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# AUTOIMMUNE DIABETES IN ADULTS

EPIDEMIOLOGICAL STUDIES OF
RISK FACTORS AND MORTALITY

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# ABSTRACT

Although autoimmune diabetes in adults is a common form of diabetes, knowledge on risk factors and long term consequences of the disease is limited. The aims of this thesis were to investigate the influence of socioeconomic factors (education and occupation), sleep disturbances and psychological well-being on the risk of developing autoimmune diabetes in adults, to investigate whether genetic variation in the melatonin receptor 1B (*MTNR1B*) contributes to the association between poor sleep and type 2 diabetes which has been previously suggested, and finally to investigate the risk of mortality from all causes, cardiovascular disease and ischemic heart disease in adult-onset autoimmune diabetes, with consideration of the possible influence of metabolic risk factors, glycaemic control, lifestyle factors and socioeconomic position.

These studies are based on data from the Norwegian HUNT Study, to date the largest population-based study where incident cases of autoimmune diabetes in adults can be separated from cases of type 2 diabetes. The HUNT Study consists of three separate surveys performed on three occasions in 1984-2008 and contains information from questionnaires, clinical examinations and blood samples. Information on mortality was obtained by linkage to the national Cause of Death Registry. Individuals who were positive for antibodies against glutamic acid decarboxylase and with onset of diabetes at ≥35 years were classified as having autoimmune diabetes in adults.

The main finding of Study I was that high educational levels (university versus primary school) were associated with an increased risk of autoimmune diabetes in adults (HR 1.98, 95% CI 1.21-3.26) after adjustment for BMI, physical activity, smoking, alcohol consumption, and family history of diabetes, whereas type 2 diabetes was more common in those with low education. An increased risk of autoimmune diabetes in adults was also seen in individuals who reported having sleep disturbances and low psychological well-being (HR 1.84, 95% CI 1.10-3.09), a risk similar to that seen in type 2 diabetes (HR 1.31, 95% CI 1.13-1.50) (Study II). The results from Study III indicated that there was no influence of the *MTNR1B* genetic variant on the association between poor sleep and type 2 diabetes. The association remained after adjustment for genotype and was seen in non-carriers as well as in carriers of the risk allele. Mortality from all causes (HR 1.55, 95% CI 1.25-1.92), cardiovascular disease (HR 1.87, 95% CI 1.40-2.48) and ischemic heart disease (HR 2.39, 95% CI 1.57-3.64) was increased in autoimmune diabetes in adults compared to individuals without diabetes. Importantly, mortality risk was as high as in type 2 diabetes, despite a more favourable metabolic risk profile in patients with autoimmune diabetes. In these patients, excess mortality appeared to be primarily associated with poor glycaemic control.

These findings suggest, for the first time, that socioeconomic and psychosocial factors contribute to the development of autoimmune diabetes in adults. The results are in line with previous data indicating that the aetiology of autoimmune diabetes is partly similar to that of type 2 diabetes but suggest, also, that there are other, currently unidentified, environmental risk factors for autoimmune diabetes that remain to be explored. Finally, the results indicate that survival in individuals with autoimmune diabetes with adult onset would be improved by a more effective treatment.

# LIST OF PUBLICATIONS

I. Olsson L, Ahlbom A, Grill V, Midthjell K, Carlsson S. High levels of education are associated with an increased risk of Latent Autoimmune Diabetes in Adults. Results from the Nord-Trøndelag Health Study. Diabetes Care 34:102-107, 2011

II. Olsson L, Ahlbom A, Grill V, Midthjell K, Carlsson S. Sleep disturbances and low psychological well-being are associated with an increased risk of autoimmune diabetes in adults. Results from the Nord-Trøndelag Health Study. (Submitted)

III. Olsson L, Pettersen E, Ahlbom A, Carlsson S, Midthjell K, Grill V. No effect by the common gene variant rs10830963 of the melatonin receptor 1B on the association between sleep disturbances and type 2 diabetes: results from the Nord-Trøndelag Health Study. Diabetologia 54:1375-1378, 2011

IV. Olsson L, Grill V, Midthjell K, Ahlbom A, Andersson T, Carlsson S. Mortality in autoimmune diabetes in adults is linked to poor glycaemic control. (In manuscript)

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| Anti-GAD | Antibodies against glutamic acid decarboxylase |
| BMI | Body mass index |
| CI | Confidence interval |
| CVD | Cardiovascular disease |
| $HbA_{1C}$ | Haemoglobin $A_{1C}$ |
| HDL | High-density lipoprotein |
| HPA | Hypothalamic-pituitary-adrenal |
| HR | Hazard ratio |
| HUNT | Nord-Trøndelag Health Study |
| ICD | International Classification of Disease |
| IHD | Ischemic heart disease |
| LADA | Latent autoimmune diabetes in adults |
| MTNR1B | Melatonin receptor 1B |
| OR | Odds Ratio |
| SNP | Single –nucleotide polymorphism |
| WHO | World Health Organization |

# 1 BACKGROUND

## 1.1 DIABETES, PREVALENCE AND IMPACT ON HEALTH

Diabetes is a public health problem that is becoming increasingly important as the number of people with the disease increases at an alarming rate (1, 2). The prevalence of diabetes varies between different parts of the world. In the Scandinavian countries, the prevalence has been estimated to 3-4% in the adult population (3-5). The global prevalence of diabetes among adults has been estimated to increase from 6.4% in 2010 to 7.7% in 2030, affecting about 439 million individuals by the year 2030 (1).

In addition to demographic transition, the growing burden of diabetes is linked to increasing levels of obesity and a sedentary lifestyle (6, 7). Beside the negative impact on the health and well-being of individuals, diabetes and its complications impose a considerable economic burden on society (8, 9).

## 1.2 DIAGNOSIS AND CLASSIFICATION OF DIABETES

Diabetes is diagnosed by hyperglycaemia. The World Health Organization (WHO) diagnostic criteria for diabetes are fasting plasma glucose ≥7.0 mmol/l or 2-hour plasma glucose ≥11.1 mmol/l (measured after a standardized oral glucose tolerance test) (10). In 2011, WHO additionally stated that haemoglobin $A_{1C}$ ($HbA_{1C}$) (≥6.5%) can be used as a diagnostic test for diabetes (11).

Current WHO recommendations classify the major forms of diabetes into type 1 diabetes with beta cell destruction; type 2 diabetes with varying degrees of insulin resistance and insulin hyposecretion; and gestational diabetes, i.e. diabetes restricted to pregnancy (10).

## 1.3 TYPE 1 DIABETES

Type 1 diabetes is an autoimmune, inflammatory disease where the immune system destroys the insulin-producing pancreatic beta cells, usually leading to severe insulin deficiency. Autoimmunity in type 1 diabetes is usually manifested by antibodies against antigens related to the beta cells. Such antibodies include glutamic acid decarboxylase (anti-GAD), islet cell or insulin antibodies (12).

The Nordic countries are among the countries with the highest incidence of type 1 diabetes (13). Type 1 diabetes accounts for 10-15% of all diabetes cases in Sweden (14). Both type 2 diabetes and autoimmune diabetes are increasing and according to a new report, the number of new cases of type 1 diabetes in Europe is expected to double between 2005 and 2020 (15). To prevent this development it is important to identify common, and potentially preventable, risk factors in the population.

Genetic risk factors for type 1 diabetes are well recognized, and a major part of the genetic liability resides in certain haplotypes within the human leukocyte antigen (HLA) complex (16). Environmental factors are also thought to be important and most likely contribute to the increasing number of cases. There are few established risk factors for type 1 diabetes besides certain virus infections (17), but an increased risk has been linked to some dietary factors (e.g. cow's milk, wheat gluten and lack of vitamins D and E), perinatal (e.g. older maternal age at birth and excessive maternal weight gain) and psychosocial (e.g. stress, negative life events) factors (18, 19).

## 1.4 TYPE 2 DIABETES

Type 2 diabetes is characterized by a combination of insulin resistance and an inadequate compensatory insulin secretory response. Type 2 diabetes is the most common form of diabetes accounting for 85-95% of all cases (20). Metabolic control in type 2 diabetes tends to deteriorate with time, primarily due to a successive decrease in beta cell function. Pharmacological treatment becomes more important with increasing duration of the disease and insulin treatment is eventually initiated in many patients.

Risk factors for type 2 diabetes include genetic factors (21), and a strong impact of family history of diabetes has been demonstrated (22). The impact of environmental factors is well documented and in particular that of obesity (23) and physical inactivity (24). Also smoking (25) and certain dietary habits (26) play a negative role. Type 2 diabetes is thus largely preventable. It has been demonstrated that 90% of all new cases of type 2 diabetes can be attributed to the combination of overweight and modifiable life style factors (27, 28).

## 1.5 AUTOIMMUNE DIABETES IN ADULTS

Type 1 diabetes is the most common form of diabetes among children and adolescents. However, about half of the cases of type 1 diabetes develop in adult life (29, 30). Still, most previous epidemiological studies on autoimmune diabetes are focused on type 1 diabetes in childhood or adolescence, and data in age groups above 30-40 years are scarce. Autoimmune diabetes with adult onset can be divided into Latent Autoimmune Diabetes in Adults (LADA) and "classic" type 1 diabetes.

