possible mechanisms behind a beneficial effect of alcohol consumption could be improved insulin sensitivity (110-113), anti-inflammatory effects (115; 116), and increasing adiponectin levels (117-120). In line with this we found an inverse association between alcohol consumption and HOMA-IR both in patients with LADA and type 2 diabetes, suggesting also that the results are due to the same underlying mechanism. Some experimental studies indicate that alcohol has anti-inflammatory and immune-modulating properties (176), and this may explain the adverse association between alcohol and some autoimmune diseases like rheumatoid arthritis, Graves' hyperthyroidism, multiple sclerosis, and lupus erythematous (124-126; 177; 178). However, findings are inconsistent as other

studies did not find evidence of any beneficial effect on autoimmunity (179-181), consistent with our studies.

In line with some previous studies (121; 182), the reduced risk of LADA and type 2 diabetes was primarily associated with intake of wine rather than other alcoholic beverages. One possible explanation could be that wine contains other substances, like polyphenols and antioxidants that may also have beneficial effects, rather than only ethanol. Also, wine drinking may be related to a healthier lifestyle behavior in general, particularly with regard to diet and socioeconomic factors, compared with drinking spirit and beer (121). Our data confirm other studies (108; 182) also suggest that frequent drinking is associated with reduced risk of both LADA and type 2 diabetes, irrespective of the amount of alcohol consumption.

6.1.2 Smoking

We could confirm previous studies showing a positive and dose-dependent association between smoking and the risk of type 2 diabetes (41). With regard to LADA, results based on HUNT and ESTRID were contradictory; with prospective data from HUNT we found an inverse association between smoking and the risk of LADA. In contrast, with data from ESTRID including more than twice as many cases, we did not find any indication of beneficial effect of smoking on LADA; we even observed an increased risk of LADA in heavy smokers. Methodological differences could contribute to the conflicting results. Notably, the cut-off level of GADA positivity was higher in HUNT than in ESTRID. However, raising the cut-off level in ESTRID did not change the results. The main analysis of HUNT did not distinguish between LADA and type 1 diabetes with adult onset, but still, restricting the analysis to LADA patients (without insulin treatment during the first year after diagnosis) yielded similar result. With regard to information on smoking, due to prospective design of HUNT any misclassification of exposure can be assumed non-differential and hence unlikely to result in spurious associations. However, the use of baseline information on smoking implies that changes in smoking habits that occur during follow-up can not be taken into account. In ESTRID on the other hand, information on smoking was collected retrospectively until the year of diagnosis and can thus be expected to cover the etiologically relevant period. However, this information was retrospective and recall bias, e.g. patients overestimating past smoking could have contributed to the excess risk.

There are mechanistic evidences that are compatible with both beneficial and harmful effects of smoking on glucose homeostasis. The increased risk of type 2 diabetes in smokers has primarily been attributed to an insulin resistance-inducing effect of nicotine (139; 140). However, it has been suggested that smoking (or some compounds in cigarette smoke) can also exert immune-modulating and anti-inflammatory effects (144; 145); these potential effects are still controversial (144-147; 183) (figure 8). The seemingly contradictory findings of our two studies are compatible with the heterogeneous nature of LADA (184); a reduced risk can be attributed to exposure of nicotine and its anti-inflammatory and immune-modulating effect, and an increased risk could be linked to insulin resistance-inducing effect

of smoking. In line with this, smoking was associated with lower GADA levels both in ESTRID and in HUNT. The net effect of these possible positive and negative mechanisms may vary in different LADA patient populations with variable degrees of autoimmunity and insulin resistance.

Findings in other autoimmune disorders, including type 1 diabetes are also contradictory (124-126; 178; 185); A reduced risk of type 1 diabetes has been observed in offspring of smokers (103-105), whereas a recent Swedish study reported an increased risk of type 1 diabetes in offspring of smoking mothers, when genetic susceptibility of *HLA*-haplotype was taken into account (186). The influence of genetic factors may indeed contribute to the seemingly inconsistent results; A very strong interaction between smoking and *HLA*-haplotype has been identified in relation to rheumatoid arthritis (187). *HLA*-haplotype is also associated with the risk of type 1 diabetes and LADA, so one could speculate that the influence of smoking on autoimmune diabetes is modified by genetic factors, which remains to be explored. In this context it is noteworthy that with regard to genetic factors the LADA population in HUNT seems to be more type 1-like (90) than the Swedish LADA population in Scania (20; 188).

