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DEPRESSION, DAYLIGHT AND DIABETES: SHARED GENETIC BACKGROUND AND GENOMIC MODERATION OF ASSOCIATIONS

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ACADEMIC DISSERTATION

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Emale
To my mom

ABSTRACT

Type 2 Diabetes (T2D) is an increasingly prevalent health problem and better understanding of its etiology is vital for improved prevention and treatment. It is known that T2D has multifactorial origin and mental health problems seem to accompany this progressive disorder. Of note, there is a bidirectional association between T2D and depression. One explanation for this relationship between T2D and depression could be the presence of pleiotropic genetic variants and possibly shared biological pathways. However, the underlying mechanisms remain poorly understood.

Another possible common biological mechanism underlying T2D and depression comorbidity is related to disrupted circadian rhythms. It has been well established that many common disorders have seasonal fluctuations and it has been hypothesized that the amount of daylight might play an important role in seasonal fluctuations of both mental health conditions, glucose metabolism and T2D. The amount of daylight varies substantially throughout the year in Finland, making it one of the best locations to study this phenomenon.

This research explores the shared genetic basis of T2D and depression as limited information exists on shared genetic risk of these conditions. Furthermore, another possible biological mechanism associated with both T2D and depression – circadian rhythm – has received limited research attention and is thus one of the focus areas of current thesis in relation to glucose metabolism.

This research aims to provide better understanding in the genetic basis of increasingly prevalent health problems of T2D depression. The indicated knowledge together with understanding environmental effects of these prevalent conditions is needed in development of intervention strategies in light of the diabetes epidemic. Thus, this thesis focuses on four objectives. The first objective of this doctoral thesis is to examine whether there is a common genetic basis of T2D, glycemic indices related to T2D, and depressive symptoms. The second objective is to examine which specific genetic variants show common variation between glycemic indices and depressive symptoms. The third aim is to study if a common diabetes risk variant rs10830963 in the Melatonin Receptor 1B (*MTNR1B*) gene influences the relationship between depressive symptoms and glycemic indices. The final aim of this doctoral thesis is to investigate to what extent the amount of daylight moderates the associations between *MTNR1B* rs10830963 and glycemic indices. The first two objectives are addressed in Study I, objective three in Study II and objective four in study III.

Study I is based on the summary statistics data from previously published Genome-Wide Association Studies (GWAS) of depressive symptoms by CHARGE consortium, T2D by Diagram consortium and glycemic traits by

MAGIC consortium. GWAS on depressive symptoms involved 51,258 participants, GWAS on T2D involved 34,840 cases and 114,981 controls, GWAS on glycemic traits involved up to 58,074 participants. Studies II-III are conducted within the same Prevalence, Prediction and Prevention of Diabetes, the PPP-Botnia Study cohort. This prospective study cohort comprises 5,208 individuals at baseline visit and 3,850 individuals at follow-up, on average 6.8 years later. The analytic sample of Study II involved 4,455 non-diabetic participants from the baseline visit who were genotyped for *MTNR1B* rs10830963, had data on glycemic indices available based on an Oral Glucose Tolerance Test (OGTT) and additionally had data on depressive symptoms measured using Mental Health Inventory (MHI). The analytic sample of Study III involved 3,422 participants who had data on glycemic indices at both time points, were genotyped for *MTNR1B* rs10830963 and had no diabetes at baseline.

The results of Study I showed that there was very low SNP-based heritability of the traits of interest and no overall SNP-based genetic correlation between glycemic traits or T2D and depressive symptoms. Yet, pleiotropic genetic variation for depressive symptoms and T2D was found in the *IGF2BP2, CDKAL1, CDKNB-AS, and PLEKHA1* genes. Pleiotropic genetic variation for depressive symptoms and fasting glucose was found in the *MADD, CDKN2B-AS, PEX16, and MTNR1B* genes. The results of Study II showed that the common diabetes risk variant rs10830963 in the *MTNR1B* gene and depressive symptoms are independently associated with glycemic traits. The associations between glycemic traits and depressive symptoms were not influenced by the variation in diabetes risk variant rs10830963. The results of Study III showed that each addition of the risk allele G of rs10830963 was associated with an increasingly worse glycemic profile across the 6.8-year follow-up. Additionally, more daylight was associated with worse glycemic response across the follow-up. Finally, the risk genotype GG of the *MTNR1B* rs10830963 became more insulin resistant during the follow-up, if the amount of daylight was less at the follow-up than at the baseline.

Based on these findings, there are differences in underlying genetic background of glycemic traits, T2D and depressive symptoms. Additionally, on a candidate gene level, the known diabetes risk variant rs10830963 does not contribute significantly to the comorbidity between depression and diabetes. Yet, the rs10830963 and daylight are associated with glucose metabolism and the longitudinal glycemic profiles vary according to the amount of daylight, *MTNR1B* rs10830963 genotype and their interaction.

This study contributes to the research literature in several ways. The findings provide valuable insights into the relationship of T2D and depression by addressing the common genetic background of these conditions. Furthermore, it emphasizes the importance of the amount of daylight in glucose metabolism and consequently T2D genesis.

TIIVISTELMÄ

Tyypin 2 diabetes (T2D) on yleistynyt ja sen etiologian parempi ymmärrys on oleellista sairauden tehokkaamman ehkäisyn ja hoidon kannalta. T2D on perustaltaan monitekijäinen, etenevä sairaus, jonka kanssa yhtä aikaa esiintyy myös mielenterveyden häiriöitä, kuten masennusta. Yksi selitys T2D:n ja masennuksen väliselle kaksisuuntaiselle yhteydelle voi olla näihin molempiin vaikuttavat - pleiotrooppiset - geneettiset variantit sekä molemmille yhteiset biologiset mekanismit. Näitä taustalla olevia mekanismeja ei kuitenkaan vielä riittävästi ymmärretä. Tämä tutkimus selvittääkin T2D:n ja masennuksen yhteistä geneettistä perustaa.

Toinen mahdollinen yhteinen biologinen mekanismi T2D:n ja masennuksen komorbiditeetin taustalla liittyy häiriintyneeseen vuorokausirytmiin. Monet yleiset häiriöt vaihtelevat vuodenajan mukaan ja on esitetty, että päivänvalon määrän vaihtelu voisi olla tärkeässä osassa selittämässä vuodenaikojen mukaan tapahtuvaa mielenterveyden häiriöiden, sokeriaineenvaihdunnan ja T2D:n vaihtelua. Suomi on tämän ilmiön tutkimisen kannalta olennaisessa asemassa, koska päivänvalon määrä vaihtelee huomattavasti läpi vuoden. Vuorokausirytmin vaikutuksia on tutkittu kohtalaisen vähän, joten se on yksi tämän tutkimuksen kohdealueista suhteessa sokeriaineenvaihduntaan.