### *LADA*

The concept of LADA was first introduced to describe a group of adult patients with phenotypic type 2 diabetes, who were positive for autoantibodies related to type 1 diabetes (usually anti-GAD) but initially – as in type 2 diabetes – not needing insulin treatment to sustain acceptable metabolic control (31, 32). LADA patients constitute an important part of all diabetes. Epidemiological studies indicate that LADA accounts for 2-12% of all cases of diabetes (33).

The diagnosis of LADA is usually based on three criteria; 1) adult age at onset, 2) the presence of circulating islet autoantibodies (usually anti-GAD), and 3) insulin independence at diagnosis (lack of requirement of insulin for at least 6 months after diagnosis) (34). The second criterion distinguishes LADA from type 2 diabetes and the

third criterion is meant to distinguish LADA from type 1 diabetes. It may be surmised that LADA is characterized by a slower loss of beta cells than "classic" type 1 diabetes. However, progression of beta cell demise in LADA eventually leads, in a majority of LADA patients, to a need for insulin treatment to uphold acceptable metabolic control. Time to insulin treatment is usually shorter than in type 2 diabetes.

The concept of LADA is debated. The controversy primarily concerns whether LADA should be considered a distinct form of diabetes or rather a variant of type 1 diabetes (34-40). It has been argued that the grounds for designating LADA as a distinct aetiological entity are insubstantial and that autoimmune diabetes should be regarded as a continuum (36), but also that LADA has provided a better understanding of the grey area between type 1 and type 2 diabetes and shown that there are patients with features of both types (37). Although debated, LADA has over the last decades challenged the traditional, rather strict, classification of diabetes into two main types and given evidence for a considerable overlap between these forms of diabetes.

Throughout this thesis, the term 'autoimmune diabetes in adults' is used to describe all adult-onset autoimmune diabetes, with or without insulin dependency at diagnosis. In addition, LADA patients are also analysed as a separate entity.

## 1.6 RISK FACTORS FOR AUTOIMMUNE DIABETES IN ADULTS

Although autoimmune diabetes with adult onset is a common form of diabetes, little is known about risk factors for this form of diabetes (Figure 1). Lack of knowledge is probably due to the fact that there are few observational studies with necessary data on autoimmune markers (e.g. GAD antibodies) to separate autoimmune diabetes with adult onset from type 2 diabetes. One exception is the HUNT Study (an acronym for the Norwegian name: Helseundersøkelsen i Nord-Trøndelag), a large population-based study conducted in a Norwegian county in 1984-2008 (described in chapter 3).

*Figure 1. Risk factors for diabetes.*

Previous studies based on data from the HUNT Study indicate that traditional risk factors for type 2 diabetes, such as family history of diabetes (41), overweight and physical inactivity (42), are equally strong risk factors for autoimmune diabetes in adults. Smoking has, on the other hand, been associated with a reduced risk of autoimmune diabetes, in contrast to the increased risk seen in relation to type 2 diabetes (43). Moderate alcohol consumption has been associated with a reduced risk of both autoimmune diabetes and type 2 diabetes (44).

These studies suggest that autoimmune diabetes in adults shares risk factors with type 2 diabetes and that there may be an important role for insulin resistance in the pathogenesis of the disease. This notion is supported by previous findings of comparable insulin resistance in LADA and type 2 diabetes (45).

***Genetic factors***

Genetic variants conferring risk of type 2 diabetes are primarily related to beta cell development and function, and the strongest associations have been found for gene variants in the loci *TCF7L2* (21). Both type 1 diabetes susceptible genes (46-49), and the strongly type 2 diabetes associated gene *TCF7L2* (48, 50) have been associated with LADA. It has been proposed that LADA genetically is an admixture of type 1 and type 2 diabetes (48, 51).

## 1.7 SOCIOECONOMIC FACTORS

There is a well-established association between socioeconomic factors (e.g. education, occupation and income) and type 2 diabetes (52-54). In a recent systematic review and meta-analysis (55), the summarized results from 23 studies showed that individuals in the lowest compared to the highest socioeconomic group had a 30-40% increased risk of type 2 diabetes. The mechanisms through which socioeconomic factors could relate to type 2 diabetes are not fully understood. The association can partly be attributed to traditional risk factors such as obesity, physical inactivity and smoking (56, 57), although other factors such as access to health care services and information, available healthy foods and places to exercise (58), may also be involved.

Contrary to findings in type 2 diabetes, higher incidence rates of type 1 diabetes have been correlated to indicators of national prosperity such as low infant mortality and high gross domestic product, suggesting type 1 diabetes to be a wealth related disease (59). Accordingly, lower rates of type 1 diabetes have been seen in neighbourhoods characterized by lower income, educational and occupational levels (60-63).

Environmental risk factors, such as diet and breast feeding practices, vitamin D deficiency, factors with a potential effect on the immune system, have been hypothesised to contribute to these differences (59, 60, 62-64). Another potential explanation is the hygiene hypothesis, which posits that the immune system needs environmental stimulation early in life in order to fully develop and mature (65-68). Thus, a reduction in early exposure to infections would result in an increased risk of

autoimmune disorders. Socioeconomic factors have previously not been studied in relation to autoimmune diabetes that develops in adult life.

## 1.8 PSYCHOSOCIAL FACTORS

Accumulating studies show that psychosocial factors, in addition to traditional factors, may influence the risk of type 2 diabetes. Major stressful life events (69), work stress and low sense of coherence (i.e. inability to cope with stressors) (70) have been associated with type 2 diabetes in previous cross-sectional studies. In prospective analyses, work stress (71-73), effort-reward imbalance (74), psychological distress (75) and depression (76) have been related to type 2 diabetes.

Sleep disturbances have been associated with increased risk of type 2 diabetes in an increasing number of epidemiological studies, and in pooled analyses of 13 cohorts (77), both quantity and quality of sleep predicted the risk of type 2 diabetes. It has also been demonstrated in experimental studies that reduction of the duration or quality of sleep adversely affects metabolic and endocrine function and increase risk for type 2 diabetes (78, 79).

Although the mechanisms behind these associations are not completely clarified, both indirect effects through an unhealthy lifestyle and direct neuroendocrine effects may be involved. Sleep loss has been associated with type 2 diabetes through glucose regulation and insulin resistance (80). It has also been suggested that psychosocial stress may contribute to the development of type 2 diabetes through activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) -axis in genetically susceptible individuals. Activation of the HPA-axis causes increasing cortisol levels, which may contribute to abdominal obesity, insulin resistance and type 2 diabetes (81, 82).

It has been suggested that the aetiology of type 1 diabetes in children may include psychosocial factors, such as psychosocial stress in the family and stressful life events (83, 84). However, there are no studies on psychosocial factors such as sleep disturbances and psychological well-being in relation to the risk of developing autoimmune diabetes in adults.

In recent genome-wide association studies, a common variant in the melatonin receptor 1B (*MTNR1B*) has been linked to fasting glucose and type 2 diabetes (85-87). Melatonin is a circulating hormone mainly secreted by endocrine cells in the pineal gland, and a major regulator of circadian rhythms and pattern of sleep (88). *MTNR1B* encodes one of two known human melatonin receptors. It has been hypothesised that variation in *MTNR1B* may contribute to the association between sleep disturbances and type 2 diabetes (89).

## 1.9 COMPLICATIONS AND MORTALITY

Diabetes is a serious illness associated with increased morbidity and premature mortality (90, 91). Well-recognized complications of diabetes include damage to and failure of different organs, especially the kidneys, eyes, nerves, blood vessels and heart (92-95). Cardiovascular disease (CVD), which is a general term that describes diseases of the heart or blood vessels, is the primary cause of death in both type 1 and type 2 diabetes (96-98). A recent meta-analysis of 102 prospective studies reported that diabetes confers about a two-fold excess risk of CVD, independently from other risk factors (99). Excess mortality in type 2 diabetes is, in addition to traditional risk factors, associated with duration of diabetes (100) and degree of glycaemic control (101). Excess mortality in type 1 diabetes is also associated with poorly controlled glycaemia and CVD risk factors related to hyperglycaemia, such as renal disease (92).

There are few studies on long-term consequences of autoimmune diabetes in adults, including mortality (102, 103), and knowledge on factors influencing this risk is limited. In cross-sectional studies, it has been shown that the metabolic syndrome is less prevalent in adult-onset autoimmune diabetes than in type 2 diabetes (104-106). Despite the lower prevalence of metabolic risk factors, equally increased mortality in autoimmune diabetes and type 2 diabetes has been demonstrated in the few existing prospective studies on mortality in autoimmune diabetes (102, 103). $HbA_{1C}$ levels have been shown to be higher in GAD positive than in GAD negative individuals (102, 107). Still, the importance of glycaemic control for mortality in autoimmune diabetes is unclear.

# 2 AIMS

The overall aim of this thesis was to expand current knowledge on risk factors and mortality in autoimmune diabetes in adults.

The specific aims were:

- To investigate whether the risk of autoimmune diabetes in adults differs between socioeconomic groups and to compare such risk with that for type 2 diabetes (Study I).

