Insulin-Degrading Enzyme:
a link between insulin resistance
and Alzheimer's disease

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INSULIN-DEGRADING ENZYME:  
A LINK BETWEEN INSULIN RESISTANCE  
AND ALZHEIMER'S DISEASE  

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Nog finns det mål och mening i vår färd –
Men det är vägen, som är mödan värd.

*ut I rörelse av Karin Boye*
Populärvetenskaplig sammanfattning


Vi valde ut en grupp patienter med Alzheimers sjukdom som var ordinerade någon form av diabetsläkemedel. Vi analyserade sedan om den typ av läkemedel som patienterna var ordinerade hade betydelse för de beteendemässiga och psykiska symptom som hade registrerats.

Resultaten i avhandlingen visade att patienter med diabetes typ 2 hade högre nivåer av IDE än patienter utan påverkat blodsocker, patienter med Alzheimers sjukdom och friska frivilliga. Resultaten visade också att högre nivåer av IDE var associerade med högre ålder, högre vikt och högre blodsocker. Dessutom, i proverna från hjärnbanken fann vi att högre nivåer av IDE var associerade med högre nivåer av inflammatoriska markörer och total tau, som är en markör för nedbrytning av nervceller i hjärnan. Resultaten indikerar alltså att IDE skulle kunna vara en viktig faktor i den metabola funktionen, men också kan vara associerad med viktiga riskfaktorer för kognitiv sjukdom så som inflammation. Minskade nivåer av IDE har kopplat till en ökad risk för Alzheimers sjukdom. Om patienter med typ 2 diabetes har stigande IDE-nivåer, skulle ett sjunkande värde av IDE kunna vara en markör för att patienten löper ökad risk för att drabba av eller till och med befinner sig i en tidig fas av Alzheimers sjukdom.


Sammanfattningsvis har denna avhandling bidragit till vår kunskap om IDE i blodprover från människor och visat att det är en stabil markör som kan analyseras med analysmaterial som finns tillgängligt på marknaden. IDE synes vara en potentiell markör för att upptäcka personer som riskerar att drabba av eller som befinner sig i tidig fas av Alzheimers sjukdom, innan personen själv upplever några symptom. Det är också möjligt att enzymet kan komma att utgöra mål för behandling av Alzheimers sjukdom i framtiden. Mycket forskning kvarstår dock innan analys av IDE skulle kunna bli en del av den gängse vården.
Abstract

Background: Prior research has demonstrated an elevated risk of Alzheimer's disease (AD) among individuals diagnosed with type 2 diabetes mellitus (T2DM). Insulin resistance is a potential common link between these conditions, and it is associated with AD due to the insulin-degrading enzyme (IDE), which also degrades amyloid-β (Aβ) in the brain. It has been suggested that high insulin levels may hinder IDE's effective degradation of Aβ, contributing to plaque formation. IDE is measurable in blood and is a promising biomarker for AD risk assessment and diagnosis. IDE has been demonstrated to decrease in blood from patients with cognitive impairment, but the overall knowledge about IDE in human blood is sparse. The aim of the thesis was to increase knowledge of the relationship between insulin resistance and AD, focusing on IDE as a biomarker for risk assessment and diagnosis of AD.

Study Participants: In study I, we included patients with type 2 diabetes (n=46) and compared them with patients without metabolic diseases (n=18). Serum samples were obtained from the Sophiahemmet biobank. In study II, we used post-mortem plasma samples from patients with Alzheimer's disease (n=18) and compared them with non-demented controls (n=6). These samples were obtained from the Netherlands Brain Bank. In study III, where we had three groups, we recruited patients with Alzheimer's disease (n=9) from a dementia care facility and healthy volunteers from Sophiahemmet University and Sophiahemmet Health Center (n=64). The serum samples from these individuals were then compared with serum samples from patients diagnosed with type 2 diabetes from Study I.

In Study IV, we utilized data from the Swedish BPSD (Behavioral and Psychological Symptoms of Dementia) registry. This registry is used by healthcare units that work with patients with neurocognitive disorders and contains information about the patient's diagnosis, drug treatment, and behavioral and psychological symptoms. We selected a group of AD patients prescribed some form of antidiabetic drug treatment and analyzed whether the type of treatment impacted the registered behavioral and psychological symptoms.

Methods: In study I-III, ELISA was used to analyze blood samples for levels of IDE and other metabolic markers. In study II, Luminex multiplex was used to measure inflammatory markers. These biomarkers and clinical data were analyzed statistically to investigate differences between groups and correlations between variables. In study IV, the association between BPSD and antidiabetic drug treatment was investigated using a bivariate logistic regression model, adjusted for age, sex, specific diagnosis, and drug treatment.
**Results:** The results showed that patients with T2DM had higher serum IDE levels than patients without metabolic diseases, patients diagnosed with AD, and healthy volunteers. The results also indicated that higher IDE levels were associated with older age, higher weight, and elevated blood glucose. In the samples from the brain bank, we found that higher IDE levels were correlated to increased levels of inflammatory markers and total tau.

In the analysis of registry data, we found that the prescription of metformin, one of the most common drugs for type 2 diabetes, was associated with lower odds for symptoms of depression and anxiety. This association was not observed for any other antidiabetic drug.

**Conclusion:** The results suggest that IDE may be an essential factor in metabolic function but may also be associated with significant risk factors for neurocognitive disorders, such as inflammation. Although based on smaller studies, these findings contribute to the limited knowledge of IDE in human blood. Reduced IDE levels have been linked to an increased risk of AD. If patients diagnosed with T2DM have elevated IDE levels, a decrease in IDE levels possibly would indicate that the patient is at higher risk of or even in an early stage of AD. As interest in blood-based biomarkers grows, IDE emerges as a stable and reliable candidate in this thesis. Additionally, the research raises attractive possibilities for enhancing treatment strategies for AD patients experiencing affective symptoms. Metformin is believed to increase IDE levels and has previously been suggested to treat affective disorders and AD. Further investigation is needed to explore this promising avenue.
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<tr>
<td>Aβ</td>
<td>Amyloid-beta</td>
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<tr>
<td>Acetyl-CoA</td>
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<td>AD</td>
<td>Alzheimer's disease</td>
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<td>ADI</td>
<td>Adiponectin</td>
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<td>Apolipoprotein E</td>
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<td>APP</td>
<td>Amyloid precursor protein</td>
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<tr>
<td>ATC</td>
<td>Anatomical therapeutic chemical</td>
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<td>ATP</td>
<td>Adenosine triphosphate</td>
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<td>BACE1</td>
<td>Beta-site APP cleaving enzyme 1</td>
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<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
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<td>BMI</td>
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<td>BPSD</td>
<td>Behavioral and psychological symptoms of dementia</td>
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<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CRISPR</td>
<td>Clustered regularly interspaced short palindromic repeats technology</td>
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<td>Dipeptidyl peptidase 4</td>
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<td>fBG</td>
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<td>Human islet amyloid polypeptide</td>
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<td>IFN-γ</td>
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<tr>
<td>IQR</td>
<td>Interquartile range</td>
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<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>LOD</td>
<td>Limit of detection</td>
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<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide</td>
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<tr>
<td>NBB</td>
<td>The Netherlands Brain Bank</td>
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<tr>
<td>NDR</td>
<td>National Diabetes Register (Nationella Diabetesregistret)</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<tr>
<td>NPI-NH</td>
<td>Neuropsychiatric inventory for nursing homes</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>ROS</td>
<td>Reactive oxidative spices</td>
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<td>SGLT2</td>
<td>Sodium-glucose transport protein 2</td>
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<td>T1DM</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrotic factor alpha</td>
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Introduction

By providing patients with adequate knowledge, we can empower them to make informed choices about their illnesses, care, and health. To guide and support our patients, healthcare professionals need to be up to date with the latest evidence, how to identify individuals at risk, and what options are available through preventive measures or diagnostic tests, if more appropriate. This exploration will have to search beyond the surface, comprehending each patient's unique profile of risk factors. As a nurse and a researcher, I recognize the pivotal role I can play in this process by contributing to the continued development of knowledge.

Patients diagnosed with type 2 diabetes mellitus (T2DM) are at increased risk of severe complications, including neurocognitive disorders like Alzheimer's disease (AD) (1). They have significant health benefits to gain from taking control of their condition (2). Remarkably, T2DM and AD share several risk factors and pathological features, suggesting shared underlying biological mechanisms (3, 4).

Non-communicable conditions, like T2DM and AD, are becoming increasingly common worldwide (4, 5). These continuous illnesses significantly burden healthcare systems and cause distress for patients and their families. In addition, these progressive conditions often present with subtle initial symptoms, and individuals can be affected for a long time without recognizing it. As a result, prevention and early diagnosis have gained prominence as strategies to alleviate suffering in alignment with the Sustainable Development Goals stated by the United Nations (6). These strategies require reliable ways of identifying those with an increased risk of getting ill and those who have already developed the disease.

While diagnosing T2DM is relatively easy, diagnosing AD may involve expensive and inaccessible methods. Seeking more available options, biomarkers measurable in blood have gained significant attention (7). Blood-based biomarkers are appealing due to the relative ease of obtaining blood samples (7), contributing to the call for affordable and available healthcare solutions (6). Such advancements could revolutionize AD diagnostics and enhance the feasibility of early interventions (7). Additionally, the utilization of biomarkers could be expanded to interventions to manage AD symptoms, including drugs to improve cognitive function (8).
1 Background

1.1 METABOLISM IN BRIEF
The human metabolism is the complex network of biochemical processes within the cells to produce energy, sustain growth, and maintain bodily functions. As all living organisms need energy continuously, metabolism is essential for life (9).

The metabolism comprises catabolic reactions, as macromolecules are being degraded into more simple molecules, and anabolic responses in the synthetization of complex macromolecules, such as proteins and lipids (9). The energy derived from catabolic processes is harnessed through cell respiration (Figure 1). The process involves three main stages: glycolysis, the citric acid cycle, and oxidative phosphorylation in the electron transfer chain. Carbohydrates are converted into glucose, which enters glycolysis to produce adenosine triphosphate (ATP). ATP is a highly charged molecule that can store and release energy within the cell at the work site. Fats are broken down through beta-oxidation to yield ATP, and proteins are catabolized into amino acids that can enter various metabolic pathways (10).

Figure 1. Cell respiration is the metabolic process in which cells generate energy by breaking down glucose and other organic molecules, with oxygen (aerobic) or without (anaerobic). Abbreviations: acetyl coenzyme A (Acetyl-CoA), adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NADH). Created with BioRender.com.
In glycolysis, glucose is converted into pyruvate, releasing a small amount of ATP and electron carriers, such as nicotinamide adenine dinucleotide (NADH). Pyruvate then enters the mitochondria, which undergoes the citric acid cycle, producing more ATP, additional NADH, electron carriers, and carbon dioxide (9). Oxidative phosphorylation occurs in the inner mitochondrial membrane. Electron carriers transfer electrons along the electron transport chain. As electrons move through the chain, they release energy, which is used to pump protons across the membrane, creating an electrochemical gradient. The flow of protons back into the mitochondria through ATP synthase drives the synthesis of ATP, also referred to as oxidative phosphorylation. NADH plays a crucial role in shuttling electrons and facilitating the generation of ATP during these processes (11).

Excess glucose can be stored in the liver or muscles as glycogen (glycogenesis) or converted into triglycerides by lipogenesis, primarily in adipose tissue. To maintain adequate glucose levels in the bloodstream during low glucose availability, the body can produce glucose from glycogen through glycogenolysis or non-carbohydrate sources, such as amino acids and glycerol. This process is called gluconeogenesis and occurs mainly in the liver and, to a lesser extent, in the kidneys (12).

1.2 INSULIN

Insulin is a pivotal hormone that regulates glucose homeostasis and influences various metabolic processes. It is processed in the β-cells of the pancreas as proinsulin. Proinsulin consists of three parts: chain A, chain B, and a connecting chain C, called C-peptide (Figure 2). Proinsulin is then cleaved into two pieces: insulin and C-peptide, both of which are equally released into the circulation. One significant role of insulin is to promote glucose uptake into cells. Insulin enhances glucose transport by increasing the number of glucose transporter proteins on the cell surface, particularly glucose transporter (GLUT) 4 (13).

![Figure 2. Insulin synthesis by cleavage of proinsulin. Proinsulin is split into insulin and C-peptide by proteolysis. Disulfide bonds (S) connect chains A and B and help chain A to fold correctly. Upon stimulation, both peptides are secreted into circulation at an even ratio. As insulin has a short half-life, the concentration of insulin will be lower than C-peptide. Created with BioRender.com.](image-url)
This will allow glucose to move from the blood into the cell, reducing blood glucose levels and enabling energy utilization (14). Insulin release is strictly regulated in response to changes in blood glucose levels. The pancreas releases insulin into the portal vein, through the liver, and into the bloodstream when blood glucose rises, e.g., after a meal, and diminishes when blood glucose decreases (15). C-peptide does not regulate blood glucose but can be a valuable biomarker, providing information about the amount of insulin processed by the pancreas (16).

Nonetheless, insulin has several roles in glucose homeostasis. It stimulates glycogen synthesis in the liver and muscle cells. When blood glucose levels are high, insulin triggers the conversion of glucose into glycogen, which can be used for future energy needs (9). Insulin also suppresses gluconeogenesis to prevent excessive glucose production, further aiding glucose regulation (12).

In addition, insulin supports cell growth and differentiation, especially during development. It enhances protein synthesis in muscle cells, aiding tissue repair and growth (14). Insulin affects lipid metabolism by increasing the uptake of fatty acids and their conversion into triglycerides and inhibiting lipolysis breakdown of stored fat into fatty acids, thus reducing the release of fatty acids into the blood (17).