Tässää väitöskirjassa oli neljä tavoitetta. Ensimmäinen tavoite oli tutkia T2D:n, sokeriaineenvaihdunnan ja masennuksen yhteistä geneettistä taustaa. Toinen tavoite oli tutkia, millä geneettisillä varianteilla on yhteistä vaihtelua sekä sokeriaineenvaihdunnan että masennusoireiden kanssa. Kolmas tavoite oli tutkia, vaikuttaako melatoniinireseptori 1B (*MTNR1B*) -geenissä sijaitseva yleinen T2D riskivariantti rs10830963 masennusoireiden ja T2D:n sekä sokeriaineenvaihdunnan väliseen yhteyteen. Viimeinen tavoite oli tutkia missä määrin päivänvalon määrä muokkaa yhteyksiä *MTNR1B* rs10830963-riskivariantin ja sokeriaineenvaihdunnan välillä. Kahta ensimmäistä tavoitetta tutkittiin Tutkimuksessa I, kolmatta tavoitetta Tutkimuksessa II ja neljättä tavoitetta Tutkimuksessa III.

Tutkimus I perustuu masennusoireiden osalta CHARGE-konsortion, T2D:n osalta Diagram-konsortion ja sokeriaineenvaihdunnan osalta MAGIC-konsortion aiemmin julkaistuihin genominlaajuisiin assosiaatiotutkimuksiin (GWAS). Masennusoireita tutkinut GWAS-tutkimus sisälsi 51258 osallistujaa, T2D:sta tutkineessa GWAS-tutkimuksessa oli mukana 34840 potilasta ja 114981 verrokkia ja sokeriaineenvaihduntaa tutkinut GWAS-tutkimus sisälsi 58074 osallistujaa. Tutkimukset II-III toteutettiin PPP (Prevalence, Prediction and Prevention of Diabetes)-Botnia tutkimuskohortissa. Tämä prospektiivinen tutkimuskohortti sisälsi alun perin 5208 osallistujaa ja seurantavaiheessa, keskimäärin 6.8 vuotta myöhemmin, 3850 osallistujaa. Tutkimuksen II analyysiotos sisälsi 4455 alkuperäistä osallistujaa, joilla ei

ollut diabetesta, joiden genotyyppi *MTNR1B* rs10830963:n osalta oli selvitetty, joilla oli tietoa sokeriaineenvaihdunnasta sokerirasitustestistä (OGTT) ja joilta oli lisäksi tieto masennusoireista Mental Health Inventory (MHI)-menetelmää käyttäen. Tutkimuksen III analyysiotos käsitti 3422 osallistujaa, joilta oli tietosokeriaineenvaihdunnasta molemmissa aikapisteissä, joiden genotyyppi *MTNR1B* rs10830963:n osalta oli selvitetty ja joilla ei ollut tutkimuksen alussa diabetesta.

Tutkimus I osoitti, että tutkittujen piirteiden mitatun geneettisen vaihtelun perusteella arvioitu periytyvyys oli vähäistä. Lisäksi sokeriaineenvaihdunnan piirteiden tai T2D:n ja masennusoireiden väliset geneettiset korrelaatiot eivät olleet merkitseviä. Sen sijaan geeneistä *IGF2BP2, CDKAL1, CDKNB-AS* ja *PLEKHA1* löytyi pleiotrooppisia geneettisiä variantteja, jotka olivat yhteydessä masennusoireisiin ja T2D:hen. Lisäksi geeneistä *MADD, CDKN2B-AS, PEX16* ja *MTNR1B* löytyi pleiotrooppisia geneettisiä variantteja, jotka olivat yhteydessä masennusoireisiin ja paastosokeritasoon. Tutkimus II osoitti, että *MTNR1B*-geenissä sijaitseva yleinen diabeteksen riskivariantti rs10830963 ja masennusoireet olivat itsenäisesti yhteydessä sokeriaineenvaihdunnan piireisiin. Sen sijaan diabeteksen riskivariantti rs10830963 ei vaikuttanut sokeriaineenvaihdunnan piirteiden ja masennusoireiden välisiin yhteyksiin. Tutkimus III osoitti, että diabeteksen riskialleeli G rs10830963:ssa oli additiivisesti yhteydessä heikompaan sokeriaineenvaihduntaprofiiliin 6.8 vuoden seurannan aikana. Lisäksi seurannan aikana todettiin, että enenevä päivänvalon määrä oli yhteydessä heikompaan sokerivasteeseen. Lopuksi, diabeteksen riskigenotyyppi rs10830963 GG:n kantajilla insuliiniresistenssi lisääntyi seurannan aikana, jos päivänvalon määrä oli seurannan lopussa vähäisempi kuin tutkimuksen alussa.

Näiden tulosten perusteella sokeriaineenvaihdunnan piirteiden, T2D:n ja masennusoireiden taustalla olevassa geneettisessä perustassa on eroja. Lisäksi yleinen diabeteksen riskivariantti rs10830963 ei merkitsevästi vaikuta masennuksen ja diabeteksen komorbiditeettiin. Sen sijaan, rs10830963 ja päivänvalo ovat yhteydessä sokeriaineenvaihduntaan, ja pitkäaikaiset sokeriaineenvaihduntaprofiilit vaihtelevat päivänvalon määrän, *MTNR1B* rs10830963 -genotyypin ja niiden yhteisvaikutuksen suhteen.

Tämä tutkimus tarjoaa arvokasta tietoa T2D:n ja masennuksen yhteydestä keskittymällä näiden häiriöiden yhteiseen geneettiseen perustaan. Lisäksi tämä tutkimus korostaa päivänvalon määrän merkittävää osaa sokeriaineenvaihdunnassa ja näin ollen T2D:n synnyssä.

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would never be able to reach for the stars without you by my side. Just remember that it's always darkest before the dawn but I love you more than anything.

Thank you, next chapter!