- To investigate the influence of sleep disturbances and low psychological well-being on the risk of autoimmune diabetes in adults and to compare such risk with that for type 2 diabetes (Study II).

- To investigate whether the single-nucleotide polymorphism (SNP) rs10830963 in the melatonin receptor 1B is associated with sleep problems and whether this genetic variation contributes to the association between sleep disturbances and type 2 diabetes (Study III).

- To investigate the risk of mortality from all causes, cardiovascular disease and ischemic heart disease in autoimmune diabetes and type 2 diabetes, with consideration of the possible influence of metabolic risk factors, glycaemic control, lifestyle factors and socioeconomic position (Study IV).

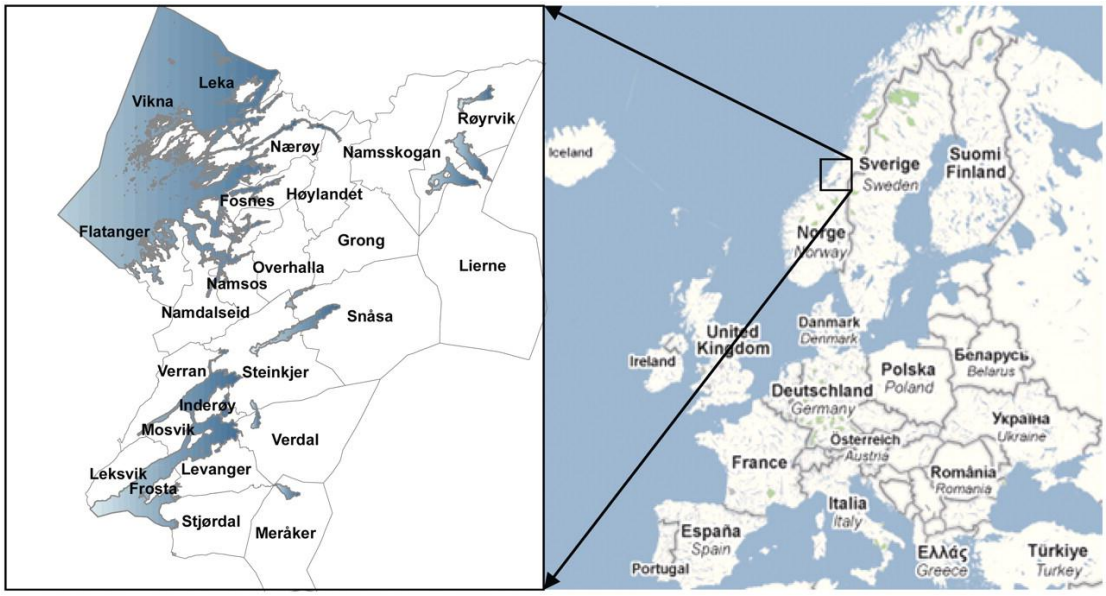
# 3 MATERIAL AND METHODS

## 3.1 THE NORD-TRØNDELAG HEALTH STUDY (HUNT)

This thesis is based on data from the Norwegian HUNT Study, one of the largest studies of its kind with detailed information collected through questionnaires, clinical examinations and blood samples from about 125,000 individuals (108). The HUNT Study is, to date, the largest population-based study where incident cases of autoimmune diabetes with adult onset can be separated from incident cases of type 2 diabetes.

The HUNT Study consists of three separate surveys, performed on three occasions between 1984 and 2008 in the Norwegian county of Nord-Trøndelag (Figure 2). All inhabitants of the county aged 20 years or older were invited to participate. The HUNT Study has been thoroughly described elsewhere (108).

*Figure 2. Norway and the Nord-Trøndelag County. Adapted from Krokstad 2012 (108).*




The clinical examinations included measurements of height, weight, waist and hip circumference and blood pressure. Blood samples, carried out whenever the individual attended, were collected at HUNT2 and HUNT3. These non-fasting serum samples were analysed for total and high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose for all participants. For participants who reported diabetes, blood was also drawn for analyses of $HbA_{1C}$. For an overview of the design of the HUNT Study see Figure 3.

Using this data, two cohort studies (Study I-II), one nested-case control study (Study III), and one registry-based study (Study IV) were performed.

## 3.2 THE COHORT STUDIES (STUDY I-II)

For Study I-II, information from all three HUNT surveys was used to form a cohort, consisting of individuals who could be followed prospectively for incidence of diabetes during 11-22 years. Eligible were individuals who participated in two or three surveys and who were free of diabetes at baseline (i.e. at HUNT1 or HUNT2 depending on when the participant entered the study).

In Study I, the study population consisted of 56,296 individuals with baseline information on educational level. During follow-up, 2,461 incident cases of diabetes were identified. In Study II, the study population consisted of 53,394 individuals with baseline information on sleep disturbances and/or psychological well-being, including 1,895 incident cases of diabetes.

## 3.3 THE NESTED CASE-CONTROL STUDY (STUDY III)

Study III was a case-control study, nested within the HUNT Study that included all cases of diabetes identified in the HUNT2 survey. For these cases and for matched controls, randomly selected among the participants of HUNT2, DNA was extracted from blood samples and genotyped for the *MTNR1B* SNP rs10830963. In total 1,322 prevalent cases of type 2 diabetes were investigated together with 1,447 controls.

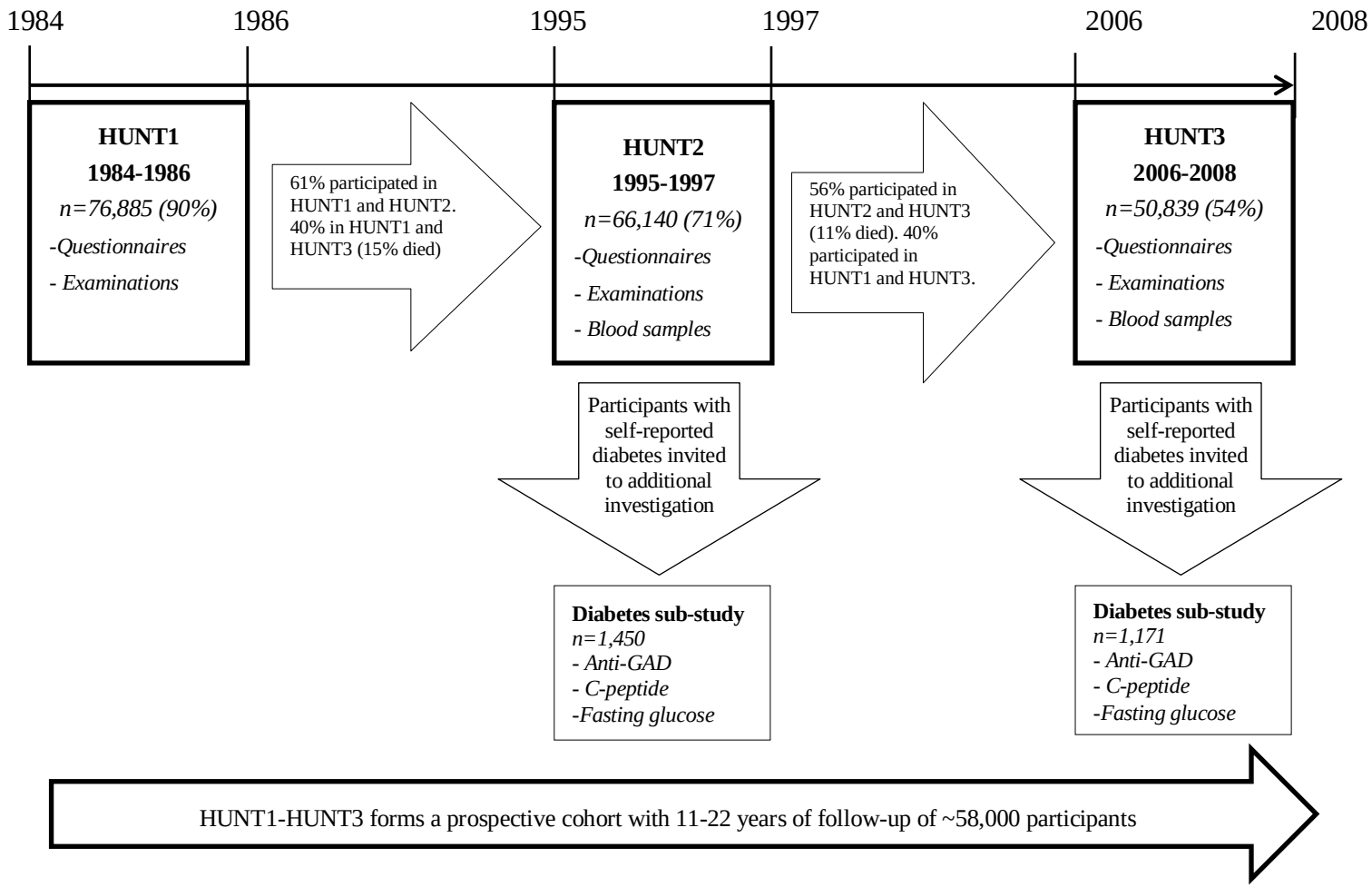
In addition, prospective analyses were performed using information on sleep status from HUNT1 from 838 cases of type 2 diabetes (who were free of diabetes at baseline, i.e. in HUNT1) and 1,133 controls from the HUNT2 survey who participated also in the HUNT1 survey.