While insulin is foremost associated with glucose regulation in peripheral tissues, recent research has demonstrated that the hormone also has a role in the brain (18). However, the glucose uptake in brain tissue has traditionally been considered not dependent on insulin. In normal glucose metabolism, insulin levels do not regulate brain glucose utilization as it does in the periphery (19). Glucose transport between the blood and the brain over the blood-brain barrier (BBB) primarily occurs with GLUT1 and GLUT3, which are not insulin-dependent glucose transporters. It has also been demonstrated that hyperinsulinemia within normal ranges does not increase glucose transport into the brain (20). Still, insulin-dependent glucose transporters, GLUT4, have been confirmed in the brain (13) and mainly in neurons (21). Insulin has been suggested as essential for glucose utilization in the hippocampus (13). In addition, insulin can modulate brain networks (22), affect the plasticity of the neurons, regulate emotions (23, 24), and feeding behavior to maintain energy homeostasis (13, 25).

It has been discussed whether insulin also is synthesized in the brain. Nevertheless, studies demonstrate that most insulin in the central nervous system originates from the pancreas and is transported to the brain by blood, passing over the BBB (19). These mechanisms still need to be fully understood but suggest being an active, saturable transport mechanism (19, 25). The fact that insulin must be transported across the BBB indicates that insulin levels in the brain do not necessarily correlate with peripheral levels (26). It has been proposed that insulin action in the hypothalamus will regulate metabolic pathways in peripheral tissues in favor of brain insulin signaling, affecting peripheral insulin sensitivity (27).
1.3 INSULIN RESISTANCE

Insulin resistance is clinically defined as the inability of exogenous or endogenous insulin to facilitate glucose uptake. This condition implies that the cells do not respond to insulin and thus reduce their energy uptake, which can be critical for several physiological processes (28). Systematic insulin resistance is characterized by hyperinsulinemia and decreased glucose tolerance (29).

Insulin resistance is accompanied by increased free fatty acids, increased oxidative stress, and overactivation of signaling pathways, which may disturb insulin secretion and pancreatic β-cell growth (30). As insulin resistance develops, the pancreas compensates by producing more insulin to overcome the cells' reduced responsiveness (31). As this overactivation becomes chronic, degeneration of β-cells will result in a relative lack of insulin over time (30).

Insulin resistance has wide-ranging effects on the body. It will disrupt glucose homeostasis and contribute to the development of metabolic disease. It increases the risk of cardiovascular diseases due to its impact on blood vessels and cholesterol metabolism. Additionally, insulin resistance affects other metabolic pathways, potentially leading to abnormal lipid levels, high blood pressure, and non-alcoholic fatty liver disease (14) (Figure 3).

Figure 3. The effects of insulin resistance. In the liver, insulin resistance increases glucose production, raising blood glucose levels. The pancreas compensates by releasing more insulin, potentially straining the organ. In adipose tissue, insulin resistance promotes fat storage and contributes to obesity. Muscle tissue becomes less responsive to insulin, hindering glucose uptake and utilization. These combined effects can lead to hyperglycemia, weight gain, and an increased risk of type 2 diabetes and cardiovascular disease. Created with BioRender.com.
Furthermore, peripheral insulin resistance may lead to central insulin resistance in the brain. It has been discussed whether brain insulin resistance, as it is hypothesized, depends on a lack of responsiveness to insulin in brain cells or is a result of inadequate insulin delivery to the brain. It could be that the transport of insulin over the BBB is impaired due to peripheral insulin resistance (27). However, this relative lack of insulin is proposed to result in a hypometabolism and energy deficit in neurons, possibly leading to difficulties with various brain functions. As the hypothalamus, hippocampus, and cerebral cortex have especially high concentrations of insulin-dependent glucose transporters (27), it has been suggested that insulin resistance in the brain affects cognitive functions, such as memory and learning (13, 19, 26, 32, 33).

Insulin resistance does not necessarily cause clinical symptoms; many individuals may be affected without knowing it. Identifying and addressing insulin resistance early is crucial for preventing its progression to more severe metabolic conditions, promoting health, and reducing the risk of complications (31).

1.4 TYPE 2 DIABETES MELLITUS
Diabetes mellitus is a condition of metabolic dysfunction occurring when the glucose level in the blood is elevated. There are two main types of diabetes with diverse etiology. These are commonly referred to as type 1 and type 2 (34, 35). In addition, there are monogenetic forms of diabetes, gestational diabetes, and diabetes due to other medical conditions. The patient needs to get the correct diagnosis, as treatments may differ. Most patients, around 90%, are diagnosed with T2DM (1).

Type 1 diabetes mellitus (T1DM) is an autoimmune disease in which the patient’s immune system attacks the insulin-producing cells in the pancreas. This results in the cessation of insulin production. T1DM can affect individuals of all ages, but it is more common to be diagnosed as a child, adolescent, or young adult. Patients typically present with symptoms such as polydipsia, polyuria, fatigue, and weight loss. There is no cure for T1DM, and patients need life-long insulin therapy to achieve glycemic control (36).

T2DM is an acquired metabolic disorder characterized by elevated blood glucose levels due to a combination of insulin resistance and impaired insulin secretion. T2DM is closely related to lifestyle factors, such as obesity, insufficient physical activity, and genetic predisposition. High blood glucose levels in T2DM may lead to similar symptoms as T1DM, i.e., excessive thirst, frequent urination, and weakness (1). Nonetheless, individuals with T2DM do not always have clinical symptoms. Elevated fasting glucose can be detected randomly during health checks or medical visits for conditions that later turn out to be diabetes-related complications (37).
1.4.1 Pathophysiology

T2DM is a complex metabolic disorder characterized by a multifaceted interplay of physiological factors. Its primary pathophysiology involves a combination of insulin resistance and β-cell dysfunction, which collectively lead to the symptom of elevated blood glucose levels, known as hyperglycemia (1). Insulin resistance is a pivotal component of T2DM and occurs when cells fail to respond effectively to the insulin hormone. Consequently, these cells struggle to take up glucose from the bloodstream, reducing glucose uptake (14). To compensate for this resistance, β-cells in the pancreas will produce more insulin to increase the circulating insulin levels, resulting in hyperinsulinemia. The β-cells become exhausted over time, further exacerbating the insulin deficit (38).

Insulin resistance, hyperinsulinemia, and subsequent hyperglycemia are the classic hallmarks of T2DM. Still, recent research has unveiled additional layers to the T2DM pathogenesis. Abnormalities in signaling pathways and hormonal dysregulation, such as imbalances in adipokines and other substances secreted by adipose tissue, can contribute to insulin resistance and further disrupt glucose homeostasis (39). Moreover, hyperglycemia and increased fat accumulation may disturb mitochondrial function and cause an overproduction of reactive oxygen species (ROS), creating an imbalance between harmful ROS and protective antioxidants. This oxidative stress can impair insulin signaling and further contribute to insulin resistance, exacerbating metabolic deterioration (40).

In T2DM, the liver's ability to respond to insulin and suppress glucose production is compromised. Consequently, the liver continues to release excessive amounts of glucose into the circulation, further aggravating the problem of elevated blood glucose levels (29). In a healthy metabolic system, gut hormones called incretins play a crucial role by enhancing insulin secretion and reducing glucagon release following meals (41). In individuals with T2DM, it has been suggested that inadequate incretin function results in an insufficient insulin response after eating and elevated glucagon levels. This hormonal imbalance further intensifies blood glucose levels. Prolonged hyperglycemia can damage blood vessels, nerves, and organs, contributing to the complications of T2DM (29).

The diagnostic criterion for diabetes is a random (non-fasting) venous blood glucose \( \geq 11.1 \text{ mmol/L} \) (\( \geq 12.2 \text{ mmol/L} \) if the sample is collected capillary) or a fasting blood glucose of 7.0 mmol/L or higher. If needed, the diagnostic procedure in T2DM can be extended with an oral glucose tolerance test (OGTT). In this case, the patient ingests 75 g of glucose, and plasma glucose is measured after two hours. The venous blood glucose value should be higher than 11 mmol/L to be consistent with a T2DM diagnosis. A value between 7.8 and 11 mmol/L is considered impaired glucose tolerance, a risk factor for T2DM (35).
It is also possible to estimate a patient's blood glucose over time. Glucose in the bloodstream will chemically bind to the hemoglobin in the blood. This process is called glycation. The higher the glucose concentration in the blood, the more glucose binds to hemoglobin, and the red blood cells are glycated in proportion to the average glucose levels in the blood. The compound formed is called hemoglobin A1c (HbA1c). Since red blood cells have a lifespan of about two to three months, a measurement of HbA1c can provide an estimate of an individual's blood glucose levels during this time (42). The normal range for HbA1c is given as 27-42 mmol/mol. A value above 48 mmol/mol on two different occasions or combined with elevated blood glucose is consistent with a T2DM diagnosis. As a result, an HbA1c value falling within the range of 42 to 47 mmol/mol can be regarded as elevated and indicative of prediabetes (35).

1.4.2 Prevalence
Globally, T2DM has reached epidemic proportions. Its prevalence has surged due to various factors, including urbanization, a more sedentary lifestyle, unhealthy diets, and increasing obesity rates (43). It has been estimated that in 2021, over 400 million individuals lived with T2DM worldwide, and the prevalence was growing. The prevalence of T2DM varies by region and population; especially low- and middle-income countries are experiencing a rapid rise (44).

In Sweden, the prevalence has been estimated to be around 5% but is likely to increase (45, 46). The incidence is higher for men, individuals with lower socioeconomic status, and international migrants. However, the rising figures mainly account for increased survival and an aging population (47). The prevalence of T2DM in the over-65 age group has been estimated to be 10-20% globally (5, 48).

1.4.3 Complications
The course of T2DM also includes several possible complications (1). Acute events in diabetes are usually related to deviant blood glucose but are much less common in T2DM than in T1DM. Hypoglycemia is one of the most feared acute complications. In patients diagnosed with T2DM, dangerously low blood glucose occurs almost exclusively during treatment with insulin or sulfonylureas (49). Patients at risk need strategies to manage abnormalities in blood glucose levels (50).

Hyperglycemia can be an acute risk but is foremost damaging over time. Macrovascular damage increases the risk of cardiovascular and cerebrovascular disease, and risk factors such as blood pressure and lipid levels are closely monitored. Microvascular damage includes impairment of the kidneys, nerves, and eyes (37). Regular foot exams are crucial to detect and manage diabetic neuropathy. Nerve damage, especially in the feet, and poor blood circulation can lead to extensive and difficult-to-heal wounds. These wounds can become so severe that amputation becomes necessary (51).
Also, T2DM significantly increases the risk of retinopathy, which can lead to visual impairment and even blindness (52). Moreover, damage to the small blood vessels and high blood pressure can affect the kidneys, and kidney function is monitored to detect signs of diabetic nephropathy (1).

In addition, psychosocial or psychological interventions may be needed to support patients in coping with the mental challenges of long-term illness. Recent research has highlighted the increased risk of depression in individuals with T2DM (53), and epidemiological studies have shown that T2DM is a risk factor for neurocognitive disorders (54-57). This suggests that healthcare should monitor possible psychiatric and cognitive symptoms, especially in elderly patients (58, 59).

1.4.4 Treatment
Treating T2DM involves a multifaceted approach that aims to regulate blood glucose levels, prevent complications, and improve overall well-being. The strategy of treatment often combines lifestyle interventions and pharmacotherapy. All treatment plans must be personalized based on age, overall health, comorbidities, and individual wishes and preferences (1).

The basis of all T2DM care is self-management. This means that patients manage parts of their care themselves, such as regular blood glucose checks, administrating drugs, examining their feet, and planning for their diet and physical activity. Self-management is a collaborative effort between the patient and the healthcare team. Healthcare providers offer guidance, education, and support, while the patient is responsible for actively implementing daily life strategies and lifestyle changes (60).

Lifestyle interventions are fundamental to managing T2DM. This includes helping patients adopt a balanced and nutritious diet, engaging in regular physical activity, maintaining a healthy weight, and avoiding tobacco and excessive alcohol consumption. This can enhance insulin sensitivity to promote glucose control and may be sufficient for patients to regain normal blood glucose levels (61).

As most patients diagnosed with T2DM find it challenging to achieve glycemic control through lifestyle changes alone, drugs are usually added to the treatment (1). A range of peroral drugs are available to help manage T2DM. These drugs work through various mechanisms, such as increasing insulin secretion, improving insulin sensitivity, and reducing glucose production by the liver, as presented in Figure 4. Classes of oral medications include metformin, sulfonylureas, thiazolidinediones, dipeptidyl peptidase 4 (DPP-4) inhibitors, and sodium-glucose-linked transporter 2 (SGLT2) inhibitors (28).

Injectable medications can be utilized if peroral treatment is insufficient in achieving glycemic control. One of the newer options is glucagon-like-peptide 1 (GLP-1) receptor analogs. This drug stimulates insulin secretion, suppresses glucagon release, slows gastric emptying, and suppresses hunger, usually leading to weight loss (41).
Nonetheless, some patients diagnosed with T2DM may eventually require insulin therapy to achieve target blood glucose levels. Insulin therapy is tailored to an individual's needs and can be administered as basal, premixed, or intensive insulin therapy (28). It is also common for drug treatment in T2DM to include different types of drugs in combination (53).

Figure 4. The mechanisms of action of the most common antidiabetic drugs. Abbreviations: Dipeptidyl peptidase 4 (DPP4), Glucagon-like peptide (GLP), Sodium-glucose transport protein 2 (SGLT2). Created with BioRender.com.