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Haljas, K., Amare, A. T., Alizadeh, B. Z., Hsu, Y. H., Mosley, T., Newman, A., Murabito, J., Tiemeier, H., Tanaka, T., van Duijn, C., Ding, J., Llewellyn, D. J., Bennett, D. A., Terracciano, A., Launer, L., Ladwig, K. H., Cornelis, M. C., Teumer, A., Grabe, H., Kardia, S. L. R., Ware, E.B., Smith, J. A., Snieder, H., Eriksson, J. G., Groop, L., Räikkönen, K., Lahti, J. (2018). Bivariate Genome-Wide Association Study of Depressive Symptoms with Type 2 Diabetes and Quantitative Glycemic Traits. *Psychosomatic Medicine,* 80(3), 242-251. doi:10.1097/PSY.0000000000000555

II Haljas, K., Lahti, J., Tuomi, T., Isomaa, B., Eriksson, J., Groop, L., Räikkönen, K. (2018). Melatonin receptor 1B gene rs10830963 polymorphism, depressive symptoms and glycemic traits. *Annals of Medicine,* 50(8), 704-712. doi:10.1080/07853890.2018.1509118

III Haljas, K., Hakaste, L., Lahti, J., Isomaa, B., Groop, L., Tuomi, T., Räikkönen, K. (2019). The associations of daylight and melatonin receptor 1B gene rs10830963 variant with glycemic traits: the prospective PPP-Botnia study. *Annals of Medicine*, doi:10.1080/07853890.2018.1564357

The publications are referred to in the text by their roman numerals. All original publications have been reprinted with the kind permission of the copyright holders.

ABBREVIATIONS

ADA	American Diabetes Association
AUC	Are Under the Curve
BDI	Beck Depression Inventory
BMI	Body Mass Index
CDKAL1	CDK5 Regulatory Subunit Associated Protein 1 Like 1
CDKN2B-AS	CDKN2B Antisense RNA 1
CES-D	Center for Epidemiological Studies Depression Scale
CHARGE	The Cohorts for Heart and Aging Research in Genomic Epidemiology consortium
CIR	Corrected Insulin Response
CNS	Central Nervous System
CVD	Cardiovascular Disease
DI	Disposition Index
DIAGRAM	DIAbetes Genetics Replication And Meta-analysis consortium
FDR	False Discovery Rate
FMI	Finnish Meteorological Institute
FPG	Fasting Plasma Glucose
GWAS	Genome wide association study
HOMA	Homeostasis Model Assessment
HPA axis	The Hypothalamic–Pituitary–Adrenal axis
IDF	International Diabetes Foundation
IFG	Impaired Fasting Glucose
IGF2BP2	Insulin Like Growth Factor 2 MRNA Binding Protein-2
IGT	Impaired Glucose Tolerance
IR	Insulin Resistance
ISI	Insulin Sensitivity Index
IVGTT	Intravenous Glucose Tolerance Test
LD	Linkage Disequilibrium
LDSC	Linkage Disequilibrium Score Regression
MADD	MAP Kinase Activating Death Domain
MAGIC	The Meta-Analyses of Glucose and Insulin-related traits Consortium
MDD	Major Depressive Disorder
MetS	Metabolic Syndrome
MHI-5	Five-item Mental Health Inventory
MTNR1A	Melatonin Receptor 1A
MTNR1B	Melatonin Receptor 1B
OGTT	Oral Glucose Tolerance Test
PEX16	Peroxisomal Biogenesis Factor 16

PLEKHA1	Pleckstrin Homology Domain Containing A1
PPP	The Prevalence, Prediction and Prevention of Type 2 Diabetes Study
SD	Standard Deviation
SCN	Suprachiasmatic Nucleus
SNP	Single Nucleotide Polymorphism
SF-36	The 36-item Short-Form Health Survey
T2D	Type 2 Diabetes
WHO	World Health Organization
25-OHD	Serum 25-hydroxyvitamin D

1 INTRODUCTION

Diabetes is one of the leading global health problems that is currently affecting more than 451 million people aged 18-99 worldwide (Cho et al., 2018). Alarmingly, a dramatic increase in diabetes prevalence has occurred globally over the past few decades. It has to be noted that the prevalence of diabetes was evaluated to be 151 million in 2000 and it is now estimated to rise over 693 million by 2045 (Cho et al., 2018). That is 4.5 times higher future prevalence rates compared to early 2000.

The increased diabetes prevalence reflects on an increased societal burden. Currently, the expenditure on diabetes already accounts for approximately 11% of the world's total health expenditure (da Rocha Fernandes et al., 2016). However, T2D patients often present several comorbidities, such as mental disorders, high blood pressure, heart disease and so on, which is why the associated healthcare costs can be considered significantly higher (Aguiree et al., 2013).

According to the current classification of diabetes, type 2 diabetes (T2D) comprises 90% of people with diabetes (WHO, 1999). Due to the very high heterogeneity of the most common diabetes, T2D, a completely new five-cluster classification of diabetes has been recently proposed including severe autoimmune diabetes, severe insulin-deficient diabetes, severe insulin-resistant diabetes, mild obesity-related diabetes and mild age-related diabetes (Ahlqvist et al., 2018). Nevertheless, independent of the classification criteria, it needs to be acknowledged that almost half of all people who have diabetes are undiagnosed (Cho et al., 2018). Unaware of their condition, people could experience persistency high glucose levels for several years, which is likely to lead to various complications (WHO, 2009). Moreover, the disease is often recognized only when complications such as cardiovascular disease (CVD), nephropathy, retinopathy or neuropathy become evident. While many somatic complications can be seen in relation to diabetes, mental health problems also seem to accompany this progressive condition. Furthermore, several environmental factors are associated to diabetes.

Taken together, in light of the growing epidemic of diabetes, a better understanding of its etiology and specific risk factors is vital for improved intervention.

2 REVIEW OF THE LITERATURE

2.1 DIABETES DEVELOPMENT AND DIAGNOSIS

Diabetes is considered to be one of the main threats to human health in the 21st century (Alberti, Zimmet, & Shaw, 2005). It is a heterogeneous syndrome that is characterized by abnormalities in carbohydrate and fat metabolism. While in healthy individuals, glucose stimulates pancreatic secretion of insulin to maintain normal blood glucose levels by facilitating cellular glucose uptake, in individuals with T2D, the secretion of insulin is insufficient for their level of insulin sensitivity. This results in individuals with T2D having at least some degree of insulin resistance.

The progression towards T2D can be seen years before the manifestation of the disease. With that said, prediabetes is a condition where liver and/or skeletal muscles are insulin resistant and the body is not able to lower blood glucose level. As a result, pancreas produces more insulin to compensate for insulin resistance, which is called hyperinsulinemia. In a prediabetic state, the body is not using insulin effectively, causing glucose to build up in the bloodstream (Wilcox, 2005). As described above, prospective studies also show that blood glucose may start to rise years or even decades before the diagnosis of T2D (Mason, Hanson, & Knowler, 2007). Shortly before the diagnosis, blood glucose values rise very steeply and it has been hypothesised that viral infections might play a role in the manifestation of T2D (Šestan et al., 2018). Recent findings show that acute infection is not only associated with the manifestation of T2D but also with insulin resistance (Šestan et al., 2018). Even though prediabetes is a condition that can last for years and is related to several complications, it does not fulfill the criteria of T2D (Tabák, Herder, Rathmann, Brunner, & Kivimäki, 2012) but is rather a high-risk state of developing T2D.