## 3.4 THE REGISTRY-BASED STUDY (STUDY IV)

In Study IV, the study population consisted of all participants with complete baseline information on diabetes status in HUNT2, n=64,815, including 3,159 cases of diabetes. The study population was followed for mortality between 1995 and 2009 by linkage to the Norwegian national Cause of Death Registry.

Causes of death were coded according to the International Classification of Disease (ICD). Cause of death from CVD was coded I00-I99 (ICD-10) and 390-459 (ICD-9), and cause of death from ischemic heart disease (IHD) was coded I20-I25 (ICD-10) and 410-414 (ICD-9). The quality of the Cause of Death Registry in Norway is high (109) and the coverage is virtually complete.

*Figure 3. Flow chart of the design of the HUNT Study 1984-2008.*



## 3.5 IDENTIFICATION AND CLASSIFICATION OF DIABETES

In the HUNT Study, cases of diabetes were identified by questionnaire and individuals with self-reported diabetes were invited to a follow-up appointment performed within a few days of the surveys (at HUNT2 and HUNT3). At this appointment, fasting blood samples were drawn and analysed with regard to levels of anti-GAD, C-peptide and glucose. Antibody levels were expressed as an antibody index relative to a standard serum and an index of ≥0.08 was considered positive. This cut-off was chosen to achieve the highest possible specificity (1.00) with an acceptable corresponding sensitivity (0.64). Anti-GAD values are reported in WHO units (0.08=43 WHO units/ml), as recommended (110).

In this thesis, diabetes was classified based on information on anti-GAD and age at onset. Anti-GAD positive individuals with age at onset of diabetes ≥35 years were classified as having autoimmune diabetes in adults. Anti-GAD negative individuals with adult onset of diabetes were classified as having type 2 diabetes. To separate LADA from autoimmune diabetes in adults, information on insulin treatment (available for 85% of the cases) was used. LADA, i.e. cases without insulin treatment within the first year after diagnosis, constituted 83% of all the incident cases of adult-onset autoimmune diabetes with available information on insulin treatment.

## 3.6 EXPOSURE INFORMATION

The HUNT questionnaires included detailed questions on health, lifestyle (including physical activity, smoking and alcohol consumption), socioeconomic factors, psychosocial situation and family history of diabetes. The questionnaires from the HUNT Study can be found at http://www.ntnu.edu/hunt/data/que.

The participants were asked to specify their highest level of education and the response options were collapsed into three categories; low (primary school), middle (upper secondary school), and high (university) education. Participants were also asked to classify themselves in one of nine occupational categories.

Sleep disturbances were measured by one question at HUNT1 and three questions at HUNT2. As suggested previously (111, 112), two questions on difficulties falling asleep and early awakening were combined to operationalize insomnia. This operationalization was constructed to meet the criteria for insomnia as specified by DSM-IV (Diagnostic and Statistical Manual for Mental Disorders) (113). Insomnia was considered as present in individuals who reported having problems with initiating sleep and/or maintaining sleep often or almost every night and absent if not.

Five questions on psychological well-being were included in both the HUNT1 and the HUNT2 questionnaires and a single measure of well-being was obtained from the total score of these five items, as suggested previously (114). These questions have also been used in previous reports based on data from the HUNT Study (115-117).

## 3.7 STATISTICAL ANALYSES

Cox proportional hazards models were used to estimate hazard ratios (HR) of diabetes together with 95% confidence intervals (CI) in relation to socioeconomic and psychosocial factors in Study I-II, and hazard ratios of mortality in relation to baseline diabetes status in Study IV.

In Study I-II, person-years were accumulated from age at baseline (either at HUNT1 or HUNT2) until age at diagnosis of diabetes, age at death, or age at end of follow-up (either at HUNT2 or HUNT3), whichever came first. In Study IV, person-years were accumulated from age at baseline (HUNT2) until age at death or age at end of follow-up, December 31$^{th}$ 2009. Age (in years) was used as the underlying time scale in the Cox model (118). The analyses were time-dependent and exposure information was updated during follow-up.

Cox proportional hazard model is a widely used model in time-to-event analysis (119, 120). This model does not make any assumption about the shape of the hazard function and the baseline hazard is allowed to vary. However, the hazards in the exposed and unexposed groups are assumed to be proportional over follow-up time (119). Violations of the proportional hazard assumption are equivalent to effect modification by time (120), in this case age, and in these analyses there were no indications of strong effect modification by age. In the present studies, all variables were included as time dependent if possible and also, the aim was to investigate the population average effect over the range of the observed time, rather than individual risks.

Logistic regression models were used to calculate odds ratios (OR) and 95% CI in the case-control study (Study III). An additive effect of the risk allele was assumed. Most risk variants can be described using additive models, which assume that on a log scale, the risk in carriers of two copies of a certain allele (e.g. GG) is twice than in carriers of one allele (e.g. CG) (121, 122). Dominant (CG and GG genotypes were pooled) and recessive (CC and CG genotypes were pooled) models were used in additional analyses.

All analyses conducted and reported in this thesis were performed in SAS 9.2 (SAS Institute, Cary, NC, USA).

## 3.8 ETHICAL CONSIDERATIONS

The HUNT Study and the mortality follow-up of the participants were approved by the Norwegian Data Inspectorate and the Regional Medical Research Ethical Committee. The participants gave written informed consent. Data were analysed anonymously and without personal identification numbers.

# 4 RESULTS

In the HUNT Study, the prevalence of diabetes in the adult population increased from 2.9% to 4.3% between 1984 and 2006. During follow-up, 165 incident cases of autoimmune diabetes with adult onset were identified, constituting 7% of all new cases of diabetes. Characteristics of incident cases of autoimmune diabetes in adults, type 2 diabetes and LADA are presented in Table 1.

Individuals with autoimmune diabetes in adults had lower BMI, lower triglycerides levels and higher HDL cholesterol compared to individuals with type 2 diabetes. On the other hand, these individuals had higher levels of fasting glucose and $HbA_{1C}$ (Table 1). As compared to the whole group of adult-onset autoimmune diabetes, LADA cases did not differ with regard to most characteristics.

*Table 1. Characteristics of incident cases of diabetes in the HUNT Study.*

| | ***Autoimmune diabetes in adults*** | ***Type 2 diabetes*** | ***LADA*** | ***p*** |
|---|---|---|---|---|
| | | | | |
| No. | 165 | 2,215 | 112 | |
| Men, % | 49.1 | 51.9 | 50.0 | 0.490 |
| Age at onset, years | 59.5 (11.4) | 61.1 (11.0) | 60.8 (10.5) | 0.070 |
| BMI | 28.7 (5.1) | 29.8 (4.9) | 29.0 (5.0) | 0.005 |
| Systolic blood pressure, mm Hg | 145.9 (22.7) | 146.4 (22.8) | 148.6 (23.0) | 0.775 |
| Diastolic blood pressure, mm Hg | 78.4 (13.2) | 79.5 (13.5) | 79.3 (13.4) | 0.291 |
| Triglycerides, mmol/l | 2.26 (1.61) | 2.63 (1.62) | 2.26 (1.32) | 0.005 |
| Cholesterol, mmol/l | 5.40 (1.23) | 5.45 (1.36) | 5.45 (1.21) | 0.636 |
| HDL-cholesterol, mmol/l | 1.29 (0.43) | 1.19 (0.34) | 1.28 (0.48) | <0.001 |
| Fasting glucose, mmol/l | 8.8 (3.2) | 8.3 (2.4) | 8.8 (3.0) | 0.058 |
| $HbA_{1C}$ , % | 8.1 (2.1) | 7.5 (1.6) | 7.9 (2.1) | <0.001 |
| C-peptide, pmol/l | 542.0 (735.5) | 850.0 (588.0) | 632.0 (674.0) | <0.001 |

Data are means (SD) or percentages. Data on C-peptide is expressed as median and interquartile range.
P-value for the difference between autoimmune diabetes in adults and type 2 diabetes.

## 4.1 STUDY I

The main finding of Study I was that high levels of education (i.e. university versus primary school) were associated with an increased risk of developing autoimmune diabetes in adults (HR 1.60, 95% CI 1.00-2.56). In contrast, the risk of type 2 diabetes was reduced in individuals with high education (HR 0.69, 95% CI 0.57-0.82).

In autoimmune diabetes, adjustment for BMI, physical activity, smoking, alcohol consumption, and family history of diabetes did not change these results, whereas in type 2 diabetes the association was, to a large extent, explained by differences in BMI and lifestyle (Table 2). The associations were seen in both men and women (results not shown). Similar, although less pronounced associations were seen for high versus low occupational position in autoimmune diabetes (HR 1.53, 95% CI 0.82-2.84) and in type 2 diabetes (HR 0.87, 95% CI 0.73-1.03).

Education and occupation were also analysed in relation to the risk of developing LADA; the adjusted HRs of LADA were 1.68 (95% CI 0.93–3.05) for individuals with high versus low education, and 1.57 (95% CI 0.77-3.22) for high versus low occupational position.