In the follow-up of antidiabetic treatment, blood glucose levels will guide possible adjustments (1). Patients can monitor their blood glucose as a part of self-management. However, the recommendation is that healthcare providers should monitor HbA1c regularly to assess glycemic control over time. HbA1c is a strong predictor of diabetes-related complications. The aim of antidiabetic treatment should be to achieve as normal blood glucose levels as possible. Still, this is difficult for many patients, and international medical guidelines recommend an HbA1c below 53 mmol/mol. (62). Patients diagnosed with T2DM have much to gain from adequate glycemic control, both in terms of short-term well-being and long-term complications. Nevertheless, treatment goals must be balanced against the patient's quality of life, expected survival, and the risk of severe side effects, such as hypoglycemia (63).
1.5 ALZHEIMER’S DISEASE

1.5.1 Pathophysiology
AD is a progressive neurodegenerative disease characterized by neuronal death and progressive cognitive neurodegeneration. Loss of neuronal synapses leads to brain atrophy, especially in the neocortex and hippocampus, which causes cognitive symptoms (64), like memory impairment and difficulties with attention, motivation, and orientation (65).

The neuronal death in AD is suggested to be caused by a combination of proteins misfolding and accumulating, activated microglia, and neuroinflammation. At a histopathological level, one of the hallmarks of AD is the abnormal accumulation of amyloid-beta (Aβ) peptides, which leads to the formation of extracellular plaques. Aβ peptides are derived from the breakdown of a larger protein called amyloid precursor protein (APP) (66).

These plaques disrupt neuron communication and trigger inflammation, contributing to neuronal dysfunction and cell death (67). Within the neurons, tau protein forms twisted neurofibrillary tangles. Tau usually helps stabilize microtubules that support cell structure (Figure 5). In AD, over-activation of glycogen synthase kinase (GSK) 3β causes abnormal tau phosphorylation and impairs transport within neurons, leading to degeneration (68).

Figure 5. The histopathological hallmarks of Alzheimer’s disease leading to brain atrophy. Created with BioRender.com.
Immune cells in the brain, microglia, become activated in response to Aβ plaques. While microglia aim to clear debris, chronic activation leads to excessive inflammation that contributes to neuronal damage (69). In addition, the energy production of the neurons is compromised in AD. Mitochondria, responsible for energy production, are impaired, affecting neuronal function and survival (70). Meanwhile, Aβ plaques and neurofibrillary tangles generate ROS, causing oxidative stress that damages neurons and their components (71). In addition, Aβ plaques disrupt synapses between neurons and contribute to impaired memory and cognitive decline. This will occur early in the AD process, and it is suggested that this ongoing synaptic dysfunction and neuronal damage will lead to neuronal death, causing brain atrophy and neurocognitive disorder (72).

Considering these complexities, it is essential to acknowledge the criticism directed toward the amyloid cascade hypothesis, which posits that Aβ accumulation is a primary driver of disease progression in AD (66). Some researchers have suggested that Aβ plaques are not the cause of the disease but rather a consequence of the ongoing process (73). The amyloid cascade hypothesis claims that AD starts with the accumulation of peptides and that the subsequent plaques and neurofibrillary tangles cause inflammation, activated microglia, and neuronal death (66). Yet, why these peptides begin to accumulate is still to be elucidated. It has been suggested that the innate immune system, inflammation, and age could be essential factors (73). Age seems to affect the immune system, causing an impaired adaptive immune response, increased production of pro-inflammatory cytokines, and a higher risk of autoimmune activity. This age-specific inflammation, or inflammaging, has been suggested as a reason for age-related diseases such as AD (74).

This alternative perspective prompts a reevaluation of therapeutic strategies and underscores the multifaceted nature of AD (66). The pathophysiological changes proceeding AD are thought to start years, if not decades, before clinical symptoms. Currently, no reliable methods exist to identify individuals at risk of developing AD or for diagnosis before the onset of symptoms (75). Today, diagnosing AD involves a collaborative effort undertaken by a multidisciplinary team. This team usually employs a combination of psychological assessments, analysis of biomarkers in both blood and cerebrospinal fluid, neuroimaging, and occasionally positron emission tomography (PET) scans. A diagnosis can be formulated when reviewing the aggregated results and the patient’s medical history (76). However, the definitive confirmation of whether an individual has AD can only be obtained through a post-mortem brain examination (77).

Blood-based biomarkers have attracted increasing interest in the diagnosis of AD. There is a need for more objective markers for diagnosis, but also cheaper and more accessible methods. The current research has mainly focused on traditional markers, such as Aβ and tau, and has presented promising results, but these analyses are not yet part of standardized care (7, 78).
1.5.2 Prevalence
AD is the most common form of neurocognitive disorder globally (4). The World Health Organization reports that approximately 55 million individuals worldwide are affected by neurocognitive disorders (65), with around 150,000 people in Sweden currently living with such diagnosis. Approximately 60-70% of these suffer from AD (79). The prevalence of AD varies among ethnic and cultural groups. Genetic variations, access to healthcare, and cultural practices may influence these differences (80), but it is a global issue, and its epidemiology reveals a growing public health concern worldwide (4). In Sweden, the care of patients with AD is estimated to cost society around SEK 80 billion, primarily in municipal home care and nursing home accommodations (79).

1.5.3 Complications
Besides memory impairment, most patients diagnosed with AD suffer from some form of behavioral and psychological symptoms of dementia (BPSD), which includes a wide range of symptoms, such as delusions, hallucinations, affective disturbance, aberrant motor behavior, and problems with sleeping and eating (81). BPSD is not always considered a part of disease progression but has been linked to brain atrophy (82) and neurodegeneration (83). Deficits in neurotransmitters like acetylcholine, serotonin, and norepinephrine are thought to contribute to psychiatric and behavioral symptoms (84).

It has been estimated that about 80-90% of patients diagnosed with AD experience one or more BPSD during their illness, and symptoms can vary significantly between individuals (83). The type of symptoms and severity may differ, which can also change over time as the disease progresses. BPSD can arise in any stage but usually becomes more pronounced in the moderate and severe stages of the disease (85). BPSD not only has a substantial impact on the quality of life for patients but also on caregiver stress (86), health care cost (83), and need for care (87). BPSD is often the reason a patient diagnosed with AD can no longer live at home and must move to a nursing home (87). Overactive or aggressive symptoms are also a common reason why these patients are prescribed unsuitable drugs, such as neuroleptics. These drugs are associated with further impairment of cognitive function and severe side effects, possibly leading to increased mortality (88).

1.5.4 Treatment
So far, there is no cure for AD. Therefore, treating AD aims to manage symptoms, slow progression, and enhance quality of life. AD treatment is highly individualized, considering factors such as the stage of the disease, the individual's overall health, and their preferences. Regular monitoring and adjustments to treatment plans are crucial as the disease progresses (65).
Drugs widely available on the market can help manage cognitive symptoms and temporarily improve quality of life. Acetylcholinesterase inhibitors, such as donepezil, rivastigmine, and galantamine, are commonly prescribed to enhance neurotransmitter function and communication between neurons (89). Memantine, an N-methyl-D-aspartate (NMDA) receptor antagonist, can regulate glutamate activity, reduce neurotoxicity, and ameliorate cognitive symptoms (90). Unfortunately, the overall effect of drug treatment is usually modest (91).

Ongoing research explores new therapeutic avenues. Experimental treatments targeting amyloid plaques have shown potential in clinical trials (92). There is currently one substance approved for the treatment of AD, Lecanemab. It is a monoclonal antibody given as an infusion at 2-week intervals. The drug has shown some effect on cognitive symptoms, even if it does not stop the neurodegeneration (93). Long-term follow-up is needed to evaluate long-term benefits, but the drug is now available for prescription in the US. Discussions are ongoing for possible approval in Europe (94).

Non-pharmacological strategies are crucial for managing cognitive symptoms and BPSD. Creating a structured routine, providing a safe and supportive environment, and engaging in activities that promote mental stimulation and social interaction can help manage mood changes, aggression, and agitation (95). Also, adopting a healthy lifestyle can potentially slow disease progression. Regular physical exercise, a balanced diet, and staying socially active may protect brain health (2).

Drug treatment for BPSD is considered when non-pharmacological interventions are ineffective or in severe cases (88). The goal of drug treatment in BPSD is to enhance the individual's well-being and quality of life while minimizing distress and risk. This approach should be part of a comprehensive care plan that includes non-pharmacological interventions and psychosocial support. Family members and caregivers should be informed about the potential risks and benefits of medications used for BPSD and be involved in the decision-making process (88).

No drugs are designed to treat BPSD, but the choice must be made according to the patient's symptom profile and past medical history (85). Treatment should start by optimizing drugs targeted to AD. Acetylcholinesterase inhibitors and NMDA receptor antagonists may have a moderate effect (96). Antidepressants can be helpful for depressive symptoms but can also be used to treat anxiety or agitation, even if evidence of its efficacy in patients diagnosed with AD is limited (97). On the other hand, anxiolytic drugs have aggravating side effects such as falls and sedation (98). In addition, antipsychotic drugs can be prescribed in severe cases of aggression or psychotic symptoms but have potentially serious side effects, including stroke and premature death (99). These drugs must be utilized with great caution and close follow-up (100). In all cases, drug treatment should focus on well-being and ensure that the benefits outweigh the risks of treatment (85, 100).
1.6 SHARED RISK FACTORS

The development of both T2DM and AD is influenced by several shared risk factors, which has shed light on the critical role of lifestyle and genetics in these interconnected conditions (101).

Lifestyle factors have long been recognized as fundamental in insulin resistance and T2DM genesis. Recently, research has extended this paradigm to AD, emphasizing that our daily lifestyle also impacts our risk of developing neurocognitive disorders (2). Unhealthy habits, such as a diet rich in sugary and processed foods, a sedentary lifestyle, and insufficient physical activity, can lead to weight gain and obesity, substantially increasing the risk of insulin resistance (1). Moreover, lifestyle factors frequently associated with poor dietary habits and physical inactivity, like hypertension and high cholesterol, have been linked to cognitive impairment (2). Conversely, adopting a well-balanced diet, engaging in regular physical activity, managing body weight, and stimulating cognitive functions through mental exercises may reduce the risk of both T2DM and AD, underscoring the importance of lifestyle modifications in preventing and managing these diseases (2, 102, 103).

Nevertheless, as we live longer globally with improved lifestyles and better access to health care, age has become a significant risk factor for numerous diseases (104). The prevalence of T2DM rises notably among older adults, making age an important contributing factor (105). In the case of AD, age is the most prominent risk factor, with the risk approximately doubling every five years after age 65. AD can manifest before age 65, even infrequently, and may result from specific genetic variants. However, most AD cases are sporadic with no known hereditary cause (106). The genetic, or familiar, form of AD and sporadic AD share similar pathological and clinical features, including cognitive decline, memory loss, and an increased need for daily assistance (107).

In sporadic AD, genetic factors, such as the apolipoprotein E (ApoE) 4 allele, contribute to risk in a complex interplay with other genetic, lifestyle, and psychosocial factors (108, 109). Interestingly, carriers of ApoE4 have also been suggested to have an increased risk of developing T2DM, possibly indicating a genetic overlap between the two conditions (101). Genetic predisposition plays a substantial role in susceptibility to T2DM, with various genetic variations impacting insulin sensitivity and β-cell function (110). Furthermore, it has been suggested that specific ethnic groups, such as Native Americans, Hispanics, African Americans, and specific Asian populations, exhibit a higher predisposition to T2DM (28).

Thus, the development of both T2DM and AD is influenced by a multifaceted interplay of lifestyle choices, genetic factors, and age (102). Understanding these shared risk factors is crucial for developing comprehensive strategies to prevent, manage, and potentially delay the onset of these debilitating diseases (2, 102).
2 Literature Review of the Research Field

2.1 IS ALZHEIMER'S DISEASE DIABETES MELLITUS TYPE 3?

There is mounting evidence that T2DM is a risk factor for cognitive impairment (54, 56, 57), and it has been demonstrated that T2DM approximately doubles the risk of developing neurocognitive disorders (111, 112). As T2DM has been linked to cardiovascular disease, the increased risk of vascular dementia is acknowledged, but the association between T2DM and AD is not as well-established (67). In the epidemiological field, there have been conflicting results. Several studies have indicated that T2DM is a risk factor for AD (54, 55, 57, 101, 105, 111, 113-118), yet studies also demonstrate no association (119-121). Nonetheless, researchers have argued that AD could be considered a metabolic disease, type 3 diabetes mellitus. This term may be controversial but has been used to describe the potential link between AD and insulin resistance (67).

In AD, there is evidence of impaired insulin signaling in the brain (122), and it has been suggested that this metabolic dysfunction can be present even in patients not diagnosed with T2DM. In normal glucose metabolism, an increase in circulating insulin levels does not affect brain glucose metabolism. However, in patients with impaired glucose tolerance, insulin seems to increase glucose utilization in the brain (123). Also, patients diagnosed with AD seem to respond to hyperinsulinemia with improved cognitive functions, even without hyperglycemia (124).

Several recent studies have indicated that insulin resistance plays a significant role in AD development (30, 33, 71, 125-127). Thus, insulin is essential for the brain’s energy metabolism. Insulin resistance may lead to reduced glucose uptake, energy deficits, and impaired neuronal function in the brain (33). Insulin helps with neurotransmitter signaling, synaptic function, and maintaining neuronal health. Also, insulin resistance can impair the function of blood vessels in the brain, affecting blood flow and nutrient delivery (25, 128).

In addition, insulin resistance can affect the permeability of the BBB, which usually protects the brain from harmful substances in the circulation. When this barrier is compromised, it may allow the entry of potentially damaging molecules into the brain, exacerbating neurodegenerative processes (40). Insulin resistance also promotes the accumulation of Aβ and tau, further disrupting brain function (125), hindering Aβ degradation (55), and activating GSK-3β in the insulin-signaling pathway. This disruption will lead to hyperphosphorylation of tau (129) and has been demonstrated in patients diagnosed with AD and T2DM (130).
Insulin resistance can trigger neuroinflammation and oxidative stress in the brain. Chronic inflammation, oxidative damage, and vascular dysfunction may further contribute to the degeneration of brain cells and cognitive decline (127) (Figure 6).