High blood glucose levels indicate impaired glucose metabolism which is affected by liver and/or muscle insulin resistance. Thus, fasting hyperglycemia (or Impaired Fasting Glucose, IFG) and impaired glucose tolerance (IGT) are closely associated with insulin resistance. As described earlier, the progress towards T2D can be seen years before the manifestation of the disease. However, not only the diagnosis is related to severe complications. Also chronic hyperglycemia is associated with a plethora of complications including long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. In order to reduce the risk of developing those complications, it is important to know about the risk factors contributing to the development of impaired glucose metabolism. Other cardiometabolic risk factors in addition to impaired glucose metabolism also exist. For example, Metabolic Syndrome (MetS) is a term used to classify a combination of impairments in glucose and lipid metabolism, obesity and

hypertension (Alberti et al., 2005). These conditions may lead to T2D and furthermore, are risk factors for mortality.

T2D is diagnosed on the basis of hyperglycemia, characterized as a progressive disorder of glucose metabolism with decreased β-cells function and insulin resistance as the dominant factors in its genesis (Ismail-Beigi, 2012). The assessment of T2D requires an oral glucose tolerance test (OGTT) which needs to be performed after an overnight fast, using a glucose load containing the equivalent of 75 grams of anhydrous glucose dissolved in water, and with samples obtained before (baseline) and 2h after the glucose load (American Diabetes Association, 2010).

Several indices can be calculated based on the OGTT. For example, various insulin sensitivity indices exist, including the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), the product of fasting glucose and insulin concentrations divided by a constant; furthermore, an updated non-linear computer based HOMA-IR2 calculation; and additionally, Insulin Sensitivity Index (ISI), which, in addition to fasting values, takes 30 min OGTT based glucose and insulin values into account. Insulin secretion and β-cell function are commonly estimated using Disposition Index (DI) and Corrected Insulin Response (CIR) indices.

In addition to OGTT-based diagnosis, several other measurements can be used to evaluate suboptimal glycemic control and in diagnosing diabetes as suggested by American Diabetes Association (ADA). For example, other highly accurate measurements of insulin sensitivity include the gold standard - hyperinsulinemic-euglycemic clamp - and intravenous glucose tolerance test (IVGTT), which also provide an assessment of insulin secretion. Additionally, diabetes can be diagnosed based on glycated hemoglobin (HbA1c) with a cut-point ≥6.5% (Sherwani, Khan, Ekhzaimy, Masood, & Sakharkar, 2016) as an alternative to those above mentioned criteria.

Nevertheless, although there are several easily accessible methods to diagnose diabetes, still around half of the individuals with T2D are left undiagnosed (Cho et al., 2018).

2.2 DIABETES ETIOLOGY AND COMMON RISK FACTORS

Diabetes is a heterogeneous condition and the origin of its most common type, T2D, is likely to be multifactorial with both environmental and genetic factors contributing to the development of the disease.

One of the main factors associated to the T2D epidemic is the worldwide increase in obesity (American Diabetes Association, 2017). Sedentary lifestyle that is very common in modern day society is contributing to the increase in body mass index (BMI) of both children and adults. It is known that the increase in adipose tissue (that is related to obesity) is triggering both

metabolic and inflammatory changes. These changes interfere with insulin action in response to glucose loading in peripheral tissues, eventually possibly culminating in β-cell failure. Taken together, this might cause the manifestation of diabetes (Qi et al., 2009).

Although obesity is one of the main risk factors in diabetes development, not all overweight or obese individuals develop T2D. According to the most recent meta-analysis on T2D risk factors, in addition to adiposity, several other factors related to unhealthy lifestyle are associated to the development of T2D such as unhealthy dietary pattern, decreased physical activity, high sedentary time and duration of television watching (Bellou, Belbasis, Tzoulaki, & Evangelou, 2018). Furthermore, some medical conditions such as high systolic blood pressure, MetS and preterm birth but also serum biomarkers (including increased level of alanine aminotransferase, gamma-glutamyl transferase, uric acid and C-reactive protein, and decreased level of adiponectin and vitamin D) increase the risk of T2D. Of note, the authors found two main psychosocial factors (lower educational attainment and lower consciousness – a personality trait of being mindful and diligent) to be associated with the risk of T2D and furthermore, highly suggestive evidence for the associations between MDD and bipolar disorder and the risk for T2D were shown (Bellou et al., 2018).

Against this background, it is important to identify other risk factors of T2D and explore the associations of these variables with glycemic traits. Thus, the following chapters focus on other common T2D risk factors relevant for this thesis – depressive symptoms, seasonality including the amount of daylight and genetics. The last section of the introduction gives an overview of how these risk factors may be associated with each other and provides insight into the novelty of current study.

2.2.1 MENTAL HEALTH

In current modern society, people experience growing number of stressful events at the societal and interpersonal level, which might have harmful consequences on health and wellbeing. Moreover, a somatic illness itself can be considered as a life stressor and is often accompanied by high rates of mental health problems (Cassem, 1995).

In general terms, it is well established that psychosocial factors are associated with chronic illnesses (Schneiderman, Ironson, & Siegel, 2005) and the relationship appears to be bidirectional. Individuals who are experiencing high levels of psychological distress might not be able to take proper care of their health that is especially necessary in the context of chronic illnesses. At the same time, as described earlier, chronic illnesses can be considered as life stressors that contribute to the development of mental health conditions. Thus, the relationship between mental and somatic health can be reciprocal.

A recent overview and clinical practice guidelines on diabetes and mental health by the Diabetes Canada Clinical Practice Guidelines Expert Committee

(Robinson, Coons, Haensel, & Yale, 2018) has pointed out the following psychiatric conditions to be most commonly related to diabetes: schizophrenia and other psychotic disorders, anxiety disorders, sleep disorders, eating disorders and stress-related disorders, depressive disorder, bipolar and related disorders. And furthermore, individuals with diabetes often experience diabetes distress, psychological insulin resistance and the persistent fear of hypoglycemic episodes (Robinson et al., 2018).

On a biological level, Björntorp has hypothesised that mental health problems such as depression or unpleasant social conditions, behavioral characteristics (such as smoking, alcohol consumption and drug abuse) and other forms of pressures on the individual, act as stressors that can cause endocrine responses (Björntorp, 1991). Indeed, as described above, on a phenotypic level psychological problems such as depression, anxiety and eating disorders, appear to accompany T2D (Ducat, Philipson, & Anderson, 2014). However, the potential underlying mediators between mental health and somatic illnesses remain unclear.