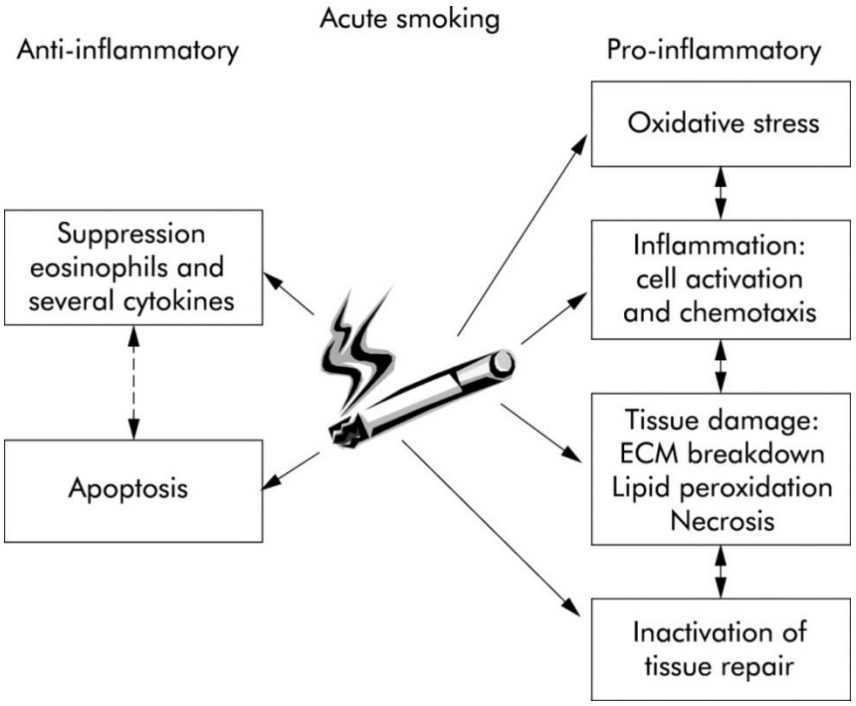


Figure 8. Summary of the acute effects of cigarette smoking; data extracted from human, animal, and in vitro studies. Adapted from van der Vaart, H. *et al*. (183)

6.1.3 Moist snuff use

In contrast to findings regarding cigarette smoking, we found no association between moist snuff use and the risk of type 2 diabetes and LADA, this supports findings of one out three small previous studies in type 2 diabetes (136; 151; 152). Our findings also fit with studies of tobacco use in relation to the risk of circulatory diseases such as stroke and myocardial infarction (189-191), where a smaller risk increase is observed in moist snuff users

compared to smokers. For LADA our findings are consistent with observations made in other autoimmune diseases including rheumatoid arthritis (192) and Crohn's disease (193) in relation to moist snuff use. This suggest that noxious substances in cigarette smoke other than nicotine, such as nitrosamine, are likely to be involved in etiology of type 2 diabetes (194), and moist snuff provides less exposure to toxins than cigarette smoking. Importantly, we did not have enough power to rule out a small effect of moist snuff use on type 2 diabetes, and this notion remains to be addressed in larger studies.

6.2 METHODOLOGICAL CONSIDERATIONS

6.2.1 Non-response

The participation rate at the first survey of HUNT was high, but it declined at HUNT 2 and HUNT3 surveys. However, the non-participations studies for HUNT surveys did not find significant difference according to health and mortality, except for elderly non-participants who had higher morbidity compared to participants in that age (102; 158).

Loss to follow-up may be a problem in longitudinal studies and lead to selection bias if there is a differential loss to follow-up related to exposure and outcome. In HUNT, participants were lost either because they died or because they discontinued their participation. Loss to follow-up could introduce bias in studies I and III, if it was related to both diabetes and alcohol consumption/smoking. In order to check the potential influence of loss to follow-up we compared the average daily alcohol consumption among those lost with those individuals remaining in the study, and no considerable difference was found (3.70 vs. 3.90 gram/day). Neither did we see any substantial difference across categories of smoking (current smoking: 34% vs 33%; heavy smoking: 8% vs. 9%) among those who re-attended compared to those lost to follow-up. However, if we assume that individuals with high alcohol consumption, if they developed diabetes, were more likely to die from it before follow-up, this could lead us to overestimate a proposed beneficial effect of alcohol consumption. Following the same line of reasoning, smokers who develop diabetes may be more likely to die, or chose not to participate in the follow-up survey. One consequence could be an overestimation of the reduced risk of LADA related to smoking. It is however hard to see how such a bias would yield contrasting results for LADA and type 2 diabetes.