*Table 2. HRs of autoimmune diabetes in adults and type 2 diabetes associated with education.*

| | ***Autoimmune diabetes in adults*** | | | ***Type 2 diabetes*** | | |
|---|---|---|---|---|---|---|
| | Cases | Model 1* | Model 2† | Cases | Model 1* | Model 2† |
| ***Education*** | | | | | | |
| Primary school | 74 | 1 | 1 | 995 | 1 | 1 |
| Upper secondary | 21 | 0.61 (0.37-1.01) | 0.60 (0.35-1.02) | 408 | 0.88 (0.78-0.99) | 0.98 (0.87-1.12) |
| University | 27 | 1.60 (1.00-2.56) | 1.98 (1.21-3.26) | 152 | 0.69 (0.57-0.82) | 0.89 (0.74-1.06) |

*Model 1 adjusted for age and sex. †Model 2 adjusted for age, sex, BMI, physical activity, smoking, alcohol consumption, and family history of diabetes.

Characteristics of cases with high versus low education were compared. Cases of autoimmune diabetes with high levels of education were more often treated with insulin (56 versus 33%, p=0.05), had longer duration of diabetes (10 versus 7 years, p=0.05) and lower levels of C-peptide (217 versus 637 pmol/l, p<0.001). There were also indications of higher levels of anti-GAD in cases with high education (363 versus 276 WHO units/ml, p=0.20). In cases of type 2 diabetes with high versus low education there were no apparent differences with regard to insulin treatment, duration of diabetes or levels of C-peptide.

## 4.2 STUDY II

The main finding of Study II was the increased risk of developing autoimmune diabetes in adults seen in individuals who reported sleep disturbances and low psychological well-being. Compared to individuals who reported no such problems, HR for autoimmune diabetes was 1.84 (95% CI 1.10-3.09). Corresponding estimate for type 2 diabetes was HR 1.31 (95% CI 1.13-1.50).

Poor sleep and low well-being were associated with increased diabetes risk also when analysed separately, although with some differences between men and women. An increased risk of autoimmune diabetes was seen in men with baseline sleep disturbances (HR 1.83, 95% CI 1.05-3.20), but not in women. These results correspond well to those seen in relation to type 2 diabetes (Table 3). An association between low well-being and risk of autoimmune diabetes was seen only in women (Table 3), whereas an increased risk of type 2 diabetes was seen in both men and women with low psychological well-being (HR 1.34, 95% CI 1.16-1.54). These results persisted after adjustment for age, sex, BMI, waist to hip ratio, physical activity, smoking, alcohol consumption, education, and family history of diabetes.

Analyses in relation to LADA showed a similarly increased risk associated with sleep disturbances and psychological well-being (HR 1.81, 95% CI 1.00-3.28). There was an increased risk of LADA in men with poor sleep (HR 1.99, 95% CI 1.05-3.77), but not in women, whereas an increased risk of LADA in relation to low well-being was seen for both men and women; HR for men and women combined was estimated at 1.75 (95% CI 0.90-3.39).

No differences in BMI, blood pressure, blood lipids, duration of diabetes or levels of C-peptide were seen in cases with and without sleep problems or low well-being. In autoimmune diabetes, mean anti-GAD levels were lower in exposed versus non-exposed cases (177 vs. 306 WHO units/ml, p=0.04). Insulin treatment also appeared to be less frequent in the exposed group, although this difference was not significant (42 versus 56%, p=0.28).

*Table 3. HRs of autoimmune diabetes in adults and type 2 diabetes associated with sleep disturbances and low psychological well-being.*

| | | ***Autoimmune diabetes in adults**** | | | ***Type 2 diabetes†*** | | |
|---|---|---|---|---|---|---|---|
| | **No diabetes** | **Men** | | **Women** | **Men** | | **Women** |
| | Person-years | Cases | HR (95% CI) | Cases | HR (95% CI) | Cases | HR (95% CI) | Cases | HR (95% CI) |
| ***Sleep disturbances*** | | | | | | | | | |
| No | 591,178 | 39 | 1 | 36 | 1 | 726 | 1 | 585 | 1 |
| Yes | 204,152 | 20 | 1.83 (1.05-3.20) | 26 | 1.32 (0.76-2.28) | 254 | 1.25 (1.08-1.44) | 312 | 0.98 (0.85-1.14) |
| ***Psychological well-being*** | | | | | | | | | |
| Very high | 198,399 | 15 | 1 | 6 | 1 | 213 | 1 | 154 | 1 |
| High | 174,463 | 18 | 1.11 (0.55-2.24) | 9 | 1.50 (0.53-4.21) | 200 | 1.01 (0.83-1.23) | 172 | 1.08 (0.86-1.35) |
| Moderate | 218,492 | 12 | 0.69 (0.32-1.47) | 19 | 1.93 (0.75-4.94) | 283 | 1.09 (0.91-1.31) | 236 | 1.13 (0.92-1.39) |
| Low | 162,469 | 13 | 1.12 (0.53-2.37) | 22 | 2.59 (1.03-6.54) | 227 | 1.29 (1.07-1.57) | 262 | 1.41 (1.15-1.73) |
| ***Sleep disturbances and low well-being combined*** | | | | | | | | | |
| Neither | 512,089 | 35 | 1 | 29 | 1 | 611 | 1 | 483 | 1 |
| Either or | 205,352 | 17 | 1.38 (0.76-2.51) | 18 | 1.16 (0.63-2.14) | 269 | 1.27 (1.10-1.47) | 278 | 1.04 (0.89-1.21) |
| Both | 80,634 | 8 | 1.90 (0.87-4.15) | 15 | 1.87 (0.94-3.74) | 106 | 1.35 (1.09-1.66) | 148 | 1.27 (1.05-1.53) |

*Adjusted for age, sex, BMI, physical activity, smoking and education. † Adjusted for age, sex and BMI.

## 4.3 STUDY III

The results from the nested case-control study showed that type 2 diabetes tended to be more prevalent in carriers than non-carriers of the risk allele of rs10830963. Among cases of type 2 diabetes, 51% were carriers of the risk allele, compared to 47% of the controls, and OR for type 2 diabetes was 1.12 (95% CI 1.00-1.27). There was also an association between the *MTNR1B* rs10830963 variant and prevalence of sleep disturbances, specifically difficulty maintaining sleep; OR for the recessive model was 1.46 (95% CI 1.03-2.06).

The main finding of this study was that the association between poor sleep and type 2 diabetes was not affected by adjustment for variation in *MTNR1B* rs10830963 (Table 4). Also, the association between sleep and diabetes was seen in non-carriers as well as in carriers of the risk allele, e.g. OR of type 2 diabetes associated with insomnia was 1.67 (95% CI 1.09-2.56) in non-carriers, and 1.79 (95% CI 1.08–2.95) in carriers. In prospective analyses of the association between baseline sleep disturbances (sleep status at HUNT1) and the risk of developing type 2 diabetes during follow-up (cases identified at HUNT2), OR was adjusted for genotype 1.35 (95% CI 0.94-1.94).

*Table 4. Odds ratios of type 2 diabetes associated with sleep disturbances.*

| | | ***Type 2 diabetes*** | |
|---|---|---|---|
| | No. of cases/controls | Adjusted for age, sex and BMI | Adjusted for age, sex, BMI and genotype |
| | | OR (95% CI) | OR (95% CI) |
| ***Difficulty maintaining sleep*** | | | |
| Never or now and again | 923/1,042 | 1 | 1 |
| Often or almost every night | 188/166 | 1.23 (0.97-1.57) | 1.23 (0.96-1.56) |
| ***Difficulty initiating sleep*** | | | |
| Never or now and again | 956/1,068 | 1 | 1 |
| Often or almost every night | 162/132 | 1.35 (1.04-1.75) | 1.36 (1.04-1.76) |
| ***Insomnia*** | | | |
| Never or occasionally | 383/518 | 1 | 1 |
| Frequently | 135/98 | 1.69 (1.22-2.33) | 1.69 (1.23-2.34) |

No. of cases/controls differ as a result of internal non-response on the sleep questions