Some possible mechanisms have been proposed to be underlying this relationship. These mechanisms include the Hypothalamic–Pituitary–Adrenal axis (HPA) activity and inflammation (Golden, 2007; Moulton, Pickup, & Ismail, 2015). Additionally, Talbot and Nouwen (2000) have proposed that psychological problems might be associated with T2D merely because of the daily burden of diabetes and its complications that have a negative effect on one's behavior (Talbot & Nouwen, 2000). Furthermore, other researchers also give support to the behavioral factors underlying the relationship between mental health and diabetes. It has been stated that poor health behaviors associated with psychological problems, such as poor diet, smoking and lack of exercise, that may lead to weight gain and obesity, major risk factors for diabetes (Renn, Feliciano, & Segal, 2011).

Sleep is significantly affecting our behavior and one of the problems in today's overscheduled society is associated with sleep. Both adults and children have increasingly more problems with attaining proper sleeping pattern. Problems with sleep are commonly seen among depressed individuals, almost 90% of patients with depression have difficulties with sleeping (Tsuno, Besset, & Ritchie, 2005). Also, associations between sleep problems and glucose metabolism have been shown before. Based on a meta-analysis, both the quantity and quality of sleep have been shown to consistently and significantly predict the risk of the development of T2D (Cappuccio, D'Elia, Strazzullo, & Miller, 2010). Spiegel (2008) has hypothesised that sleep loss and sleep disturbances could contribute to the development of insulin resistance and T2D either directly by having a deleterious effect on components of glucose regulation or indirectly, via behavioral factors such as dysregulation of appetite, leading to weight gain and obesity, a major risk factor for insulin resistance and T2D (Spiegel, 2008).

There have been some animal studies that explore the associations between mental health and glucose metabolism. On a molecular level, mice study shows

that mice lacking the insulin receptor in the brain are displaying increased depression- and anxiety-like behaviors. Furthermore, these findings suggest that mood disorders in diabetes are caused by insulin resistance in the central nervous system (CNS) and involve disruption of dopaminergic pathways (Kleinridders et al., 2015).

Based on the current knowledge, it is important to study T2D related indices in relation to mental health because, among other issues, individuals with T2D with comorbid psychological problems are at increased risk for complications, poorer quality of life, and increased mortality (de Groot, Anderson, Freedland, Clouse, & Lustman, 2001). Among other complications, patients with comorbid mental health conditions and T2D have the highest relative risk for developing dementia later in life (Katon et al., 2015).

In the next chapter, the association between depression, one of the most common psychological risk factors of diabetes, and T2D are discussed.

2.2.1.1 Depression and its subclinical symptoms

Depression is a condition that carries a high global prevalence with similarly rapid increase in disease incidence as T2D. Depression is estimated to affect 350 million people worldwide and it is characterized by persistent sadness and a loss of interest in activities that you normally enjoy, accompanied by an inability to carry out daily activities (WHO, 2012). Lifetime prevalence rates suggest that up to 30% of adults experience a depressive episode sometime during their lives and estimates of 12-month prevalence rates vary from 2.9 to 8.3% (Kessler & Wang, 2008; WHO, 2012; Wittchen & Jacobi, 2005). Finnish estimates are even higher and show that the prevalence of major depressive disorder (MDD) in Finland has significantly increased and reached 9.6% (Markkula et al., 2015). However, like diabetes, depression is common condition that is among the leading causes of disability adjusted life years: by 2030 it is estimated to rank second in the world and first in middle and high-income countries (Mathers & Loncar, 2006). Similarly to T2D, many individuals with depression have not been diagnosed with the condition. Moreover, subclinical depressive symptoms might be even more common than previous estimates show.

Like T2D, depression is also a heterogeneous disease that imposes a heavy burden on people with the condition. Those who suffer from depression commonly experience feelings of sadness and hopelessness and lose interest in activities they once enjoyed. According the the diagnostic criteria, these symptoms must be present for at least two weeks (American Psychiatric Association, 2013). However, as mentioned above, depression is a heterogeneous condition and it has been recently proposed that it can be characterized as a complex dynamic system explained through evolving symptom-symptom relations (Cramer et al., 2016). Thus, depression is unique for every individual and it has been shown that those people with strong

connections between the symptoms are in the highest risk of developing depression, especially if their vulnerable network is subject to external stress (Cramer et al., 2016). T2D could potentially be considered as an external stressor to the depressive symptoms network and influence the development of depression.

To continue, evidence strongly suggests that depression and T2D often co-occur more often that would occur only by chance. The bidirectional relationship between T2D and depression has been confirmed in several meta-analyses (Campayo et al., 2010; Demakakos, Pierce, & Hardy, 2010; Knol et al., 2006; Mezuk, Eaton, Albrecht, & Golden, 2008). Research literature has demonstrated that MDD is at least twice as prevalent among people with T2D as compared to the general population (Anderson, Freedland, Clouse, & Lustman, 2001). Furthermore, Knol and colleagues concluded in their meta–analysis that depression is a risk factor for the onset of T2D, comparable in effect to smoking and physical activity (Knol et al., 2006). This makes depression one of the leading risk factors contributing to the development of T2D. However, as the relationship between depression and T2D is reciprocal and bidirectional, it can also be considered as a common complication of diabetes, affecting 10–30% of people with T2D (Anderson et al., 2001). Furthermore, a meta-analysis including 27 studies showed that an increase in depressive symptoms is associated with an increase in the severity or number of diabetes complications (de Groot et al., 2001). Based on the above, it is important to study the associations between depression and T2D because if those conditions co-occur, the individuals who are experiencing both disorders are at a higher risk of developing diabetes-related complications.

On the level of symptomatology, depressive symptoms have been shown to influence glucose metabolism (Musselman, Betan, Larsen, & Phillips, 2003). Pyykkönen and colleagues have found that depressive symptoms are associated with insulin resistance based on several indices such as fasting insulin and HOMA-IR, with antidepressant medication is not modifying these associations (Pyykkonen et al., 2011). Another study has found a significant association between depression and higher levels of insulin resistance as measured with HOMA-IR (Everson-Rose et al., 2004). Additionally, it has been shown that individuals with depression develop glucose intolerance, also in high correlation with insulin secretion, suggestive of insulin resistance (Lustman & Clouse, 2005). Importantly, it has been shown that impaired insulin sensitivity might get resolved after recovery from depression based on a small prospective study of 20 non-diabetic patients with depression (Okamura et al., 2000). Behavioral factors might play a role in this potentially reversible process as individuals are able to adhere better to the treatment after recovery from mental health conditions (Dixon, Holoshitz, & Nossel, 2016).