In case-control studies, controls are used to estimate the prevalence of exposure in the population which produced the cases. In ESTRID, we selected controls randomly and continuously from the same population as the cases originated from according to classical case-control methodology (168). Participation rates were relatively high among cases and controls (81% vs. 66%). Still, if consumption of alcohol, cigarettes, or moist snuff among participating controls was different from those who did not choose to participate, this would bias our findings. In the ESTRID controls prevalence of smoking, moist snuff use and alcohol consumption was comparable with what has been reported by the Public Health Agency of Sweden for the population living in Scania (148). Furthermore, the similarities of our findings

regarding type 2 diabetes with previous prospective studies (41; 182), is an important indicator of the validity of the controls.

6.2.2 Misclassification of Exposure

A high correlation between self-reported smoking data and cotinine levels (biomarker of exposure to tobacco) has been shown (195; 196). It has also been demonstrated that self-reported alcohol consumption correlates with biomarkers, and furthermore, that using beverage-specific questions increases the validity of the self-reports (200). Still, the probability of bias in self-reported information on lifestyle cannot be ruled out (197; 198). In HUNT, misclassification can be assumed to be non-differential, i.e. not affected by disease status, and will hence tend to dilute a true association, distort dose-response relationships, but is unlikely to result in spurious excess risks (199). A baseline assessment of alcohol intake may not be enough to get an accurate picture of exposure status over the whole time at risk and this may dilute the associations. In our study population, abstainers at least did not change their consumption: 86% of abstainers at the beginning of HUNT1 remained abstainers at HUNT2. With regard to smoking we had the advantage of repeated assessments in HUNT, which limits misclassification.

In ESTRID, information on alcohol and smoking was based on retrospective self-reporting. This implies that bias is introduced e.g. if people with diabetes changed their consumption after diagnosis and reported accordingly. To minimize this potential bias, cases received the questionnaire close to diagnosis with instructions to report their habits as they were before diagnosis. Importantly, in current dietary guidelines in Sweden, there is no recommendation about changing alcohol consumption for patients with diabetes (40). For smoking and moist snuff use, to minimize the problem of reverse causation (disease affecting smoking habits), we only included information on tobacco use until the index year, which was one year before diagnosis. It should also be noted that our results for type 2 diabetes in relation to both alcohol and smoking were in close agreement with findings from several prospective studies (41; 182), where exposure information was collected several years before onset of disease.

In study V, we used cross-sectional HUNT data, hence, recall bias is a potential problem as patients may have quit using tobacco several years ago following diagnosis or shift from smoking to snuffing. We did use information about previous tobacco habits, but it is possible that those who quit underestimate their previous use.

One of the concerns in alcohol consumption related observational studies is known as “Sick quitter” bias. According to this notion abstainers are not an appropriate reference group because it is possible that some people abstain from alcohol for health reasons, or because they are taking some prescribed drugs that interact with alcohol or, because they are former heavy drinkers (200). Notably, we found a reduced diabetes risk of in moderate consumers, irrespective of whether abstainers or very low consumers were used as reference category.

6.2.3 Misclassification of disease

An important requirement for any epidemiological study is a set of well-defined diagnostic criteria. In HUNT, diabetes cases were identified by self-report. A validation done previously among HUNT participants indicated a very high concordance between questionnaire data and medical records (159). Still, cases of undiagnosed diabetes will be missed. If undiagnosed diabetes is more common in individuals with low socioeconomic status which is also related to behaviors like high alcohol consumption and smoking, potential bias could occur. However, it has been reported that undiagnosed diabetes is uncommon (0.3%) in participants of HUNT (201), and the influence of this potential bias is probably minor. Cases included in ESTRID were identified and diagnosed through the public health system. It is possible that some of the cases may have undiagnosed diabetes, and if so, this will tend to make them more similar to the cases and most probably lead to a dilution of the associations between lifestyle and diabetes risk.