Also, specific inflammatory markers appear to affect both conditions. One of the most investigated cytokines is interleukin (IL) 6. Increased levels of IL-6 have been associated with cognitive impairment, neurodegeneration (131), and alterations in glucose homeostasis (132).

Risk factors for AD, such as carrying the ApoE4 allele (113, 117, 120, 133) and older age (55), have been demonstrated to increase the risk of AD for patients diagnosed with T2DM. Older age also increases the comorbidity in cardiovascular and cerebrovascular diseases, independent risk factors for AD (2). On the other hand, longer duration of diabetes (114, 115, 118), deteriorating glycemic control (116), and insulin treatment in T2DM have all been associated with a higher risk of developing AD. In addition, elevated blood glucose and increased insulin resistance have been associated with poorer cognitive performance in individuals not diagnosed with T2DM (26). Possibly supporting the association between glycemic dysregulation and neurodegeneration (116).

**Figure 6.** The Metabolic Hypothesis in AD. Metabolic disease, obesity, and peripheral insulin resistance lead to central insulin resistance, which causes inflammation, oxidative stress, and the accumulation of misfolded proteins. This will impair neuronal function, resulting in cognitive impairment. Created with BioRender.com
Notably, aging is associated with insulin resistance, a lower expression of insulin receptors in the BBB and brain, and impaired insulin receptor bindings (13, 134). This may decrease brain glucose uptake, causing hypometabolism linked to cognitive impairment (25, 33).

Brain insulin resistance is associated with decreased GLUT4 expression, impaired insulin signaling, and alterations in neurotransmission (72). Compensatory overactivation of signaling pathways increases the secretion of Aβ and phosphorylated tau (30). Aβ can alter the sensitivity of insulin receptors on neuron cells (135). The disruption in insulin signaling is causing an increase of beta-site APP cleaving enzyme 1 (BACE1), promoting the production of Aβ42, the type of Aβ most prone to aggregate (30). It can impair tau expression and phosphorylation, promoting neurofibrillary tangles formation (129). Also, it has been reported that hyperglycemia could further increase Aβ and negatively affect neuronal metabolism (135, 136).

Hence, insulin resistance is connected to both Aβ and tau pathologies in AD. Furthermore, Aβ and insulin are being degraded by the same enzyme, insulin-degrading enzyme (IDE). As IDE has a higher affinity for insulin, the possibility for clearance of Aβ might be reduced in the presence of hyperinsulinemia, indirectly contributing to the formation of plaques (137).

2.2 INSULIN-DEGRADING ENZYME
IDE is a zinc-metalloprotease that has been highly conserved, and its equivalents are present in various organisms (138). IDE was discovered in 1949 and was initially observed to degrade insulin with high affinity (139). It is expressed in most cells in the human body and can be found in blood, cerebrospinal fluid, wound fluid, and tissues (140).

The enzyme has different functions inside the cell and in the extracellular space. In plasma, IDE degrades active insulin, and an increase in IDE could result in higher blood glucose, as IDE reduces insulin levels accessible for glucose transport (141). Intracellular IDE degrades inactive insulin used in the glucose transporter pathway. This degradation is crucial as it resets the insulin signaling mechanism, making IDE essential in both the development of insulin resistance and in maintaining insulin sensitivity (141).

Even if insulin degradation was the first function to be demonstrated for IDE, researchers found that the deletion of IDE in the liver of rodents did not impact insulin levels significantly (142, 143). Also, IDE is expressed in tissues not generally associated with insulin-dependent glucose uptake, such as the brain, suggesting that IDE has organ-specific functions other than degrading insulin (139).
Consequently, later research has shown that IDE degrades several β-structure-forming proteins, including human islet amyloid polypeptide (hIAPP) in the pancreas and Aβ in the brain. It is the central protease in Aβ degradation and a culprit against aggregation and formation of plaques (139).

The multi-location, widespread expression, and long list of substrates speaking in favor of IDE have a role in maintaining protein quality and eliminating proteins prone to aggregation. IDE also functions as a heat shock protein, a dead-end chaperone, and a modulator of the ubiquitin proteasomal system (Figure 7). This has made it a biomarker of interest in T2DM and AD (143).

**Figure 7.** The structure of Insulin-Degrading Enzyme (purple) in complex with insulin (green and yellow). The enzyme opens like a clam and encloses the substrate. The substrate is then degraded or irreversibly retained, like a dead-end chaperone. ©Shutterstock.

### 2.2.1 Insulin-Degrading Enzyme in Type 2 Diabetes Mellitus
IDE has been extensively studied in the context of T2DM. It has been suggested that IDE might have a more significant role in the presence of impaired glucose metabolism (144). In T2DM, hIAPP aggregates in the pancreas, promoting the death of β-cells and adding to the dysfunction in the metabolic system (145). Moreover, it has been demonstrated in vivo that IDE is necessary for glucose-stimulated insulin secretion in the pancreas (146).

In addition, patients diagnosed with T2DM seem to have lower IDE expression in the liver (147) and degrade insulin more slowly in fat cells (148) than people without T2DM. Similarly, people of African-American origin have been found to have lower IDE activity in the liver than non-Hispanic whites, which could be part of why they are also at greater risk of developing T2DM (149). At the same time, individuals with metabolic syndrome have been found to have higher levels of IDE in their blood than metabolically healthy individuals (150, 151).

Since its discovery, research has been interested in IDE as a target for treating T2DM. In this area, inhibition of IDE has been of interest. The hypothesis has been that if the patient's insulin would remain in the bloodstream longer, exogenous
insulin would not need to be added for the same effect (152). Ideally, a drug could be found that allowed inhibition of insulin clearance but did not affect other molecules, such as glucagon. Previous IDE inhibitors have been non-specific (138), and the challenge has been getting the same good results in humans as in animal models (153). The removal of IDE further increases the risk of inflammation (154), which indicates that IDE has a role in clearing pro-inflammatory markers (155).

Expression of IDE seems to be affected by anti-diabetic treatment. The first step in anti-diabetic treatment is often increased physical activity. Interestingly, studies have shown that physical activity is increasing IDE activity, conceivably accounting for parts of the positive effects of physical activity on glucose metabolism and cognition (64). Oral hypoglycemic agents, such as sulfonylurea, have been observed to decrease the expression of IDE in β-cells (142). In contrast, insulin treatment has been demonstrated to increase IDE levels, favoring an upregulation in hyperinsulinemia (142). Also, it has been argued that the insulin-sensitizing effect of metformin (biguanides) is due to an increase in the expression of IDE (156).

### 2.2.2 Insulin-Degrading Enzyme in Alzheimer’s Disease

Since the finding that IDE also degrades Aβ, most research has focused on AD. Thus, IDE is proposed to have a cell protective role. It can degrade amyloidogenic peptides, facilitate proper folding, and prevent proteins from clogging into plaques. Later, it was observed that IDE could trap Aβ monomers without degradation, acting like a dead-end chaperone (143, 157). It has been repeatedly shown in experimental animal models that upregulation of IDE reduces Aβ plaques and improves cognitive ability (158). It has also been demonstrated that the genes encoding IDE are genetically associated with AD (11-13), further increasing interest. Researchers have suggested that AD patients would be helped by up-regulation or activation of IDE as a disease-modifying treatment. Still, the challenge has been to upregulate IDE activity directed at Aβ to avoid metabolic side effects (159).

Earlier studies have shown that individuals with cognitive impairment have lower IDE activity in cerebrospinal fluid than people with intact cognition (55). This has drawn attention to IDE as a possible marker for detecting AD before clinical symptom onset (140). Nonetheless, clinical studies have shown more varied results. It has been demonstrated that IDE levels and activity decrease in patients diagnosed with AD (160-163) but also that levels and activity are unchanged or slightly increased (164). On the other hand, there are also studies with no significant differences in IDE expression or activity in individuals with and without AD (134, 161, 164, 165). A meta-analysis from 2018 showed that IDE was significantly lower in the brain tissue of patients diagnosed with AD compared to non-demented controls (140). Still, it may be challenging to compare studies, as methods and types of samples vary (161).
It has been suggested that IDE is elevated early in AD, decreasing as the disease progresses. This could be due to increased levels of Aβ creating feedback for IDE to clear toxic peptides. Findings of IDE in and around plaques and neurofibrillary tangles point in this direction (139). Also, several studies have found an association between specific genetic variation in IDE and the risk for AD (166-170), and decreased levels of IDE in serum have been linked to an increased risk of AD in T2DM (171), and prediabetes (172).

IDE has mainly been studied as a predictive marker for AD development. However, genetic variation in IDE has also been linked to BPSD in AD, which means that IDE may impact the symptoms of the disease. In a small study, it was mainly affective symptoms (i.e., depression and anxiety) that were affected, which the authors related to altered glucose metabolism in the brain (173).

2.2.3 The Link Between Insulin Resistance and Alzheimer’s Disease

Experiments in rodents have demonstrated that the absence of IDE triggers glucose intolerance, insulin resistance, and the accumulation of Aβ (8). IDE knockout mice have increased levels of Aβ in the brain, and conversely, overexpression of IDE improves cognition and prevents pathological Aβ formations (137). In addition, the gene for IDE is found in chromosome 10q, which has been linked to sporadic AD (168) and T2DM (174).

Therefore, IDE is suggested as a significant component in the mechanism of insulin resistance and AD. The most widely accepted hypothesis is that chronic hyperinsulinemia creates negative feedback for IDE to prioritize insulin degradation, allowing Aβ to accumulate (159), as illustrated in Figure 8. Nevertheless, it has been pointed out that peripheral insulin levels are significantly higher than insulin levels in the brain, which contradicts the idea that insulin competitively inhibits Aβ degradation to a sufficient extent (175). It is possible that IDE can regulate the amount of insulin available in the brain, as it does in the peripheral. In addition, IDE has been detected at the BBB, suggesting IDE may also regulate insulin receptor signaling in the BBB, possibly altering the insulin transport from the blood (176). Insulin transport over the BBB is crucial for brain function (26).

As IDE has a multifunction, it is reasonable to assume that several factors affect this enzyme's expression and activity (143). Age is known to be a risk factor for T2DM and AD (4). With age, the clearance of potentially harmful proteins is decreased, e.g., due to kidney failure, possibly contributing to the pathological changes observed in AD (134). There are conflicting results on whether age affects IDE, but IDE levels and activity are believed to decrease with age (134, 139).

Nonetheless, the importance of investigating possible interactions or synergistic effects of IDE and other biomarkers has recently been pointed out (177). A biomarker of interest could be adiponectin (178). Adipokines play a role in many
metabolic functions and can cross the BBB. Adiponectin is a peptide released from adipocytes and improves the lipid balance in the body by increasing the sensitivity to insulin (178). Several studies have demonstrated adiponectin as a biomarker for insulin sensitivity (179).

Adiponectin promotes glucose uptake, helps cells convert glucose into energy, and is thought to have a significant role in glucose utilization and oxidation of fatty acids (179). In addition, it has been demonstrated that adiponectin has neuroprotective effects against Aβ formation (180) induced by high glucose concentrations (181). Hence, IDE and adiponectin are involved in glucose metabolism, but evidence of how they interact is limited (182-184). Adiponectin is also attributed to an anti-inflammatory effect by decreasing pro-inflammatory cytokines, such as tumor necrotic factor alfa (TNF-α), and increasing anti-inflammatory markers, such as IL-10 (185). High adiponectin levels have been linked to a lower risk for T2DM (186).

In addition, inflammatory markers, such as IL-6, have been linked to IDE expression. In skeletal muscle and liver and after physical activity, elevated IL-6 levels seem to mediate the increase in IDE expression (132). Low-grade inflammation, Aβ accumulation, and tau hyperphosphorylation will induce oxidative stress, which has been demonstrated to impair activity in IDE (71). This can also be linked to the pathophysiology of AD since IDE has been suggested to be more oxidized in the hippocampus than, for instance, in the cerebellum, which is not a typical region for Aβ accumulation (139).

![Figure 8](https://www.biorender.com/image/25c6ea59db5c865a8d2aeb43c198db8f.png)

**Figure 8.** In normal glucose metabolism, insulin-degrading enzyme (IDE) degrades insulin and amyloid beta. In brain insulin resistance, insulin will stay in the extracellular space, creating feedback for IDE to favor insulin degradation and allowing amyloid beta to accumulate. Created with BioRender.com.
3 Rationale

Studying IDE is essential in understanding critical health issues such as T2DM and AD (143). The human metabolism is essential for life and regulated by pivotal hormones like insulin. When this intricate system is impaired, as in insulin resistance, it will lead to a metabolic dysfunction that affects the whole body, including the brain (33).

Insulin resistance is a hallmark of T2DM. Patients diagnosed with T2DM have significant health benefits from adequate treatment and efforts to reduce the risk of complications (61). In recent years, the increased risk of AD in patients diagnosed with T2DM has been recognized (67).

The relationship between T2DM and AD becomes increasingly evident as shared risk factors, including lifestyle and genetics, are unveiled (102). Moreover, growing evidence shows that T2DM and AD may share pathological features, further linking these conditions (67). As a molecular link between them, IDE has been proposed. What makes the study of IDE particularly intriguing is its vital role in regulating insulin and Aβ. The absence of IDE, as demonstrated in rodent experiments, leads to glucose intolerance, insulin resistance, and the accumulation of Aβ in the brain (139). In addition, IDE has been suggested as an indicator of non-normal insulin metabolism (150) and insulin resistance seems closely intertwined with AD (67).

IDE has been investigated for decades in both T2DM and AD. However, most research has been done in experimental models and brain tissue from diseased humans, but the evidence for IDE in human blood is sparser, and there are conflicting results (140, 150). Increased knowledge of the enzyme’s systematic correlation to other markers may provide clues about its role in T2DM and AD and their possible pathological overlap (171).
4 Research Aims

4.1 OVERALL AIM
The aim of this thesis was to increase knowledge of the relationship between insulin resistance and AD, focusing on IDE as a biomarker for risk assessment and diagnosis of AD.