However, the underlying mechanisms of the phenotypic association between depression and T2D are not extensively studied. One of the possible underlying mechanisms of this relationship could be the common genetic

background. It has to be noted that the rationale of the study comes from the fairly little research attention that the common genetic background of the of the bidirectional relationship between depression and diabetes has received (Renn et al., 2011). Only one population-representative Nordic twin study suggested a moderate genetic correlation between depression and diabetes (Kan et al., 2016). Thus, the common genetic background of depression and diabetes on a genome-wide and candidate gene level was one of the main avenues to explore in current research.

2.2.2 SEASONALITY AND CIRCADIAN RHYTHM

As established earlier, mental and somatic health conditions tend to co-occur. One of the likely underlying mechanisms that influence both, in addition to the potential common biological background or behavioral factors, is circadian rhythm. It is known that both mental health conditions and glucose metabolism show circadian pattern (Coimbra et al., 2016; Doró, Benko, Matuz, & Soós, 2006; Postolache et al., 2010; Shore-Lorenti et al., 2014). Even more interestingly, there is a seasonal pattern of people searching for information on (mental) health that appears to be similar to the periodic pattern of seasonal affective disorder (Ayers, Althouse, Allem, Rosenquist, & Ford, 2013).

In general, circadian rhythms are universal and affect almost every biological action, including both mental and somatic processes. A wealth of evidence shows that numerous human physiologic and pathophysiologic processes also vary seasonally, including glucose metabolism which is of most interest in the context of current research. It is known that typically better metabolic profile is displayed during summer compared to winter (Mavri et al., 2001). For example, more individuals meet the criteria of MetS in winter than in summer, which has also been linked to insulin resistance and increased blood pressure (Kamezaki et al., 2013).

Furthermore, on a molecular level, a functional circadian clock is necessary for proper insulin secretion by pancreatic islet cells. Thus, the rhythmic behavior can be seen in pancreatic islet cells (Rakshit, Qian, Colwell, & Matveyenko, 2015). Circadian regulation of glucose homeostasis is controlled by the suprachiasmatic nucleus (SCN) of the hypothalamus, that is an essential component of the master biological clock (Asher & Schibler, 2011; Marcheva et al., 2010). Taken together, among other functions, the circadian mechanisms have been found to regulate blood glucose levels, glucose sensing, insulin gene expression and insulin secretion (Rudic et al., 2004).

Aside assisting in the regulation of glucose metabolism, it is also established that disturbances in circadian rhythms are therefore associated with increased risk of T2D (Depner, Stothard, Wright, & Jr., 2014). For example, chronic misalignment between internal and environmental rhythms is typically found in night-shift workers who are exposed to increased light at

night (Davis, Mirick, Chen, & Stanczyk, 2012; Dumont, Benhaberou-Brun, & Paquet, 2001; Vetter et al., 2018). Furthermore, based on extensive epidemiologic data, these night-shift workers are at considerably higher risk of developing T2D (Morikawa et al., 2005; Pan, Schernhammer, Sun, & Hu, 2011). Of note, night-shift workers on the other hand are not at higher risk of developing MDD according to a systematic review even though some of the individual studies give support to the increased risk (Angerer, Schmook, Elfantel, & Li, 2017).

The next chapter discusses the effect of daylight in relation to circadian disturbances and glucose metabolism.

2.2.2.1 The amount of daylight

Circadian oscillations in the human body are characteristic of nearly every hormone. It is important to note that these hormonal profiles are a product of interaction between many factors, including the light exposure. According to bioclimatic hypothesis, changes in sunlight and climate affect variations in hormones and may explain the seasonal variation in disease incidences (Lambert, Reid, Kaye, Jennings, & Esler, 2003). It has been hypothesized that seasonal variation in glucose metabolism might be driven by the changes in daylight, with increased exposure linked to reduced risk of developing T2D (Shore-Lorenti et al., 2014).

Importantly, light is the strongest environmental cue for all circadian systems (Duffy & Czeisler, 2009). External environmental timing that comes mainly from the light signals the regulation of the internal clocks. It is known that internal timing systems influence all physiological processes and energy homeostasis to maximise adaptation and fitness (Green, Takahashi, & Bass, 2008).

Taken together, the amount of daylight hours appears to affect our energy levels. It has been shown that during summer, when more daylight is available, typically better glycemic profile and lower body mass index (BMI) is displayed (Ishii, Suzuki, Baba, Nakamura, & Watanabe, 2001; Mavri et al., 2001). However, the relationship between daylight and glucose metabolism is more complex. For example, previous research findings regarding the relationship between the amount of daylight and insulin secretion and sensitivity have been scarce and inconsistent (Berglund et al., 2012; Chen, Chuang, Lin, Tsai, & Chou, 2008).

However, as it has been at least partly validated that the amount of environmental light is associated with glucose metabolism, previous research has raised the question of the pathways through which light exposure could act to influence T2D and its related outcomes. A potential pathway through which daylight exposure may influence T2D related outcomes, is circadian rhythm and its misalignment, as briefly mentioned in the previous chapter. The master clock in the brain's suprachiasmatic nucleus synchronises the

tissue specific intrinsic clocks to the photoperiod (the time between sunrise and sunset) in response to signals from retinal photoreceptors communicating the presence of daylight (Patton & Hastings, 2018). It synchronizes a wide range of complex biological processes via neurological pathways, through the central nervous system and into peripheral tissues (Morris, Yang, & Scheer, 2012).

Disturbances in hormonal profiles might arise from major changes in daylight potentially contributing to circadian misalignment. Very long and very short days might challenge the network within the circadian pacemaker as it is known that the principal circadian clock entrains to the sun light (Roenneberg, Kumar, & Merrow, 2007). Entrainment errors during the period of rapid daylight changes might challenge the circadian pacemaker and predispose to circadian misalignment especially during equinoxes when dark light transitions are most rapid.

From that perspective, the association between daylight and glucose metabolism has received some research interest. A Swedish study shows that fasting glucose concentrations are higher in participants examined during the dark season (Nov-April) compared with the light season (Renström et al., 2015). Furthermore, there is also evidence that higher level of recreational sunshine exposure (sunbathing) reduces the odds of T2D incidence, yet the findings regarding glycemic traits are not consistent (Shore-Lorenti et al., 2014).