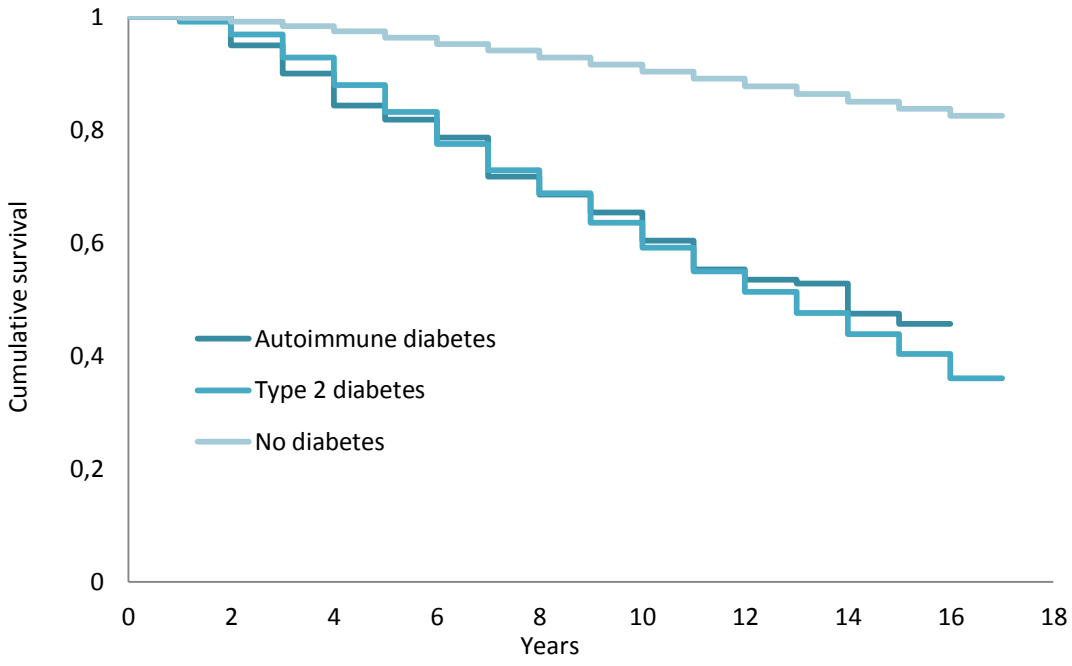
The analyses were also performed in relation to autoimmune diabetes in adults (unpublished data), but because of the low number of cases definitive conclusions could not be drawn. There were, however, no indications of an influence of the risk genotype on the association between sleep disturbances and autoimmune diabetes in adults. Prospective analyses corresponding to those for type 2 diabetes show that OR of autoimmune diabetes adjusted for genotype was 1.39 (95% CI 0.63-3.05), as compared to the unadjusted OR of 1.38 (95% CI 0.63-3.05). No association between autoimmune diabetes in adults and the risk allele was seen OR 0.94 (95% CI 0.67-1.32).

## 4.4 STUDY IV

During the 14-years of follow-up (1995-2009), 10,537 people died. Mortality was 33% among participants with diabetes and 15% in participants without diabetes. Individuals with autoimmune diabetes in adults had longer duration of diabetes (15 years versus 11 years, p<0.001), higher levels of $HbA_{1C}$ (8.3% versus 7.7%, p<0.001) and were also more likely to be treated with insulin (57% versus 24%, p<0.001), compared to individuals with type 2 diabetes. Prevalence of the metabolic syndrome was lower in autoimmune diabetes than in type 2 diabetes (55% versus 77%, p<0.001).

Autoimmune diabetes was associated with increased mortality from all causes, CVD, and IHD, similar to the mortality risks seen in type 2 diabetes (Table 5). The excess mortality was seen in both men and women, and remained after adjustment for age, sex, BMI, waist to hip ratio, physical activity, smoking, alcohol consumption, educational level, family history of diabetes and the metabolic syndrome. When analyses of autoimmune diabetes were restricted to LADA, similar excess mortality was seen (Table 5). All-cause mortality in autoimmune diabetes in adults, type 2 diabetes, and individuals without diabetes is illustrated by the Kaplan-Meier curves in Figure 4.

*Figure 4. Kaplan-Meier curves of all-cause mortality.*




Excess mortality in autoimmune diabetes and type 2 diabetes was seen across levels of BMI, and also irrespective of socioeconomic group. Mortality risk did not increase with increased duration of diabetes, but did increase with increasing levels of $HbA_{1C}$. In both autoimmune diabetes and type 2 diabetes excess mortality was seen mainly in cases with $HbA_{1C}$ ≥7%; e.g. HR of CVD mortality in autoimmune diabetes was 2.17 (1.56-3.03) in cases with $HbA_{1C}$ ≥7% and 1.36 (0.73-2.55) in cases with $HbA_{1C}$ <7%.

*Table 5. HRs of mortality from all causes, CVD and IHD in autoimmune diabetes in adults, type 2 diabetes and LADA, compared to individuals without diabetes.*

| | | | **Model 1*** | **Model 2†** | **Model 3‡** |
|---|---|---|---|---|---|
| | no. deaths/person years in individuals without diabetes | no. deaths/person years in individuals with diabetes | HR (95% CI) | HR (95% CI) | HR (95% CI) |
| ***Autoimmune diabetes in adults versus no diabetes*** | | | | | |
| All-cause mortality | 9,478/812,301 | 83/2,048 | 1.56 (1.26-1.93) | 1.61 (1.28-2.03) | 1.55 (1.25-1.92) |
| CVD mortality | 4,552/812,301 | 48/2,048 | 1.89 (1.42-2.52) | 1.92 (1.39-2.64) | 1.87 (1.40-2.48) |
| IHD mortality | 1,973/812,301 | 22/2,048 | 2.44 (1.61-3.72) | 2.49 (1.58-3.93) | 2.39 (1.57-3.64) |
| ***Type 2 diabetes in adults versus no diabetes*** | | | | | |
| All-cause mortality | 9,478/812,301 | 830/21,820 | 1.43 (1.33-1.53) | 1.41 (1.30-1.53) | 1.41 (1.31-1.51) |
| CVD mortality | 4,552/812,301 | 502/21,820 | 1.73 (1.58-1.90) | 1.69 (1.53-1.87) | 1.67 (1.52-1.84) |
| IHD mortality | 1,973/812,301 | 260/21,820 | 2.17 (1.91-2.47) | 2.08 (1.80-2.40) | 2.06 (1.81-2.35) |
| ***LADA versus no diabetes*** | | | | | |
| All-cause mortality | 9,478/812,301 | 57/1,437 | 1.50 (1.16-1.95) | 1.58 (1.20-2.08) | 1.49 (1.15-1.93) |
| CVD mortality | 4,552/812,301 | 31/1,437 | 1.68 (1.18-2.40) | 1.74 (1.18-2.57) | 1.65 (1.16-2.36) |
| IHD mortality | 1,973/812,301 | 18/1,437 | 2.54 (1.60-4.05) | 2.56 (1.56-4.21) | 2.46 (1.55-3.92) |

*Model 1 adjusted for age and sex. †Model 2 adjusted for age, sex, BMI, waist to hip ratio, physical activity, smoking, alcohol consumption, educational level and family history of diabetes. ‡Model 3 adjusted for age, sex and the metabolic syndrome.

# 5 DISCUSSION

## 5.1 MAIN FINDINGS

The overall aim of this thesis was to provide new insights into the aetiology and long-term consequences of autoimmune diabetes with adult onset.

These studies suggest that socioeconomic and psychosocial factors contribute to the development of autoimmune diabetes in adults. An increased risk of autoimmune diabetes was seen in individuals with poor sleep and low psychological well-being, findings which are similar to those in type 2 diabetes. These results are in line with data from the limited number of previous studies on risk factors for adult-onset autoimmune diabetes (41, 42), suggesting that the aetiology is partly similar to that of type 2 diabetes. High socioeconomic position was associated with increased risk of autoimmune diabetes in adults, in contrast to the results seen in relation to type 2 diabetes. The excess risk was not explained by traditional risk factors suggesting that there are other, currently unidentified, environmental risk factors for autoimmune diabetes that remain to be explored.

Finally, the results presented in this thesis showed that autoimmune diabetes with adult onset was associated with increased mortality, especially from CVD. The excess risk was found to be as high as in type 2 diabetes, despite a clearly more favourable baseline metabolic risk profile, including a lower prevalence of the metabolic syndrome. Increased mortality in autoimmune diabetes was associated with poor glycaemic control rather than metabolic risk factors.

### 5.1.1 Socioeconomic position and risk of autoimmune diabetes

There are no previous studies on the impact of socioeconomic position on the risk of adult-onset autoimmune diabetes. However, the present findings can be considered as in line with previous reports of an association between early life socioeconomic conditions and the development of type 1 diabetes in children (59-63). Factors linked to autoimmunity and beta cell destruction seemed to be involved; there was an association between high education and reduced beta cell function, i.e. more frequent use of insulin, lower levels of C-peptide and indications of higher levels of anti-GAD.

The mechanisms through which socioeconomic position could influence the development of autoimmune diabetes are not clear. A potential explanation is a lack of early exposure to infections, affecting the maturation of the immune system, as laid out in the hygiene hypothesis (65-68). Information on other factors of potential importance, such as dietary factors, e.g. early introduction of cow's milk and breastfeeding practices, vitamin D deficiency (60, 62-64), was not available in this project.

### 5.1.2 Psychosocial factors and risk of autoimmune diabetes

Sleep disturbances and low psychological well-being were associated with an increased risk of autoimmune diabetes and type 2 diabetes. The results in type 2 diabetes are consistent with previous findings of an association between psychosocial factors and type 2 diabetes (71-75, 77). This allows for relevant comparisons with risk for developing autoimmune diabetes. For this form of diabetes sleep disturbances and low psychological well-being have previously not been analysed.

Potential mechanisms linking psychosocial factors to risk of type 2 diabetes, possibly by way of inducing insulin resistance, include both indirect effects of an unhealthy lifestyle, but also direct effects, e.g. through stress-related activation of the HPA axis resulting in excessive cortisol production (81, 82). Insulin resistance is a primary component in the pathophysiology of type 2 diabetes, and although previous studies have reported insulin resistance to be significantly lower in adult-onset autoimmune diabetes than in type 2 diabetes (123, 124), comparable insulin resistance has also been reported (45). A role for insulin resistance also in autoimmune diabetes with adult onset is further supported by previous findings of aetiological similarities with regard to risk factors such as overweight and physical inactivity (42).