4.2 SPECIFIC AIMS
Study I: To explore serum levels of IDE in patients diagnosed with T2DM compared to metabolically healthy subjects.

Study II: To explore the associations between IDE, total tau, and cytokine levels in plasma from subjects with AD and non-demented controls.

Study III: To explore the correlation between IDE and biological markers important in metabolic function, such as age, weight, blood glucose, C-peptide, and lipids, in patients diagnosed with T2DM or AD compared to metabolically healthy volunteers.

Study IV: To study whether metformin could be associated with BPSD for AD patients with antidiabetic drug treatment and compare this to the association between BPSD and other anti-diabetic drugs, such as insulin, sulfonylurea, and DPP-4 inhibitors. In addition, we aimed to investigate if there were interaction effects between metformin and other antidiabetic drugs.
5 Materials and Methods

5.1 OVERVIEW
This thesis is based on four studies. Three studies are based on laboratory analyses of blood samples. The fourth study is based on data from a quality register. All studies are to be regarded as cross-sectional. Table 1 summarizes these studies, the type of data, the main variables, and the main statistical methods.

Table 1. Overview of studies included in this thesis.

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<tr>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
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<tr>
<td>Sample</td>
<td>Patients diagnosed with T2DM (n=47) and healthy controls (n=18)</td>
<td>Patients with post-mortem diagnosis of AD (n=18) and non-demented controls (n=6)</td>
<td>Patients diagnosed with T2DM (n=47), AD (n=9) and healthy controls (n=64)</td>
</tr>
<tr>
<td>Data Source</td>
<td>Biobank 925 at Sophiahemmet Hospital</td>
<td>The Netherlands Brain Bank</td>
<td>Biobank 925 at Sophiahemmet Hospital. Recruitment at nursing homes, Sophiahemmet University, and Sophiahemmet Health Center.</td>
</tr>
<tr>
<td>Type of Data</td>
<td>Measurements from laboratory analyses of serum samples and clinical data from biobank</td>
<td>Measurements from laboratory analyses of plasma samples and clinical data from brain bank</td>
<td>Measurements from laboratory analyses of serum samples, clinical data from biobank, and self-reported clinical data</td>
</tr>
<tr>
<td>Main Variables</td>
<td>IDE, disease duration, drug treatment</td>
<td>IDE, total tau, IL-6, IL-8, IL-10 and TNFα</td>
<td>IDE, age, sex, fBG, HbA1c, C-peptide</td>
</tr>
<tr>
<td>Main Data Analyses</td>
<td>Pearson’s Chi² Student’s t-test Mann-Whitney Kruskal-Wallis</td>
<td>Pearson’s Chi² Mann-Whitney Spearman’s rank correlation</td>
<td>Quade analysis Spearman’s rank correlation Non-parametric partial correlation</td>
</tr>
</tbody>
</table>

Abbreviations: Fasting blood glucose (fBG), hemoglobin A1c (HbA1c), Insulin-degrading enzyme (IDE), Interleukin (IL), Neuropsychiatric Inventory for Nursing Homes (NPI-NH), Tumor Necrosis Factor Alpha (TNFα).
5.2 STUDY PARTICIPANTS

5.2.1 Study I
In study I, we included blood serum samples from patients diagnosed with T2DM from the biobank at Sophiahemmet Hospital. These patients had been recruited for an earlier study, and all participants were diagnosed with T2DM at a primary healthcare facility. We selected all patients in the biobank with a diagnosis of T2DM. No exclusion criteria were applied.

For comparison, we included blood serum samples from metabolically healthy individuals from the biobank. These participants were patients diagnosed with various forms of intestinal or nasal problems. Inclusion criteria were applied: fasting blood glucose < 5.6 mmol/L, triglycerides < 1.7 mmol/L, and HDL > 1.3 mmol/L for women and >1.0 mmol/L for men.

5.2.2 Study II
For study II, post-mortem samples from individuals diagnosed with AD were retrieved from the Netherlands Brain Bank (NBB). We selected donors over 65 with a post-mortem diagnosis of AD, with plasma samples available. No other selection criteria were applied.

Post-mortem samples from NBB for non-demented control patients were used as control subjects. We selected individuals over 65 who had been assessed as cognitively healthy (clinically before death and by post-mortem examination) and had plasma samples available.

5.2.3 Study III
In study III, a more extensive recruitment was conducted to include healthy volunteers. This recruitment was conducted at Sophiahemmet University and Sophiahemmet Health Center. Information about the study was provided during lectures and visits to the health center. We also distributed brochures and posters in the university, hospital, and health center common areas.

As we aimed to recruit metabolically healthy controls, the following exclusion criteria were applied: blood pressure exceeding 140/90 mmHg, fasting blood glucose above 5.6 mmol/L, HbA1c above 39 mmol/mol, and body mass index (BMI) of 30 kg/m² or higher (187).

In addition, we recruited patients diagnosed with AD in collaboration with a nursing home. All patients diagnosed with AD were invited to participate if they had not received a diagnosis of T1DM, prediabetes, or T2DM.

Patients diagnosed with AD and healthy volunteers were in analyses compared to those diagnosed with T2DM from study I.
5.2.4 Study IV
In study IV, all participants were patients registered in the Swedish BPSD registry, a national Swedish quality register, as having a diagnosis of AD, receiving antidiabetic drug treatment (i.e., any drug within Anatomical Therapeutic Chemical (ATC) code A10A-X), and having a registered assessment of BPSD within 30 days of drug registration. In this study, we considered all patients receiving antidiabetic drug treatment as patients diagnosed with T2DM.

We included all registered patients diagnosed with early-onset AD, late-onset AD, or a combination of AD and vascular dementia (VD). We also included patients registered with an unspecific dementia diagnosis (UNS) or no diagnosis if prescribed acetylcholinesterase inhibitors or NMDA receptor antagonists. Patients registered with VD, Lewy body dementia, frontotemporal dementia, dementia in Parkinson’s disease, or another specific diagnosis were excluded regardless of drug prescription.

5.3 BIOLOGICAL SAMPLES
5.3.1 Biobank Samples
Studies I and III used serum samples from the biobank at Sophiahemmet Hospital. These samples had been collected as part of other projects at Sophiahemmet University and Sophiahemmet Hospital. The participants had given informed consent for the samples to be used in subsequent studies, provided that a new ethical approval was obtained.

5.3.2 Post-mortem Samples
For study II, post-mortem plasma samples were used from the NBB. These samples were collected from donors after their death, from whom written informed consent for a brain autopsy and the use of tissues and clinical information for research purposes had been obtained by the NBB. Researchers worldwide can apply to use samples from the NBB in research. All samples are provided anonymously, with limited clinical data.

5.3.3 Blood Sample Collection
As a part of study III, fasting blood samples were collected from healthy volunteers and patients diagnosed with AD. Fasting blood samples were drawn from a decubital vein and collected in serum tubes and as whole blood in tubes with EDTA. Whole blood was used to conduct point-of-care analyses at the time of blood collection. Serum tubes were left at room temperature to clot for 30 minutes before centrifuging at 3000 rpm for 15 minutes. After that, serum was collected and frozen at -80 degrees until laboratory analyses.
5.4 LABORATORY ANALYSES
For all biochemical analyses, we decided to continue with statistical analyses for biomarkers where at least 90% of included samples exceeded the detection limit, i.e., gave measurable results. This means that all analyzed biomarkers were not presented as a part of the results. All analyses included in the results have had a coefficient of variability (CV) value of no more than 10.

5.4.1 Point-of-Care Analyses
We performed point-of-care analyses for fasting blood glucose and lipids in all included laboratory studies. In study III, we had access to unfrozen whole blood and could also include analyses of hemoglobin and HbA1c.

Fasting blood glucose and hemoglobin were analyzed using point-of-care platforms from HemoCue (HemoCue AB, Ängelholm, Sweden)

Lipids (triglycerides, high-density lipoproteins (HDL), and low-density lipoproteins (LDL)), and HbA1c were analyzed in serum with the point-of-care platform Afinion™ 2 (Abbott Rapid Diagnostics, Maidenhead, UK).

5.4.2 Enzyme-Linked Immunosorbent Assays
The biochemical measurement of levels of IDE was the basis of this thesis. As IDE is a relatively new biomarker, there are no golden standards regarding methods, even if several commercial analysis kits are available. Also, knowledge of how this enzyme is affected by handling and storage is limited. For this reason, the choice of kit and sample medium was preceded by several rounds of testing. We tested serum and plasma with different anticoagulation agents in the initial analyses. Serum and plasma were assessed as essentially equivalent in terms of stability and reproducibility of analytical results.

In this project, we have used sandwich enzyme-linked immunosorbent assays (ELISA) kits for IDE (Human Insulin-degrading enzyme ELISA Kit from AH Diagnostics, Solna, Sweden, limit of detection (LOD) 0.037 ng/mL). Sandwich ELISA was also used to measure C-peptide (Human C-peptide ELISA from Merck, Stockholm, Sweden, LOD 0.05 ng/mL), adiponectin (Human ELISA kit from Invitrogen, Thermo Fisher Scientific, Stockholm, Sweden, LOD 0.23 ng/mL) and total tau (Total tau assay from Abcam, Oxford, United Kingdom, LOD 16 pg/mL).

All samples were diluted, and regents and buffers were prepared following the manufacturer’s instructions. The required wash steps used an automated HydroWash® wash station from Tecan.
ELISA is a laboratory method for quantifying proteins, peptides, and antibodies. The method is available as a direct, indirect, sandwich, and competitive ELISA. In a sandwich ELISA, the first step involves coating a solid surface, usually a plate with multiple wells, with a capture antibody. This antibody is specific to the antigen you want to detect and quantify. Then, the sample containing the biomarker we want to measure is added. The antigen in the sample will bind to the capture antibody in the wells. After this, a secondary antibody, also known as a detection antibody, is added. This secondary antibody is linked to an enzyme. This enzyme-linked antibody recognizes a different epitope on the antigen, allowing it to bind to the antigen that is already captured by the capture antibody. When the secondary antibody binds, an enzyme substrate is added. The enzyme-linked to the secondary antibody catalyzes a reaction that converts a colorless substrate into a colored product. The intensity of the color can be measured and is proportional to the amount of antigen present in the sample. A standard curve with known concentrations converts the absorbance values into measurements (188).

5.4.3 Luminex
To measure cytokines, we used Luminex. Luminex uses color-coded beads, each coated with a unique capture molecule specific to a particular biomarker we want to measure. The beads can be coated with a mixture of capture molecules, making measuring multiple biomarkers in one sample possible. The beads are labeled with different ratios of fluorochromes, creating a unique spectral address for each bead type (189).

The samples are added to the bead mixture, and each analyte binds to its respective capture molecule on the bead. After incubation, unbound components are washed away, leaving only the analyte-captured beads. Then, fluorescently labeled detection molecules are added. These reporter probes bind to the analytes captured on the beads, creating a sandwich-like complex. The bead mixture is passed through a Luminex instrument, which uses lasers to excite the fluorochromes on the beads. The beads radiate fluorescence at different wavelengths based on their spectral addresses, and the intensity is proportional to the amount of analyte present in the sample. Specialized software is used to analyze the fluorescence signals from each bead set and correlate them with the concentration of the analytes (189). Standard curves generated from known concentrations of analytes are used for quantification.

In study II, we used the Bio-Plex Pro Human Cytokines 8-Plex assay (Bio-Rad Laboratories, Stockholm, Sweden), including granulocyte-macrophage colony-stimulating factor (GM-CSF) (LOD 0.19 pg/mL), interferon-gamma (IFN-γ) (LOD 1.05 pg/mL), IL-2 (LOD of 0.19 pg/mL), IL-4 (LOD 0.09 pg/mL), IL-6 (LOD 0.34 pg/mL), IL-8 (LOD 0.36 pg/mL), IL-10 (LOD 0.69 pg/mL), and TNF-α (LOD 1.33 pg/mL).
5.5 CLINICAL DATA
For samples from patients diagnosed with T2DM retrieved from Sophiahemmet Hospital biobank, information on age, sex, height, weight, fasting blood glucose, HbA1c, and drugs were available in the biobank database. For samples retrieved from the NBB, we had information on sex, age, time between death and autopsy, and estimated degree of neurodegeneration (expressed as Braak stage (190)).

In Study III, information on age, sex, height, and weight was self-reported. For patients diagnosed with AD, the staff nurse helped to fill out the form with age, sex, height, and weight and provided a list of prescribed drugs. BMI was calculated by dividing body weight in kilograms by the squared height in meters (kg/m2).

5.5.1 Estimation of Insulin Resistance
Hemostatic model assessment (HOMA) is a widely used tool in clinical and research settings to assess insulin resistance and β-cell function, understand its role in various health conditions, and monitor changes in insulin sensitivity over time (191). In the original model, fasting plasma insulin and fasting plasma glucose were used to estimate the relationship between hepatic glucose output and insulin secretion at a basal state (192).

Since 1996, a computerized model (HOMA2) has been available. The expanded model considers the non-linear relationship in which insulin secretion increases with increasing blood glucose levels and renal glucose loss. This allows insulin sensitivity and β-cell function to be estimated even in people with high blood glucose levels. In addition, the calculator can use C-peptide in the estimation (191). The computer model calculates a value for insulin resistance, where 1 is considered normal compared to a reference population, and a higher value indicates less sensitivity to insulin. The computer model calculates the function in percent for the β-cell function, and 100 is considered a normal function.

In this thesis, we used the computerized calculator HOMA2 (193) to estimate insulin resistance (HOMA2-IR) and β-cell function (HOMA2-β) by applying fasting blood glucose (mmol/L) and fasting serum C-peptide (ng/L).