The diabetes patients were classified into different types based on information about age at onset and GADA levels. Sensitivity of the GADA assays were 64-84% which means that some cases of LADA was missed and this reduces power but is otherwise unlikely to affect the results. With regard to specificity, it was 100% in HUNT which means that cases of type 2 diabetes are unlikely to be classified as LADA. Hence, this potential misclassification does not seem to be a probably explanation for the similarity of alcohol results seen for type 2 diabetes and LADA. However, in ESTRID the specificity of the GADA assay was 98%, implying the possibility of false-positive LADA cases (type 2 diabetes patients misclassified as having LADA), which could account for some of the similarities of alcohol consumption results seen between LADA with low GADA and type 2 diabetes. It is however unlikely to explain the lack of association observed between alcohol intake and LADA with high GADA. Also, misclassification of cases may have contributed to the similar smoking results for LADA and type 2 diabetes; If we assume that 24 (2%) of the type 2 diabetes patients are misclassified as having LADA, and all of them were heavy smokers, this would result in an attenuation of the association (OR of LADA for ≥15 pack-year estimated at 1.10). Still, this seems highly unlikely and furthermore, this potential misclassification could not explain why we find no indication of a beneficial effect of smoking on LADA in ESTRID.

In our studies, only GADA measurements were available as an indicator of autoimmunity and we may therefore be missing LADA patients who are positive for other autoantibodies such as like IAA, and ICA. GADA is however the most sensitive maker for autoimmune diabetes in adults and is present in 70–80% of patients with autoimmune diabetes (94). Furthermore, it was recently shown in the HUNT Study that only 10% of LADA cases were positive for other antibodies than GADA (202)

6.2.4 Confounding

An advantage was that we had access to detailed information about many potential confounders including smoking, BMI, physical activity, family history of diabetes, alcohol

consumption, and education. The possibility of remaining residual confounding or confounding from unmeasured factors still needs to be considered while interpreting the results. In this context it is noteworthy that with regard to analyses of alcohol in ESTRID, further adjustment for intake of coffee and soft drinks, as well as total energy intake (data now shown in the paper), did not alter the results.

7 CONCLUSION

This thesis includes studies of alcohol consumption and tobacco use, two common, modifiable lifestyle factors, in relation to the risk of LADA. The findings indicate that alcohol may have a beneficial effect on the risk of LADA particularly for more type 2-like LADA, as shown previously for type 2 diabetes. The influence of smoking was contradictory with a reduced risk of LADA in the Norwegian study, and an increased risk of LADA in the Swedish study. With regard to moist snuff use, no association was found with type 2 diabetes and LADA, this supports data suggesting that moist snuff is less harmful than other tobacco products (189-191; 194; 203).

Overall these findings indicate that LADA and type 2 diabetes may have a partly shared etiology, including insulin resistance. It is possible that in the presence of mild autoimmunity, improvements (alcohol) or impairment (smoking) of insulin sensitivity will accelerate or postpone development of manifest diabetes. The contradictory findings of smoking and the different results seen for LADA with high and low GADA levels support the concept of heterogeneity of LADA. Hence, it is possible that the effect of environmental triggers on the risk of LADA depends on interaction with genetic factors and/or the severity of the underlying autoimmune process.

The findings of the thesis fit with previous observations in LADA (79-86) and indicate that lifestyle factors may be important for prevention of LADA. Confirmation of these findings in other populations are warranted and there are many questions that remain to be addressed including both the role of individual lifestyle factors, the interaction between sets of potential risk factors and the interaction between lifestyle and genetic factors.

8 FUTURE DIRECTIONS

- Most studies on risk factors of LADA are based on Scandinavian populations. Studies in other ethnic groups and populations with different genetic disposition and clinical characteristics are clearly needed.

- The risk factors of LADA are largely unexplored, and many aspects remain to be addressed, including factors such as early life exposures, dietary factors and exposure to environmental toxins.

- Environmental factors are likely to interact with genetic factors in development of LADA but this remains to be explored, including interaction between smoking and genetic risk factors

- Factors that lead to epigenetic variation over time through DNA methylation (e.g. exposure to toxins), are also interesting to explore in future studies.

- There is a lack of studies on long term consequences of LADA including the risk of macro- and microvascular complications.

- The optimal treatment of LADA remains to be established.

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