The other potential pathways through which daylight could act to influence glucose metabolism are related to vitamin D, photo immunomodulation, thermogenesis but also cellular stress (Lindqvist, Olsson, & Landin-Olsson, 2010; Shore-Lorenti et al., 2014). It has been proposed that vitamin D might underlie relationship between daylight and glucose metabolism, as a large part of vitamin D is endogenously produced when solar rays trigger its synthesis (Holick, 2007). Low serum 25-hydroxyvitamin D (25-OHD) is the index of vitamin D nutritional status and its low levels have been associated with increased risk of several cardiometabolic outcomes including T2D and MetS (Osei, 2010). Parker and colleagues showed in their meta-analysis of 28 independent studies a 43% of decrease in cardiometabolic disorders for those of highest levels of 25-OHD (Parker et al., 2010), however experimental vitamin D supplementation studies have been inconclusive. For example, it has been shown that vitamin D supplementation does not have an effect on glucose concentrations, insulin level and HOMA-IR values based on a systematic review of randomized controlled trials in overweight and obese populations (Jamka et al., 2015; Pittas et al., 2010). Taken together, vitamin D supplementation might not be effective treatment for improvement of glucose metabolism, thus other avenues shall be explored to unravel the potential underlying mechanisms between daylight and glucose metabolism.

2.2.2.2 Melatonin

At the epicentre of circadian rhythms is melatonin, thus the association between daylight and glucose metabolism might be affected by melatonin pathway. A recent review of melatonin action emphasized that the effect of melatonin needs to receive even further research interest to reduce the existing uncertainties in relation to various health conditions given the technological and lifestyle changes that are associated with chronodisruption (such as the overwhelming use of LED lights at night) (Posadzki et al., 2018).

Melatonin is a neurohormone that is mainly associated with sleep, yet involved with a broad spectrum of functions in the human body including anti-inflammatory and immune-enhancing effects (Hardeland, Pandi-Perumal, & Cardinali, 2006). Mainly via these effects on health, melatonin is related to the development of several conditions ranging from acute coronary syndrome, metabolic diseases to various cancers (Posadzki et al., 2018). Melatonin is also involved in the development of depression (Hickie & Rogers, 2011) and diabetes (McMullan, Schernhammer, Rimm, Hu, & Forman, 2013), relevant in the context of this current thesis.

Melatonin production is under the control of the biological clock and, importantly, is directly responsive to daylight (Bonmati-Carrion et al., 2014). It is secreted from the pineal gland and produced from serotonin, which is derived from the amino acid tryptophan (Claustrat, Brun, & Chazot, 2005). Melatonin is a neurohormone that has a very short half-life. Once it is secreted, it is immediately released into the general circulation, thus plasma concentrations of melatonin are accurate and reflect its pineal synthesis (Pevet & Challet, 2011). At the same time, these measurements are time-critical due to the short half-life of melatonin in the bloodstream. Melatonin release is triggered by a loss of light exposure to the retina and its levels are high in the evening and low in the morning, thus melatonin is often referred to as a sleep hormone and also most often studied in relation to sleep disorders (Posadzki et al., 2018). However, melatonin acts in concert with other neurotransmitters, glucocorticoids and thyroid hormones.

As discussed above, melatonin is directly responsive to environmental light and exerts its effect through its receptors. In humans, there are two subtypes of melatonin receptors, MTNR1A and MTNR1B. Both of these receptors are G protein-coupled receptors and are expressed in various tissues of the body including the brain and pancreatic islets (Tosini, Owino, Guillaume, & Jockers, 2014). The presence of melatonin receptors MTNR1B and MTNR1A in various tissues explains the direct action of melatonin in many organs such as the brain, suprachiasmatic nucleus (SCN), retina and pancreatic α- and β-cells (Hardeland, 2012).

Melatonin is a hormone that has several functions in the human body as described above. Thus far, both the production and administration and supplementation (ranging from oral and intravenous to sublingual) of melatonin has been studies previously in the context of glucose metabolism.

First of all, it is known that insulin secretion follows a circadian pattern opposite to melatonin secretion with a nocturnal fall (Haus, 2007). Furthermore, it has been proposed that the phase of the insulin secretion rhythm might be modulated by melatonin (Mulder, Nagorny, Lyssenko, & Groop, 2009) and recent findings show that melatonin physiologically serves as an inhibitor of nocturnal insulin release (Tuomi et al., 2016). However, the relationship between melatonin and glucose metabolism is not that clear although endocrine rhythms and circadian rhythms are closely interconnected. Both inhibitory and stimulatory effects of melatonin on insulin secretion and action have been reported (Kemp, Ubeda, & Habener, 2002; Peschke, Bach, & Muhlbauer, 2006; Tuomi et al., 2016). However, a recent meta-analysis of the effects of melatonin supplementation on glucose metabolism concluded that melatonin administration is related to improved glucose metabolism but not with insulin levels and insulin sensitivity (Doosti-Irani et al., 2018). Furthermore, the findings of humans and of preclinical studies are conflicting – animal models show that melatonin administration improves lipid metabolism in diabetic rats, probably through restored insulin resistance (Nishida, Segawa, Murai, & Nakagawa, 2002).

Taken together, previous research literature points towards the potential influence of melatonin pathway in glucose metabolism although the findings are scarce and inconsistent.

2.2.3 GENETICS OF TYPE 2 DIABETES

In addition to previously discussed environmental and mental health associations (and potential molecular mechanisms) with glucose metabolism, it is widely known that T2D and glucose metabolism also has genetic component. T2D is polygenic disease which means that the development of this condition is influenced by several genes.

Based on family studies, it has been estimated that the lifetime risk of developing T2D is 40% for individuals who have one parent with T2D and 70% if both parents have T2D (Creutzfeldt, Köbberling, & Neel, 1976). Based on twin studies, the heritability estimates of T2D are around 76% after the 15-year follow-up; with rather astonishing concordance rates of 96% in abnormal glucose metabolism after the 15-year follow-up (Medici, Hawa, Ianari, Pyke, & Leslie, 1999). On the other hand, SNP-based heritability estimates are much lower (around 10%) because these estimates rely on common genetic variation (Bulik-Sullivan et al., 2015) as GWA studies are not capturing rare variants that might account for a sizable proportion of heritability of this complex disease and its development. Furthermore, mutations have higher effect compared to common variants which penetrance is low or indeed zero.

Nevertheless, hundreds of variants have been identified using several methods in relation to T2D and while each variant explains only a very small proportion of the disease risk, these variants have significantly contributed to

the understanding of the genesis of T2D (Prasad & Groop, 2015). Most coherently associated genetic variants are briefly described below.