The results could potentially be explained by reverse causation, if pre- or undiagnosed diabetes affected sleep patterns and well-being already at baseline. Poor sleep and low well-being would then be expected to be more common in cases diagnosed closer to baseline, which was not the case. Also, the results were not changed in analyses where cases diagnosed with diabetes during the first three years of follow-up were excluded.

It has also been suggested that the impact of sleep restriction on type 2 diabetes to some extent may be explained by genetic variation in *MTNR1B* (89). However, such an explanation is not supported by the findings presented in this thesis. The association between sleep disturbances and type 2 diabetes persisted after adjustment for genotype, and was seen in non-carriers as well as in carriers of the risk allele. These results further emphasise the importance of sleep as a risk factors for diabetes in general.

### 5.1.3 Mortality in autoimmune diabetes in adults

These results suggest that individuals with autoimmune diabetes with adult onset have a more than 50% higher risk of dying compared to people without diabetes. Importantly, even though a lower mortality risk in autoimmune diabetes could have been expected based on the more favourable metabolic risk profile, mortality was equally high as in type 2 diabetes. The excess risk was seen independently from traditional risk factors, and the association between diabetes and mortality did not differ between socioeconomic groups.

There was, however, a clear association with poor glycaemic control. The results suggest that glycaemic control is a more important risk factor for mortality in autoimmune diabetes in adults than traditional metabolic risk factors, which is

consistent with previous findings (103). This leads to the questions whether treatment of autoimmune diabetes in adults is optimal or not. As concluded in a recent Cochrane Review (125), there is little evidence, and no general agreement, regarding treatment strategies for individuals with LADA (which constitute the major part of adult-onset autoimmune diabetes). Findings from the UK Prospective Diabetes Study showed that individuals with LADA that were randomized to diet or sulfonylurea therapy did not differ with regard to glycaemic control from those randomised to insulin after a follow-up period of 10 years (126). On the other hand, it has also recently been shown that early insulin treatment may lead to better preservation of metabolic control (127, 128).

### 5.1.4 LADA

In the HUNT Study, the major part of cases of autoimmune diabetes with adult onset could be classified as LADA. Accordingly, similar results were seen in relation to LADA as for the whole group of autoimmune diabetes in adults, i.e. an increased risk associated with a high socioeconomic position and adverse psychosocial factors, and increased risk of all-cause and CVD mortality. There is only one previous prospective study on mortality in LADA (102). The results presented in this thesis confirm and extend these previous findings in a larger sample and with a longer follow-up.

Existing data indicate that some genetic factors are shared by type 2 diabetes and LADA (46, 48-50). However, no association between the risk allele of rs10830963 and autoimmune diabetes in adults was seen in the analyses performed during the work with this thesis. Although somewhat hampered by small numbers, these results were in line with recent findings of a lack of association between type 2 diabetes susceptibility genotypes, including a risk allele at the *MTNR1B*, and development of islet autoantibodies and progression to type 1 diabetes in children (129).

The definition of LADA was applied as previously suggested (34), i.e. as cases of anti-GAD positive diabetes with adult onset (≥35 years) and without insulin treatment within the first year after diagnosis. These criteria are, however, not unproblematic. Both the age and the insulin criteria have been questioned and argued to be arbitrary and not based on disease progress (36, 38, 130). It has also been shown that knowledge of autoantibody positivity leads to an earlier initiation of insulin treatment than if GAD status was not known (130). Furthermore, in the HUNT Study information on insulin treatment is available on yearly, and not monthly, basis.

## 5.2 METHODOLOGICAL CONSIDERATIONS

### 5.2.1 Non-response

A major strength of this thesis is the use of data from a large and comprehensive health study, the largest population-based study where incident cases of autoimmune diabetes in adults can be analysed separately from type 2 diabetes. All adult men and women in the county were invited to participate in the three surveys that constitute the HUNT Study. Despite the high participation rate at start of the project, a decline was seen with time. However, comprehensive non-participation studies were performed after the

surveys and there were no reports of significant selection according to health or mortality, with the exception of a tendency for a higher morbidity among elderly non-participants compared to participants of the same age (131, 132).

All subjects could not be followed with regard to diabetes status, either because they died before follow-up or declined to participate. This problem pertains primarily to Study I-II where the results may be influenced, if loss to follow-up was associated with both exposure and outcome. Since diabetes is related to excess mortality diabetes may be more common in subjects who died before follow-up. With regard to the results of Study II, individuals lost to follow-up were more likely to report poor sleep and low well-being (e.g. 17% versus 13% with insomnia and 10% versus 7% reported having feelings of nervousness at baseline). It thus seems possible that more people with diabetes in the category with low well-being may have been lost to follow-up. The most probable consequence seems to be an underestimation of the association between psychosocial factors and diabetes, rather than the opposite.

Conversely, the association between socioeconomic factors and risk of autoimmune diabetes (Study I) may have been overestimated since educational level was higher in those who re-attended, compared to those lost to follow-up (23 versus 16% with high education). However, if we assume that all individuals lost to follow-up were unexposed (i.e. had a low educational level), autoimmune diabetes in adults would have to be almost seven times more common in these individuals than in re-attenders, for this bias to explain the observed association between education and autoimmune diabetes. Furthermore, the results in type 2 diabetes would then have been expected to be attenuated. Importantly, the findings in type 2 diabetes were consistent with previous reports of an association with socioeconomic position (55).

To further address the potential problem of loss to follow-up, separate analyses were performed in the cohort consisting of individuals who participated in HUNT1 and HUNT2 (where re-attendance was higher than in HUNT2-HUNT3; 72% versus 64%), and in the HUNT2-HUNT3 cohort. Importantly, similar associations were seen in both cohorts.

### 5.2.2 Misclassification of exposure

Self-reported information on exposure may have introduced bias due to misclassification of exposure. Educational level, main measure of exposure in Study I, has the advantage that it is relatively easy to measure in self-administered questionnaires. Education has also been shown to be a good indicator of socioeconomic position in relation to health and survival (133, 134), and is the most commonly used measure of socioeconomic position in analyses of association with type 2 diabetes risk (55). Education is also relevant regardless of working circumstances, and it can capture the transition from parental to adult socioeconomic position and the socioeconomic situation early in the life of the individual (134, 135). Also, one strength was that information on both education and occupation was available and similar results from analyses of the association with both exposures were seen.

The psychological well-being index used in Study II has shown a high correlation with the General Health Questionnaire ($r=0.75$, $p<0.001$) (114). Instruments intended to measure subjective well-being often include one cognitive component of life satisfaction and two affective components (i.e. the presence of positive affect and absence of negative affect) (136). Such instruments are thought to capture long-term mood rather than momentary emotions (136). The well-being index includes questions on life satisfaction (i.e. a cognitive aspect), feelings of cheerfulness and strength (i.e. positive affect) and feelings of nervousness and anxiety (i.e. negative affect) and thus conforms to previously suggested operationalization of well-being (136).

Sleep and psychological well-being were, however, measured with relatively few questions. Even though this self-reported information may be considered relatively crude, the prospective design of these studies implies that any misclassification is likely to be non-differential, i.e. that classification does not depend on diabetes status. Non-differential misclassification will in most cases bias the risk estimates towards the null value since it increases the similarity of the exposure groups (137). Another strength of these studies was that exposure information was up-dated during follow-up for individuals who participated in more than one survey.

### 5.2.3 Misclassification of disease

Identification of cases of diabetes by questionnaire has proven to be a valid method, showing a high concordance between the questionnaire information and medical records (96% of the diabetes cases were verified) (138). However, undiagnosed cases of diabetes will be missed. If undiagnosed diabetes was more common in subjects with low socioeconomic position, self-reporting of diabetes could have resulted in an overestimation of the association between high levels of education and autoimmune diabetes in Study I. The association with type 2 diabetes would then have been expected to be attenuated. Our findings are, however, consistent with previous reports (55). We have also no reason to believe that under-diagnosis would be related to a more favourable psychosocial situation and this potential bias therefore seems unlikely to explain the findings in Study II.

The presence of GAD antibodies was used to differentiate between autoimmune diabetes and type 2 diabetes. Although individuals with adult-onset autoimmune diabetes may have other diabetes associated autoantibodies, it has been shown that the presence of more than one positive antibody is far less common in LADA than in type 1 diabetes (139, 140). It has been shown that anti-GAD (present in 70-80% of all cases of autoimmune diabetes) has the highest penetration, and it was recently shown in the HUNT Study that only 10% of LADA cases were positive for antibodies other than GAD (141, 142).

A cut-off of 43 WHO units/ml was chosen for the anti-GAD assay to achieve the highest possible specificity (1.0) with an acceptable corresponding sensitivity (0.64). A high specificity is particularly important since the proportion of autoimmune diabetes is

much lower than that of type 2 diabetes. The 100% specificity implies that cases of type 2 diabetes were not falsely classified as autoimmune diabetes. However, less than 100% sensitivity means that some cases of autoimmune diabetes were classified as type 2 diabetes. This misclassification reduces the power of the analyses of autoimmune diabetes, but is unlikely to explain why the findings in autoimmune diabetes in many respects are similar to those seen in type 2 diabetes.