5.6 REGISTER DATA
5.6.1 Data Source
In study IV, we used data from the Swedish BPSD registry. This national quality registry was initially designed to help healthcare staff assess neurocognitive symptoms in patients with neurocognitive disorders and provide a structured method for planning and evaluating the care. The registry is mainly used in nursing homes, but there are daycare facilities, home care groups, and a few hospital wards. The registry coverage is indefinite, as the number of care units may vary, and the...
number of patients with neurocognitive disorders who have yet to receive a diagnosis is unknown. Nonetheless, in 2021, 7453 care units from 288 of 290 municipalities in Sweden had joined the register, and more than 90,000 unique patients had been included (194).

Before a unit starts working with the registry, staff undergoes mandatory training. A selected group of staff members also undergo extended training on neurocognitive disorders, assessment of BPSD, and what can be done to alleviate symptoms. This group can then assist their colleagues in assessments and the computer program. All data is then entered into the registry by multidisciplinary teams. Usually, nurses and nursing assistants collaborate with physicians, physiotherapists, occupational therapists, and next-of-kin, as needed.

Data on the medical diagnosis and drug prescriptions are collected from the medical records. The assessment of BPSD is done with the neuropsychiatric inventory for nursing homes (NPI-NH). The team also evaluates whether practical, somatic, or social needs must be met. The overall assessment is summarized in a care plan, updated as often as necessary for the individual patient.

### 5.6.2 Assessment of BPSD

When working with the Swedish BPSD registry, the healthcare staff uses the NPI-NH to detect, assess, and evaluate BPSD. The instrument is an assessor's assessment and does not consider the patient's experience (81).

NPI-NH is divided into 12 symptom domains (delusions, hallucinations, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behavior, sleep and nighttime disturbance, and appetite and eating changes) based on common non-cognitive symptoms in patients diagnosed with neurocognitive disorders (81). Each domain starts with a screening question. If the symptom is present, the assessor goes on to answer how often the symptom occurs (1 point: occasionally, 2 points: less than once a week, 3 points: several times a week, but not every day, 4 points: at least once a day) and how severe the symptoms are (1 point: mild, 2 points: moderate, 3 points: severe). The points are multiplied to give a symptom score (1-12 points). A non-occurring symptom gives 0 points. The scores for the 12 domains are then added to provide a total score between 0 and 144. The higher the score, the more symptoms (195).

The NPI-NH has been criticized for being difficult to use in parametric statistical tests. As the frequency and severity scores are multiplied, the symptom score cannot have all values (e.g., 5 or 7), and therefore, the score cannot be considered a continuous scale (196). To get around this, we chose to dichotomize the symptom scores, indicating clinically significant symptoms (a score of 4 or above) or not (a score under 4). In a clinical setting, a score of 4 or more is usually linked with the need for interventions to manage care and everyday activities (99).
5.6.3 Information on Drug Treatment
In study IV, we investigated metformin as the primary exposure. Prescription
information was collected from the Swedish BPSD registry. As the registry does not
contain information on the dose or duration of treatment, patients were divided into
users or non-users.

For comparison, we also analyzed the association for the use of other antidiabetic
drugs (in separate models) divided into categories according to ATC classification
codes as follows: insulin (A10A), sulfonylurea (A10BB), DPP-4 inhibitors (A10BH)
and all other antidiabetic drugs as one category. None of the drug categories were
mutually exclusive (i.e., one patient could be in the metformin and insulin groups).

5.7 STATISTICAL ANALYSES
All analyses in this thesis were performed in SPSS v. 27 (IBM, New York, USA),
and statistical figures were produced in GraphPad Prism 10.0.3 (GraphPad
Software, CA, USA) or Microsoft Excel 2019. Across all studies, a \( p \)-value < 0.05
was considered statistically significant and reported \( p \)-values were two-tailed.

5.7.1 Descriptive and Comparative Analyses
Descriptive statistics and simple tests for differences between groups have been a
feature of all studies. Throughout the thesis, descriptive variables were presented as
count and percent, or median and interquartile range as suitable.

Pearson’s chi-squared test was used in all studies to test for differences in
proportions between groups. To test differences for continuous variables, the
choice of test depended on the distribution, the number of participants in each
study, and the number of groups compared.

In study IV, we used a parametric test for differences between groups, Student’s \( t \)-
test. In studies I-III, we used the non-parametric Mann-Whitney \( U \)-test or the
Kruskal-Wallis test as suitable. If needed, the effect of multiple testing was corrected
using the Bonferroni correction.

In study III, we wanted to adjust the analysis of differences in serum IDE levels for
the uneven distribution of age and gender between the groups. This was done using
a non-parametric version of the one-way analysis of covariance (ANCOVA) or
Quade analysis.
5.7.2 Correlation Analysis
In studies II–III, we investigated correlations between IDE and other variables. We used the non-parametric Spearman’s rank correlation to analyze unadjusted bivariate correlations.

In study III, we also used a non-parametric partial correlation. This test allowed us to adjust correlations for age and sex, which were uneven between groups. As treatment with insulin has been suggested to upregulate IDE (17) and is generally connected to lower levels of C-peptide (28), we decided to adjust for use of insulin.

5.7.3 Logistic Regression Analysis
In study IV, the associations between the use of metformin and clinically significant BPSD were investigated using bivariate logistic regression calculating odds ratios (OR) with confidence intervals (CI) of 95%. The crude model was adjusted in four steps: 1 – adjusting for age, sex, and specific forms of AD, 2 – adjusting for a combination of antidiabetic drug treatment, 3 – adjusting for the use of cognitive drugs, and last, fully adjusted model also including adjustment for all other psychotropic drugs. Covariates for adjustment were chosen based on availability and earlier research. Age, sex, and type of neurocognitive disorder are significant for the clinical presentation of BPSD (197), and psychotropic drugs are often prescribed to treat difficult symptoms (85).

Previous studies have demonstrated associations between BPSD and impaired blood glucose control. However, the Swedish BPSD registry has information about patients’ blood glucose, but not in numerical values. The healthcare staff assesses blood glucose and categorizes it as normal, high, low, or fluctuating. Specific values do not delimit the different categories and could vary significantly between patients. In addition, blood glucose could be a mediator, as antidiabetic drugs are intended to influence blood glucose levels. Based on this, together with the uncertainty in the registry of blood glucose, we decided not to include blood glucose in the logistic regression models.

To compare metformin to other antidiabetic drug treatments, we performed the same logistic models for users of insulin, sulfonylurea, DPP-4 inhibitors, and all other drugs as one group. Again, none of the drug treatment groups were mutually exclusive, i.e., one patient could be in more than one group.

To investigate possible interactions between metformin and other antidiabetic drugs, we added interaction terms for the three most used combinations (metformin in combination with insulin, sulfonylurea, or DPP-4 inhibitors) and all other combinations (as one variable). In the logistic regression model, the interaction terms were set to 1 if the patient had a prescription for both drugs.
6 Ethical Considerations

In this thesis, the main ethical issue was including individuals with neurocognitive disorders in the studies. Research involving humans requires that potential participants be given adequate information and the possibility to assent or dissent (198). Participation in research studies is regarded as an opportunity, and the participant can decline without it affecting care or treatment. The main rule is that voluntariness and informed consent are necessary for the research to be ethically and legally acceptable (199).

An informed consent presumes that the person has received information about the study, its risks, and benefits and can decide on participation based on this. Consequently, informed consent requires cognitive capacity (200). The Declaration of Helsinki (198) states that if the participant in a medical research study cannot consent, consent should be obtained from a relative who, according to national law, is competent to represent the participant. In Swedish legislation, unlike in many other countries, relatives or legal representatives do not have that authority but can only contribute with the wishes the person has previously expressed. This means that the participant’s consent is given great importance, and close relatives can admit consensus with what the person would probably have decided if the cognitive ability had been intact. The criticism has been that this assumes that the proxy person knows what the participant previously wanted, not just voicing their opinions (201). Also, the group of individuals diagnosed with neurocognitive disorders who do not have someone to represent them is growing (202).

The Swedish Ethics Review Act (203) stipulates that research studies that include participants who cannot give informed consent should be limited to a minimum. There must be clear benefits from the research, at the same time, as the participants are only exposed to minimal risks (203). Individuals diagnosed with neurocognitive disorders may be included in many different types of studies, and the risks can vary significantly. The conflict stands between protecting individuals with reduced decision-making capacity and allowing them to maintain their autonomy as much as possible (199). It can be argued that excluding individuals diagnosed with neurocognitive disorders and depriving them of the opportunity to participate and contribute to research could further disempower an already vulnerable group (204).

In this thesis, patients diagnosed with AD were included in Study III and Study IV. All participants recruited to study III, i.e., patients diagnosed with AD and healthy volunteers, received oral and written information about the study and data handling in accordance with the study’s ethical approval. Individuals with cognitive difficulties can vary significantly in decision-making capacity, so a family member or legal representative was asked to support the decision to participate.
In study IV, we used data from a national quality registry. Registry-based research does not require individual consent from participants for the specific study. However, consent was obtained to some extent when the patient was included in the registry. This allows future research to use registered data provided the study is granted ethical approval (205). The Swedish Ethical Review Authority approved the present study.

For healthcare professionals to include a patient in a quality registry, the patient's explicit consent is not required. All patients and relatives in units working with the Swedish BPSD registry are informed orally and in writing that staff are recording data, how data is collected, and how it may be used (194). If it is possible, the patient should consent to the registration. As patients diagnosed with neurocognitive disorders might have difficulties understanding and comprehending information, it is encouraged to have a family member or other representative to support the decision. Registration of a patient without decision-making capacity is possible if the patient does not actively oppose registration and the person supporting the decision believes that the patient would have agreed if being able to decide. Patients have the right to refuse registration or have their data deleted (206).

Regarding the use of biobank samples, participants in this thesis have given informed consent for samples to be saved in the biobank and used in subsequent studies. The use of samples collected in Sweden requires renewed ethical approval, which has been obtained for studies included in this thesis. Still, using samples from foreign brain banks does not require specific ethical approval under Swedish law (203). Nevertheless, samples in the NBB are collected from donors who have given informed consent before their death. The procedures for collecting tissues were approved by the University Medical Center (Amsterdam, the Netherlands). Also, the brain bank is not allowed to disclose personal information about the donors. Therefore, all samples were received anonymously with limited information about the individual's age, sex, and health background.
7 Main Results and Conclusions

This section summarizes study participants, primary results, and conclusions for the included studies. The author has provided comments that may be important when interpreting the results. For more detailed information and additional data, please refer to the respective publication.

7.1 STUDY I

7.1.1 Study Sample
This study included 47 patients diagnosed with T2DM and 18 metabolically healthy individuals. We divided the T2DM participants into three subgroups based on the type of antidiabetic treatment: only lifestyle treatment (n=10), peroral drug treatment (n=17), or insulin treatment (n=20). For 20 participants diagnosed with T2DM, we had access to serum samples from a 12-month follow-up. Patients treated with insulin had the most extended duration of disease and the highest HbA1c. Patients without drug treatment had the shortest duration of disease and the least impaired glucose control. Still, they had the highest level of insulin resistance (HOMA2-IR) (Figure 9).

![Graphs showing duration of disease, glycated hemoglobin (HbA1c), computerized Homeostatic Model Assessment of insulin resistance (HOMA2-IR), and insulin-degrading enzyme (IDE) for the three treatment groups. Data are presented as the median and interquartile range. Statistical analysis was performed using a Kruskal Wallis test (**p <0.01, ***p <0.001).]
7.1.2 Results
Patients diagnosed with T2DM had higher serum IDE levels than metabolically healthy individuals (33.7 ng/mL vs. 27.6 ng/mL, p=0.033). Still, we found no significant differences in serum IDE levels between the treatment groups.

For patients diagnosed with T2DM and available follow-up samples, serum IDE levels were significantly increased after 12 months (Figure 10). Other metabolic variables were essentially unchanged, as seen in Table 2. Notably, serum adiponectin levels were significantly decreased after 12 months (Figure 10).

**Figure 10.** Serum insulin-degrading enzyme (IDE) and adiponectin (ADI) levels in follow-up samples at the timepoint 0 months and 12 months (n=20). Serum IDE levels increased from 23.7 (19.6-30.3) to 30.4 (25.6-35.3) ng/mL. ADI decreased from 11.28 (7.77-19.54) to 6.52 (5.01-9.22) mg/mL. Data are presented as median (interquartile range), and differences between time points were tested with the Wilcoxon Signed Rank. (*p <0.05, **p <0.01)

**Table 2.** Metabolic marker at 0 and 12 months in patients diagnosed with T2DM (n=20).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Baseline</th>
<th>12 months</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>29.2 (27.7-34.3)</td>
<td>30.7 (28.3-33.3)</td>
<td>n.s</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>2.47 (1.85-3.21)</td>
<td>2.55 (1.85-3.62)</td>
<td>n.s</td>
</tr>
<tr>
<td>FG (mmol/L)</td>
<td>7.40 (6.80-9.80)</td>
<td>7.90 (6.80-8.70)</td>
<td>n.s</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>53 (48-59)</td>
<td>55 (53-65)</td>
<td>n.s</td>
</tr>
<tr>
<td>C-peptide (nmol/L)</td>
<td>0.96 (0.75-1.20)</td>
<td>1.10 (0.66-1.20)</td>
<td>n.s</td>
</tr>
</tbody>
</table>

All variables presented as median (interquartile range), and differences between time points were tested with the Wilcoxon Signed Rank test. Abbreviations: Body mass index (BMI), computerized Homeostatic model assessment of insulin resistance (HOMA2-IR), fasting blood glucose (FG), glycated hemoglobin (HbA1c), non-significant (n.s).

No significant differences were found in the sub-analyses of the 12-month samples from subjects without medical treatment (n=7), peroral treatment (n=5), and insulin treatment (n=8) in any of the variables.
7.1.3 Conclusion
The result indicates that patients diagnosed with T2DM have increased serum IDE levels compared to metabolically healthy individuals, and IDE may increase over 12 months.

7.1.4 Comments
In this study, we demonstrated significantly higher serum IDE levels in patients diagnosed with T2DM compared to metabolically healthy individuals. At this point, there was, to the best of our knowledge, no other publication on this. Nonetheless, in this study, the sample size was limited, and more extensive studies are needed to further elucidate the function of IDE.

Analyses of follow-up samples have not previously been published. Given the small sample size and the fact that we did not have data for potentially important influential factors of insulin metabolism (for instance, insulin levels, kidney function, complete medical history, and total drug prescription), it was not possible in this study to draw conclusions considering these increased levels of serum IDE. Still, the data is interesting and may give prospects for subsequent studies.

7.2 STUDY II

7.2.1 Study Sample
This study included 18 subjects with AD and 6 non-demented controls. The classification of AD or not was defined by the NBB in accordance with international guidelines (76) and adopted in the statistical analyses of this data.

There was no significant difference in age at death or post-mortem time between subjects with AD and non-demented controls. The distribution of men and women was even.

7.2.2 Results
Patients diagnosed with AD had statistically significantly increased plasma levels of total tau compared to non-demented controls. Although, we could not demonstrate any difference in serum IDE levels between the groups. Nor were there statistically significant differences between subjects with AD and non-demented controls regarding plasma levels of IL-6, IL-8, IL-10, or TNF-α (Figure 11).
Figure 11. Serum levels of insulin-degrading enzyme (IDE), total tau (t-tau), interleukin (IL) 6, IL-8, IL-10, and tumor necrosis factor-alpha (TNF-α) in patients diagnosed with Alzheimer’s disease (AD) and non-demented controls—data presented as median and interquartile range. Differences between groups were analyzed using the Mann-Whitney U-test. *p > 0.05

Figure 12. Spearman’s rank Correlations between plasma levels of IDE and cytokines in subjects with AD (n=18). Abbreviations: Alzheimer’s disease (AD), insulin-degrading enzyme (IDE), interleukin (IL), tumor necrosis factor-alpha (TNF-α).
Plasma levels of IDE were correlated with plasma levels of IL-6, IL-8, IL-10, and TNF-α in the whole sample and the group of subjects diagnosed with AD (Figure 12). These correlations could not be demonstrated in the group of non-demented controls.

In addition, we could demonstrate a statistically significant correlation between plasma levels of IDE and total tau in all subjects ($r = 0.49, p = 0.017$) but not in the group diagnosed with AD ($r = 0.14, p = 0.377$) or non-demented controls ($r = 0.77, p = 0.072$) separately.

7.2.3 Conclusion

The results suggest that levels of IDE in human plasma may be correlated with markers of inflammation and neurodegeneration. This could represent a target in future strategies for diagnosing and treating AD.

7.2.4 Comments

As far as we know, no earlier study has presented a correlation between IDE and total tau in human plasma. A correlation between IDE and total tau could further indicate a connection between insulin metabolism and neurodegeneration. However, the limitation is that a simple correlation cannot give us any extended knowledge of the relationship between these variables, as a more advanced statistical model could have. Although, the data in this study was not suitable for further analysis.

Nonetheless, diagnosing neurocognitive disorders clinically is challenging, and some studies suggest that contradictory results of clinical studies could be due to misdiagnosis (77). Using post-mortem samples, we could ensure that the study subjects fulfilled the AD diagnosis and that control subjects were non-demented. This could be considered a strength.

Still, the use of post-mortem samples is subject to certain limitations. The NBB has a limited supply of samples, and we depended on what could be made available for this study. The sample size may not have been sufficient to detect group differences. Also, the onset of death is likely to impact most biomarkers. It is recognized that blood glucose and insulin levels are challenging to interpret post-mortem (207). In addition, cytokines may be affected by the cause of death (208), which we did not have information on in this study. This means there is uncertainty about whether these results are transferable to living individuals, and the result must be interpreted with caution.
7.3 STUDY III

7.3.1 Study Sample

In this study, we included 47 patients diagnosed with T2DM (same participants as in study I), 9 patients diagnosed with AD, and 64 healthy volunteers as controls.

Healthy volunteers were significantly younger than patients diagnosed with T2DM and AD. Also, patients diagnosed with T2DM were predominantly men, unlike patients diagnosed with AD and healthy volunteers. Patients diagnosed with T2DM had statistically significantly higher fasting blood glucose, HbA1c, and BMI but lower adiponectin and HDL cholesterol levels than patients diagnosed with AD and healthy volunteers. However, there was no statistically significant difference between patients diagnosed with T2DM or AD regarding levels of C-peptide, HOMA2-IR, or triglycerides. Patients diagnosed with T2DM, or AD, had significantly higher C-peptide and HOMA2-IR than healthy volunteers (Figure 13).

Figure 13. Serum levels of insulin-degrading enzyme (IDE), glycated hemoglobin (HbA1c), C-peptide, fasting blood glucose (f-glucose), and computerized homeostatic model assessment of insulin resistance (HOMA2-IR) in patients diagnosed with T2DM or AD, and healthy volunteers. Data presented as median and interquartile range. Differences between groups were analyzed with the Kruska-Wallis test. \( * * * \ p < 0.001 \).
7.3.2 Results
Patients diagnosed with T2DM had higher serum IDE levels than those diagnosed with AD and healthy volunteers (Figure 12). The difference remained after adjustment for age and sex.

In the whole sample, age, and sex correlated with several metabolic variables and serum IDE. Nevertheless, after stratification, sex was not correlated with IDE in any group, and age was only correlated with IDE in the group of healthy volunteers ($r = 0.27, p = 0.034$).

In the adjusted correlation analyses for the whole study sample, we could demonstrate positive partial correlations between serum IDE and HbA1c ($r = 0.47$, $p < 0.001$), fasting blood glucose ($r = 0.50, p < 0.001$), C-peptide ($r = 0.56, p < 0.001$), HOMA2-IR ($r = 0.57, p < 0.001$), BMI ($r = 0.45, p < 0.001$), and triglycerides ($r = 0.33, p = 0.001$). There was also a negative partial correlation between serum IDE and HDL cholesterol ($r = -0.29, p = 0.005$).

After stratification, we found a negative partial correlation between serum IDE and C-peptide in the group of healthy volunteers ($r = -0.34, p = 0.008$). In addition, an unadjusted correlation between IDE and HOMA-IR in this group ($r = 0.28, p = 0.028$) did not remain after adjustment.

In the group of patients diagnosed with AD, we could demonstrate a strong negative partial correlation between IDE and HbA1c ($r = -0.95, p = 0.004$). An unadjusted correlation between IDE and triglycerides ($r = -0.79, p = 0.036$) was eliminated after adjustment.

In the group of patients diagnosed with T2DM, analyses did not demonstrate statistically significant correlations between IDE and any other variables.

7.3.3 Conclusion
The results further indicate that patients diagnosed with T2DM have higher serum IDE levels than patients diagnosed with AD and healthy controls. IDE was correlated with most metabolic markers in this study, suggesting that IDE is associated with metabolic function.

7.3.4 Comments
In this study, we demonstrated that patients diagnosed with T2DM had higher levels of serum IDE than patients diagnosed with AD and healthy volunteers, also after we had adjusted for differences in age and sex between the groups. This reinforces the results from Study I.

As the healthy volunteers were recruited through Sophiahemmet University, they were significantly younger than patients diagnosed with T2DM and AD.
Consequently, this was a limitation in this study, as a statistical adjustment cannot fully consider what could have influenced the correlation if the groups were similar. In earlier research, it has been suggested that IDE decreases over the lifespan, possibly explaining the relationship observed between T2DM and AD (134, 143). Yet, in this study, IDE was increasingly correlated with age. Age, on the other hand, was correlated with almost all the metabolic markers measured. We intended to build a regression model to elucidate the nature of this relationship. However, because of an extensive multicollinearity, building and adjusting a reliable regression model to thoroughly investigate age as an independent factor for serum IDE levels was impossible.

7.4 STUDY IV

7.4.1 Study Sample
We selected 3548 patients diagnosed with AD and antidiabetic drug treatment for this study. Patients with a prescription for antidiabetic drug treatment were considered patients with T2DM, and 1818 patients were prescribed metformin.

There was no difference between the groups regarding the distribution of specific AD forms or total scores on NPI-NH. Patients with metformin did have a higher prescription of neuroleptic and cognitive drugs but a lower prescription of painkillers. For other psychotropic drugs, there were no differences.

7.4.2 Results
Metformin was associated with lower odds of clinically significant depressive and anxious symptoms. The odds for depressive symptoms were significantly decreased in the unadjusted model (OR 0.79, CI (95%) 0.67-0.93, p = 0.004) and the fully adjusted (OR 0.77, CI (95%) 0.61-0.96, p = 0.022). Metformin was not associated with anxiety in the unadjusted model (OR 0.87, CI (95%) 0.75-1.05, p = 0.152) but turned out statistically significant for a decreased association in the fully adjusted model (OR 0.74, CI (95%) 0.58-0.94, p = 0.015) (Figure 14).

Symptoms of euphoria (OR 1.46, CI (95%) 1.08-1.96, p = 0.013) and disinhibition (OR 1.28, CI (95%) 1.08-1.53, p = 0.005) showed increasing odds in the unadjusted model, whereas apathy showed a decreasing association (OR 0.83, CI (95%) 0.70-0.98, p = 0.031). None of these withstand the adjustment in the continued model. We could not demonstrate any association with metformin for other symptoms (i.e., delusions, hallucinations, agitation, irritability, aberrant motor behavior, sleep and nighttime disturbance, appetite, and eating changes) (Figure 14).
For other antidiabetic drugs, the only association to be statistically significant was in the domain of appetite and eating changes. For these symptoms, sulfonylurea was associated with increasing odds (OR 1.48, CI (95%) 1.08-2.03, \( p = 0.015 \)), and DPP-4 inhibitors with decreasing odds (OR 0.68, CI (95%) 0.51-0.92, \( p = 0.011 \)).

In addition, in the unadjusted model, DPP-4 inhibitors were associated with decreasing odds of disinhibition (OR 0.74, CI (95%) 0.56-0.99, \( p = 0.039 \)), and insulin was associated with decreasing odds of aberrant motor behavior (OR 0.86, CI (95%) 0.75-0.99, \( p = 0.043 \)). These associations did not remain after adjustment. There were no statistically significant associations for the group with other antidiabetic drugs.

Interaction analyses did not demonstrate statistically significant interactions between metformin and the most common combinations. However, the combined use of metformin and the variable other antidiabetic drugs was associated with increased odds of clinically significant symptoms in the domain of sleep and nighttime disturbance (OR 2.27, CI (95%) 1.01-5.08, \( p = 0.047 \)).

7.4.3 Conclusion

Metformin was associated with lower odds of depressive or anxiety-related symptoms in AD. These results suggest that metformin may present a novel opportunity to help patients diagnosed with AD, even if more research is needed before this could be applied in clinical practice.
7.4.4 Comments
The results of this study were perhaps not surprising, as metformin has been suggested as a treatment for affective disorders in psychiatry (209) and AD (130). Still, we have not found another study investigating the association between metformin and these symptoms as a part of BPSD.

Nevertheless, it should be noted that almost half of the patients in this study were prescribed anti-depressants, which suggests that it is not an unknown problem in this patient group. However, we need to remember that the NPI-NH was not designed to diagnose depression, which means that the lower odds of depressive symptoms in patients with metformin may correspond to something else rather than lower levels of clinical depression. Assessing symptoms with the NPI-NH has limitations, as a patient diagnosed with AD may exhibit varying symptoms over time, influenced by the presence of different individuals and the context. This underscores the importance of the healthcare staff’s solid understanding of the assessment tool, its intended purpose, and the patient under evaluation. Additionally, the assessments are conducted by healthcare professionals with varying levels of education and experience, potentially impacting the reproducibility and reliability of the assessments. To decrease the risk of errors, clear instructions are provided with the NPI-NH form (210). All staff working with the registry must complete a 2-day course to ensure uniform and accurate assessment (194). In addition, the instrument has been tested, and satisfactory results were obtained in test-retest and inter-rater agreement (211).

Moreover, the classification of patients with T2DM in this study is an additional limitation. Prescription of antidiabetic drugs was considered a diagnosis of T2DM, which is not necessarily true. To improve the classification, we could have used additional data from the Swedish qualitative registry, the National Diabetes Registry (NDR). In NDR, around 80% of all patients diagnosed with diabetes in Sweden are registered, and it is possible to have data on the specific form of diabetes the patient has been diagnosed with (46). Time was the limiting factor in this case, but future studies should use multiple registries to improve reliability. To further improve classification, there are also national registers for neurocognitive disorders and the prescription of drugs.

In addition, we did not have information on the dose of antidiabetic drugs or duration of treatment, and we could not adjust for other important factors, such as physical or psychiatric comorbidity or cognitive function. It is essential to acknowledge that these possible uncertainties in the input data and classification, together with the absence of information concerning potentially significant confounding variables, could impact the applicability of these findings. Therefore, it is advisable to be cautious when interpreting the results.
8 Discussion

Increasing our knowledge of IDE in the blood may provide clues to its role in T2DM and AD. The enzyme has also been suggested as a biomarker for altered insulin metabolism (150) and ongoing neurodegeneration (171). Nonetheless, the knowledge of IDE levels in human blood is limited. The aim of this thesis was to increase the knowledge of the link between insulin resistance and AD. The focus of the research has been IDE as a biomarker and its possible use in identifying patients at risk for AD or patients in an early stage of the disease.

The result indicates that patients diagnosed with T2DM express elevated levels of serum IDE compared to metabolically healthy individuals. Also, we did find a significant increase in serum IDE levels over 12 months in patients diagnosed with T2DM. However, if, and in that case, how, other metabolic markers are contributing to these increased levels remains uncertain. In contrast, patients diagnosed with AD appear to have serum IDE levels like those of healthy volunteers despite their insulin resistance levels resembling those of T2DM patients. Intriguingly, the study suggests that metformin, a common drug in the treatment of T2DM, may benefit AD patients by reducing the odds of depressive and anxiety-related symptoms, potentially enhancing IDE.