To investigate the underlying mechanisms of the associations studied, anti-GAD was used as marker of autoimmune activity and C-peptide as marker of insulin secretion. One concern is that C-peptide levels may be affected by duration of diabetes, and also that levels of anti-GAD change over time and therefore do not reflect degree of autoimmune activity. Previous data suggest, however, that fluctuations in anti-GAD levels may be more common in autoimmune diabetes in childhood and adolescence (141, 143). The results regarding the underlying mechanisms should, however, be interpreted with caution.

### 5.2.4 Confounding

A strength of these studies was the opportunity to take many potential confounding factors into account, e.g. family history of diabetes and various life style factors. Nonetheless, the possibility of confounding from unmeasured factors cannot be excluded. Detailed information on for instance diet is lacking. Such information, together with information on infections and social conditions in early life, would have enabled further investigations into the potential pathways through which socioeconomic factors influence the development of autoimmune diabetes.

In Study II, one important factor that we were unable to account for was obstructive sleep apnoea, which is related to both sleep disturbances and type 2 diabetes (144). However, overweight and obesity are important contributors to obstructive sleep apnoea (145) and the results remained after adjustment for BMI. Sleep restriction has also been related to increased food intake and it has been shown that sleep restriction may attenuate the effect of calorie restriction (146, 147). Since limited information on dietary factors and calorie intake was available, such factors could unfortunately not be taken into account.

## 5.3 SIGNIFICANCE

Risk factors for adult-onset autoimmune diabetes are far less understood than those for type 2 diabetes. The present studies indicate that risk factors for autoimmune diabetes with adult onset include adverse psychosocial factors such as sleep disturbances and low psychological well-being, in addition to traditional risk factors such as overweight, physical activity and family history of diabetes. Taken together, these findings support the notion that autoimmune diabetes in adults shares some risk factors with type 2 diabetes, suggesting that autoimmune diabetes to some extent may be preventable through the same modifiable risk factors as type 2 diabetes.

In contrast to type 2 diabetes, autoimmune diabetes was more common in subjects with a high socioeconomic position. This association was not explained by traditional risk factors, indicating that there are currently unknown environmental factors, potentially with an effect on the immune system that may affect the risk of adult-onset autoimmune diabetes.

The results show that adult-onset autoimmune diabetes is associated with substantially increased mortality. The results also indicate that survival would be improved by more effective treatment. Notably, treatment guidelines for this group of individuals are lacking, and these findings further emphasize the importance of developing such guidelines.

# 6 CONCLUSIONS

The present studies provide new insights into the aetiology of autoimmune diabetes in adults and the long-term consequences of this form of diabetes. A high socioeconomic position was associated with an increased risk of developing autoimmune diabetes in adults, which contrast to the decreased risk seen in relation to type 2 diabetes. The excess risk was not explained by traditional risk factors such as family history or overweight and appeared to be linked to autoimmune activity. This suggests that other, currently unidentified environmental factors may contribute to the development of autoimmune diabetes in adults, possibly trough effect on autoimmunity. Individuals reporting sleep disturbances and low psychological well-being had an increased risk of developing autoimmune diabetes in adults, corresponding to the risk increase seen in type 2 diabetes. Even though there was an association between genetic variation in *MTNR1B* and type 2 diabetes and also with reports of disturbed sleep, this genetic variant did not influence the association between poor sleep and diabetes.

The findings in adult-onset autoimmune diabetes indicate that the aetiology, in addition to autoimmunity, involves insulin resistance, and add to the limited number of existing studies that indicate that some risk factors for autoimmune diabetes with adult onset are shared with type 2 diabetes. The results also suggest that there are other, still unknown environmental risk factors which remain to be explored.

Autoimmune diabetes with adult onset was associated with increased mortality, especially from CVD. An important finding was that increased mortality was seen mainly in individuals with the poorest diabetes control. These results emphasise the importance of developing treatment guidelines, which are currently lacking, to improve survival in individuals with adult-onset autoimmune diabetes.

# 7 FUTURE RESEARCH

Knowledge on risk factors for autoimmune diabetes with adult onset is still scarce, and existing studies are based on one single material (the HUNT Study). To what extent these results can be extrapolated to other populations that may differ from the HUNT population with regard to factors such as genetic disposition, levels of obesity and prevalence of autoimmune diabetes is not clear. Additional studies, preferably with a larger number of cases and detailed information on potential risk factors, are warranted to clarify the aetiology of adult-onset autoimmune diabetes. Important potential risk factors that remain to be investigated include dietary factors and infections, and with regard to the role of psychosocial factors, the influence of psychosocial work stress, stressful life events and depression needs to be addressed. Another important area for future research is the potential interactions between environmental factors and genetic predisposition.

To date, there are few studies on long-term consequences of adult-onset autoimmune diabetes. Detailed studies on to what extent individuals with this form of diabetes suffer from complications, both macrovascular such as heart disease and stroke, and microvascular complications such as diabetic foot ulcers and damage to the eyes, and which factors that influence this comorbidity, are needed. There is also a need for studies on the impact of different treatment strategies on metabolic control and long-term complications.

Should LADA be considered a distinct type of diabetes or not? This last question remains to be answered, but the similarities of adult-onset autoimmune diabetes to type 2 diabetes demonstrated in this thesis and in previous studies, do not support the idea that LADA is primarily a variant of type 1 diabetes. Future studies on the aetiology of LADA will be one way of clarifying the role of LADA in the diabetes panorama.

# 8 SAMMANFATTNING [SUMMARY IN SWEDISH]

Trots att vuxendebuterande autoimmun diabetes är en vanlig form av diabetes är kunskapen om riskfaktorer mycket begränsad och det finns få studier om långsiktiga konsekvenser av sjukdomen. Syftet med den här avhandlingen var att undersöka huruvida socioekonomiska (utbildning och yrke) och psykosociala (sömnproblem och lågt psykiskt välbefinnande) faktorer påverkar risken att utveckla autoimmun diabetes hos vuxna (Studie I-II), att studera huruvida genetisk variation i melatonin receptorn 1B (*MTNR1B*) påverkar sambandet mellan sömnproblem och typ 2 diabetes (Studie III), samt att studera risken för ökad dödlighet hos personer med autoimmun diabetes med debut i vuxen ålder och vilka faktorer (metabola riskfaktorer, glykemisk kontroll, livsstilsfaktorer och socioekonomi) som påverkar denna risk.

Dessa studier baseras på data från den norska HUNT-studien, en totalundersökning av invånarna i det norska fylket Nord-Trøndelag. I HUNT finns information tillgänglig från frågeformulär, kliniska undersökningar och blodprover samt, genom registerkoppling till dödsorsakregistret, information om samtliga dödsfall och dödsorsaker. HUNT är så vitt vi vet världens största befolkningsbaserade studie där incidenta fall av autoimmun diabetes hos vuxna kan skiljas från fall av typ 2 diabetes.

Studie I visade att personer med en hög utbildningsnivå (universitet jämfört med grundskola) hade en ökad risk att utveckla autoimmun diabetes (HR 1,98 95% CI 1,21-3,26), efter justering för BMI, fysisk aktivitet, rökning, alkoholbruk och ärftlighet. Däremot sågs en minskad risk för typ 2 diabetes hos personer med högre utbildningsnivå. Personer med sömnsvårigheter och lågt psykiskt välbefinnande hade en ökad risk för autoimmun diabetes (HR 1,84 95% CI 1,10-3,09) och typ 2 diabetes (HR 1,31 95% CI 1,13-1,50) (Studie II). Resultaten från Studie III tydde inte på att sambandet mellan sömnproblem och typ 2 diabetes påverkades av genetisk variation i *MTNR1B*; sambandet kvarstod efter justering för genotyp och sågs både hos personer med och utan den aktuella risk allelen. Slutligen sågs en ökad risk för total (HR 1,55 95% CI 1,25-1,92) och kardiovaskulär död (HR 1,87 95% CI 1,40-2,48) hos personer med autoimmun diabetes, jämfört med personer utan diabetes (Studie IV). Dödligheten vid autoimmun diabetes var lika hög som vid typ 2 diabetes, trots en mer fördelaktig metabol riskprofil. Den ökade dödligheten tycktes vara kopplad till bristande glykemisk kontroll.

Sammantaget visar resultaten att psykosociala faktorer, vid sidan av traditionella riskfaktorer, kan påverka risken att utveckla autoimmun diabetes hos vuxna och att vissa riskfaktorer delas med typ 2 diabetes. Den ökade risken bland personer med hög utbildning förklarades inte av traditionella riskfaktorer som övervikt och ärftlighet vilket tyder på att det finns ytterligare, ännu okända miljöfaktorer som påverkar risken för vuxendebuterande autoimmun diabetes. Resultaten visar på en ökad dödlighet vid autoimmun diabetes med vuxen debut och talar för att överlevnaden för dessa personer skulle förbättras genom mer effektiv behandling.

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