The results of this thesis are in line with the results from Sofer and colleagues (150). They demonstrated that patients with metabolic syndrome expressed higher serum IDE levels than metabolically healthy individuals. They also found correlations between IDE and metabolic markers (150), in line with our results. These findings would possibly speak in favor of IDE being upregulated in hyperinsulinemia. Still, we did not find a difference in IDE levels between patients with lifestyle, peroral, or insulin treatments. In addition, patients diagnosed with AD expressed serum IDE levels comparable to healthy controls despite having C-peptide levels and HOMA2-IR comparable to patients diagnosed with T2DM. Admittedly, the group with AD was small, but even so, this could indicate that high insulin levels alone cannot explain the increase in IDE seen in patients diagnosed with T2DM.

On the other hand, it must be considered that patients treated with insulin had been diagnosed with T2DM for longer and had the least optimal glycemic control. This was expected. Still, patients with only lifestyle treatment had the highest level of insulin resistance. Given this, we assumed these patients would have the highest serum IDE levels, but they did not. We did not find any differences between the treatment groups. These results suggest that patients with shorter disease duration may have higher IDE activity, therefore not needing to compensate for their elevated insulin resistance with higher serum IDE levels. Of course, this could be due to the small sample size, but it has been discussed whether IDE levels and IDE
activity are equivalent (212, 213). Similarly, the repeated measurements showed no difference in IDE levels over 12 months between the different treatment groups. This further suggests a compensatory effect related to insulin resistance or high insulin levels (155), possibly explaining why there was no significant change in other variables.

The fact that patients diagnosed with T2DM showed higher levels of IDE compared to healthy volunteers and patients diagnosed with AD could possibly be explained by drug treatment. Several antidiabetic drugs have been suggested to increase activity and expression in IDE, such as insulin (142) and metformin (156, 214). These were also the most utilized drugs in the group included in our study.

Metformin has been of interest in AD research for a long time (215). It has been suggested to decrease inflammation (216), improve cognition (130), and decrease the risk of affective disorders (217). In patients diagnosed with refractory depressive disorder, researchers demonstrated improved effects from antidepressant drug treatment after adding metformin in patients not diagnosed with T2DM. It may be that a reduction in insulin resistance increases the effectiveness of antidepressant drugs (209). In addition, it has been demonstrated that metformin increases the expression of IDE, possibly explaining the effect of increased sensitivity to insulin (156). The suggestion that metformin potentially augments the effects of antidepressant drug treatment introduces a dynamic interplay between metabolic pathways and neurocognitive well-being.

Whether AD patients could have the effect on affective symptoms from metformin is still unclear, but the results of this thesis point in that direction. It seems like patients diagnosed with AD may benefit from metformin for affective symptoms like cognitively healthy patients, even though their disease is advanced enough to live in nursing homes. On the other hand, metformin has been suggested as a cognitive enhancement drug in patients diagnosed with late-stage AD (218). Also, metformin has been demonstrated to increase brain metabolism by improving the integrity of the neurons and their interactions with other cells (219), possibly indicating that reduced affective discomfort in the AD patients prescribed metformin could be due to amelioration of cognitive symptoms. However, it has been pointed out that metformin may also have adverse effects (130, 214). It has been suggested that a higher dose and a more extended treatment period could increase the risk of depression (221) and aggravate neurodegeneration (214), indicating that more knowledge is needed before introduction in clinical praxis.

Nonetheless, about half of the patients included in the fourth study in this thesis were prescribed antidepressant drugs. It can, therefore, be assumed that depression, or at least depressive symptoms, is a common problem in this patient group. If metformin can improve the effectiveness of antidepressant drugs and possibly improve the quality of life of these patients, significant gains would be made.
It may not be realistic to think that people without T2DM would be prescribed metformin on this basis. Still, it is common to discontinue antidiabetic drug treatment in older patients, especially if they have a neurocognitive disorder, as glycemic targets may no longer be a priority (220). Considering these results, it may be reasonable for healthcare professionals to consider not to stop prescribing these drugs, although side effects must be weighed against this theoretical benefit. In the future, it may turn out that other drugs with insulin-potentiating effects may be equally, or even more, helpful. For instance, epidemiological studies suggest that GLP-1 receptor analogs offer a protective effect by delaying neurodegeneration. Clinical trials are ongoing and may provide disease-modifying treatment for AD in the future (221).

It is sensible to think that the anti-inflammatory effect of these drugs may be substantial. Both insulin resistance and neurodegeneration are associated with inflammation. Neuroinflammation is mediated by cytokines such as IL-6, IL-8, and TNF-α (69), and IL-6 has also been demonstrated to correlate with memory impairment (222) and Aβ plaques in AD (223). Inflammation has been suggested as a target for treating T2DM and AD (224, 225). Still, trials with anti-inflammatory drugs have not been successful (69). If IDE is correlated with pro-inflammatory cytokines, as in the results of this thesis, it could potentiate IDE as a target for treating AD, which has also been proposed earlier (132). However, there is likely an intricate interaction between several molecules, and IDE may be one of them.

Intriguingly, in the first study of this thesis, we observed lower serum adiponectin levels in individuals diagnosed with T2DM compared to metabolically healthy individuals. In addition, there was a significant decrease in serum adiponectin levels over 12 months. Adiponectin is known to enhance insulin sensitivity (178). It is plausible that inadequate IDE function, leading to less effective insulin clearance, and reduced adiponectin levels contributing to decreased insulin sensitivity collectively exacerbate glucose metabolism deterioration. Adiponectin has been suggested to have neuroprotective effects against high glucose levels (181) and Aβ (180). It has also been proposed to have a role in ameliorating insulin resistance in the brain (226). Given this, a decreasing serum adiponectin level could imply an added risk of cognitive impairment in patients diagnosed with T2DM.

In addition, the results of this thesis demonstrated a correlation between IDE and total tau, which indicates a possible correlation between IDE and neurodegeneration. It has been suggested that patients with cognitive impairment express decreased levels of serum IDE compared to patients with no cognitive symptoms (171, 172). If that is true, and the higher serum IDE levels in patients diagnosed with T2DM can be confirmed, it is possible that IDE could be a helpful marker.
In theory, if healthcare professionals were to identify patients diagnosed with T2DM and decreasing serum IDE levels, this could be individuals in the early stages of AD. If we could identify AD patients before clinical symptoms, it would possibly allow for a more favorable effect of already available Aβ-targeted drugs (227).

In the future, researchers will possibly be interested in other features of IDE than Aβ degradation. Hypometabolism and insulin sensitization are emerging areas of neurodegenerative research (33). Insulin signaling pathways are essential for neuronal survival and memory function in the cortical and hippocampus regions (33). In fact, intranasal insulin administration has demonstrated promising results. It seems to improve cognitive functions in patients diagnosed with mild cognitive impairment. Though, it has not affected Aβ pathology (228).

Nonetheless, the brain constitutes about 2% of the human body weight but consumes 20% of the used energy (21). Impaired insulin signaling affects the cells' basic energy homeostasis, as glucose is essential for cellular respiration and its functions. AD has been associated with decreased glucose uptake, dysregulation of the citric acid cycle, impaired mitochondrial function, and lower electron transport chain activity, limiting the production of ATP (33). It is essential for normal brain function that energy homeostasis can be maintained. Insulin signaling has been suggested as a treatment target in AD (229). There are reasons to believe that hypometabolism is present in an early stage of AD, likely many years before clinical symptoms. If energy metabolism can be restored, this promises to possibly delay or slow down an ongoing neurodegeneration (33). IDE is strongly associated with insulin signaling and clearance and may also have a role in this part of the relationship between T2DM and AD (230).

However, much research must be done before we can unveil the complete functions and possibilities of IDE. Preclinical studies, like in this thesis, are essential for knowledge of the biological mechanisms underpinning health and disease. Studies like these lay the foundation for understanding the molecular and biochemical aspects of conditions such as T2DM and AD. Still, the bridge between the work in the laboratory and the clinical setting is where the impact on patient well-being is achieved.

Healthcare professionals have a mission to keep up with the emerging knowledge and to sift out what can benefit each patient. Although IDE does not currently have a place in clinical practice, research shows a close link between metabolic function and brain health. By communicating this to our patients in an educational and empowering way, patients can be better equipped to make informed life choices. So, while much work remains to be done in the preclinical setting, well-informed clinical staff ensure that the benefits of scientific discovery are realized by those who need it most - patients on their journey through disease, care, and health.
9 Concluding Remarks

In conclusion, this thesis sheds light on the elevated serum IDE levels in patients diagnosed with T2DM and that a wide range of factors, such as glycemic control, age, and drug use, may influence the enzyme. Additionally, it highlights the potential role of metformin in ameliorating depressive and anxiety symptoms in AD patients.

As the research describing IDE levels in human blood is limited, the results of this thesis constitute an important addition to the knowledge in the field despite the small size of the studies. We could not find any study to compare IDE levels in patients diagnosed with T2DM or correlations between IDE and inflammatory markers in blood. It remains to be seen whether these results stand up to comparison when more comprehensive studies will be available. Nonetheless, there is a growing interest in exploring blood-based biomarkers due to their accessibility and cost-effectiveness (7). In this thesis, IDE has emerged as a stable and analyzable biomarker in blood, with commercially available kits demonstrating reliability, even if large-scale testing remains.

In addition, we have not found a study that examines associations between metformin and BPSD. However, several well-designed large-scale studies have included other patient groups to compare with. Metformin has been demonstrated to ameliorate affective symptoms (209), decrease the risk of depression (219), and has been suggested as a treatment for behavioral symptoms in neuropsychiatric illnesses (231). What is interesting in the results of this thesis is that it seems that patients diagnosed with moderate and advanced-stage AD have similar effects of metformin as patients without cognitive difficulties. This needs to be investigated further but could potentially mean that many patients currently diagnosed with AD and persistent affective symptoms could receive a more appropriate treatment.

Lastly, working with blood samples in the laboratory has made me aware of all the mistakes that can be made between the patient and the final analysis result. It cannot be overemphasized how important it is for nurses and other clinical professionals to learn the correct sampling techniques and how to handle samples to avoid potential errors, which can impact care and research results.
Clinical Implications

The current understanding of the clinical implications of IDE is limited. Investigating the presence and activity of IDE in blood can perhaps explain its interactions with various markers and the signaling pathways it participates in (232). Understanding the systematic functions of IDE can have significant implications for metabolic disease and conditions influenced by insulin metabolism and regulation (150), including neurodegeneration. Understanding the dynamics of blood IDE levels may be essential in this context.

Research has shown that reduced IDE levels are associated with a higher risk of developing a neurocognitive disorder (171, 172). In this thesis, patients diagnosed with T2DM had elevated serum IDE levels compared to metabolically healthy individuals. This result raises the prospect of identifying individuals susceptible to AD or in an early phase of the disease. Nonetheless, as IDE levels appear to be influenced by many factors (139), there will be difficulties in identifying what is considered a standard value. Feasibly, rather than comparing individual IDE values to a reference interval, continuous monitoring of IDE levels in patients diagnosed with T2DM may prove more valuable. This approach could account for various factors, such as age, ethnicity, genetic variations, and drug treatments, and possibly have the potential to find individuals at risk or in the preclinical phase of AD.

The ethical justification of actively seeking methods to identify individuals at risk of developing AD could be questioned (233). The reality is that, at present, there are no available treatments or lifestyle modifications proven to prevent the onset of this disease. Nevertheless, there are significant benefits in preventing T2DM and its complications. As T2DM affects individuals at younger ages and childhood obesity rates continue to rise, the impact of these conditions on public health cannot be underestimated. Patients diagnosed with T2DM at a young age will endure the challenges of the condition for a large part of their lives, contributing to an increased risk of complications, including AD.

In addition, the potential use of metformin to alleviate affective symptoms in BPSD is interesting. While its immediate adoption may require further investigation and clinical trials, its proven effectiveness in other groups of patients hints at the possibility of a new therapeutic avenue. As research advances, metformin or similar medications may evolve into tailored treatments for individuals facing the complex challenges of BPSD.
11 Future Research

Although IDE has been investigated for decades, there is still a lot we do not know. Also, much of our knowledge about IDE is based on research that is becoming outdated, which underscores the need for further exploration and modernization in this field (177). However, research on IDE continues to evolve, and the interplay between this enzyme and other molecules, its systemic effects, and its function in different contexts remain subjects of active exploration.

Future research on blood levels of IDE needs to study larger groups and preferably follow these individuals to increase knowledge of how IDE changes over time. We need more knowledge about IDE in patients diagnosed with T2DM or AD and in healthy individuals to evaluate the ability of IDE to identify those at increased risk of AD or in the early stages of the disease.

It is worth noting that most IDE research has been conducted in experimental animal models, mainly in rodents (177). While these models have provided valuable insights, they come with limitations. The differences between rodents and humans in physiology, genetics, and disease responses mean that findings in rodent studies may not always be directly transferable to humans. For instance, when studying topics like inflammation and neurodegeneration, it is known that rodent models may not fully replicate the complexities of human neuroinflammation (69).

To complement more comprehensive clinical studies, emerging technologies offer promising methods to uncover the mechanisms of IDE. For instance, the development of clustered regularly interspaced short palindromic repeats technology (CRISPR) has revolutionized genetic research (234). It allows researchers to selectively modify genes, including those encoding for IDE, which could provide critical insights into the enzyme's functions. Additionally, machine learning and computational approaches can help analyze the vast amount of biological data and identify patterns we have previously been unable to detect (235). These tools can assist in comprehending the full function of IDE and its intricate relationships with other biomolecules.
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