TCF7L2 is one of the most successfully replicated variant that has been associated with T2D. This variant was first found to be associated with T2D in linkage studies. The association between T2D and *TCF7L2* has been confirmed across various populations, conferring a relative risk of approximately 1.4 (Tong et al., 2009). Other genetic variants (single nucleotide polymorphisms - SNPs) in or near *PPARγ, KCNJ11, TCF2, TCF7L2, CDKAL1, CDKN2A-CDKN2B, IDE-KIF11-HHEX, IGF2BP2, MTNR1B, SLC30A8, KCNQ1, CDC123, GLIS3, HNF1B, DUSP9* have been identified in Caucasian populations and also replicated elsewhere (Cho et al., 2012; Imamura et al., 2012; Shu et al., 2010).

In current thesis, the most important findings arise from the collaborative effort of DIAGRAM and MAGIC consortia that are described below as these summary statistics were used in the analyses of the Study I. DIAGRAM investigators were exploring the associations between common variants on a genome-wide level and T2D diagnosis (Morris et al., 2012). They had data on 34,840 cases and 114,981 controls combining Stage 1 and 2. Based on this extensive data, these researchers found eight new loci associated with T2D. These signals were from *ZMIZ1, ANK1, KLHDC5, HMG20A, GRB14* genes. Furthermore, rs7177055 and rs13389219 were not previously reported in European ancestry populations. The authors discuss that several of these signals were mapped to loci that had been previously implicated in T2D-related metabolic traits (Morris et al., 2012).

In the analysis of glycemic traits, GWAS from MAGIC investigators has resulted in identification several new loci using the data of 46,186 individuals without diabetes and following up among 76,558 additional subjects. Following up the 25 initially identified loci resulted in 16 loci associated with glycemic traits. These included nine loci newly associated with fasting glucose (*ADCY5, MADD, ADRA2A, CRY2, FADS1, GLIS3, SLC2A2, PROX1 and C2CD4B*), one locus influencing fasting insulin and HOMA-IR (*IGF1*), and five loci with T2D (*ADCY5, PROX1, GCK, GCKR, DGKB-TMEM195)* (Dupuis et al., 2010).

These findings are not any more the most recent to date. Scott and colleagues have since published an expanded GWAS of T2D as a follow-up to the initial research effort involving individuals with European ancestry (Scott et al., 2017). They analysed the samples of 26,676 cases of T2D and 132,532 controls and found several novel loci associated to T2D. These variants were near the *GLP2R, GIP,* and *HLA-DQA1* genes; all together they found 128 independent variants to be associated with T2D belonging to 113 loci. They state that they causality and biological plausibility need to receive further research attention (Scott et al., 2017).

2.2.3.1 Melatonin Receptor 1B gene

MTNR1B is a member of the melatonin receptor family expressed in many tissues, including pancreatic islets and brain (Hardeland, 2012) that is encoded by the *MTNR1B* gene (Reppert et al., 1995). *MTNR1B* is one of the genes which variants – specifically rs10830963 - have been most consistently shown to be associated with T2D and glucose metabolism. *MTNR1B* is often described in the research literature as a diabetes risk gene. Molecular studies have shown increased expression of the MTNR1B receptors in β-cells in pancreatic islets of nondiabetic and diabetic individuals carrying the *MTNR1B* risk variant (Lyssenko et al., 2009).

Furthermore, genome-wide association studies have shown that a common variant of the *MTNR1B* gene is associated with altered insulin and glucose concentrations and risk of future T2D (Prokopenko et al., 2009). Lyssenko and colleagues showed that the risk genotype of *MTNR1B* SNP rs10830963 predicts future T2D in two large prospective studies (Lyssenko et al., 2009). It is important to point out that rs10830963 in the *MTNR1B* gene has been proved to be the causal variant in functional studies (Gaulton et al., 2015).

The *MTNR1B* gene has been studied in relation to lifestyle intervention in the context of gestational diabetes (Grotenfelt et al., 2016). Findings from the study by Grotenfelt and colleagues (2016) show that genetic variants might influence the effectiveness of lifestyle interventions although they were not able to show that diabetes risk allele G carriers would have poorer response to the intervention (Grotenfelt et al., 2016).

2.2.4 THE LINKS BETWEEN DIABETES RISK FACTORS

This literature review has given an overview of the individual contribution of depressive symptoms, circadian rhythm that is affected by the amount of daylight and genetics in the development of T2D and impaired glucose metabolism. However, these factors are all closely linked. Yet, the interactions between these factors have received very little research attention. Against this background, the current study focuses on genetic associations, filling in the gap in the research literature as the genetic moderation of the relationship between 1) depressive symptoms or 2) the amount of daylight and glucose metabolism has not not received enough scientific attention.

Pleiotropic genetic variants that are shared between the traits could unravel potential novel pathways through which these conditions are associated. Due to the limited information on these shared genetic risks, this current study was conducted, allowing the researchers to fill in the gap in the research literature. Indeed, previous research has suggested that there is abundant pleiotropy among genetic variants related to complex traits (Sivakumaran et al., 2011). Sivakumaran and colleagues showed that 16.9% of

the genes have pleiotropic effects on complex diseases (Sivakumaran et al., 2011).

There have been numerous studies exploring the genetic background of complex diseases but these have been conducted on univariate level – involving one single disease. However, these studies using both genome-wide approach or candidate gene analyses in relation to metabolic traits and mental health have found variants related to both mental and somatic health. Recent meta-analysis exploring the associations between MDD, bipolar disorder, coronary artery diseases, T2D and hypertension gave an overview of these recent findings and they identified 24 potentially pleiotropic variants shared between the traits of interest (Amare, Schubert, Klingler-Hoffmann, Cohen-Woods, & Baune, 2017). These variants included SNPs in or near *MTHFR, CACNA1D, CACNB2, GNAS, ADRB1, NCAN, REST, FTO, POMC, BDNF, CREB, ITIH4, LEP, GSK3B, SLC18A1, TLR4, PPP1R1B, APOE, CRY2, HTR1A, ADRA2A, TCF7L2, MTNR1B, IGF1*. The authors also completed pathway analysis that revealed several biologically plausible and significant pathways supporting these associations and concluded the following: "Overall, genes that encode for molecules involved in HPA-axis activity, circadian rhythm, inflammation, neurotransmission, metabolism and energy balance were found to have a central role to link mood disorders with cardiometabolic diseases. It is also worth noting the gene-environment interaction that might contribute to the diseases" (Amare et al., 2017). This study by Amare and colleagues has been the most recent to provide insights into the shared genetic mechanisms of mental health and cardiometabolic traits.

In addition to the view where depression and T2D are two distinct phenomena that might share a common genetic background, gene-environment interaction should be explained further, especially since the concept of genetic moderation of associations was mentioned above. Gene-envionment interaction is commonly described as a phenomenon where different genotypes are responding differently to environmental variation. In the case of T2D, environment is most commonly referred to as lifestyle factors (Franks, Pearson, Bchir, & Florez, 2013). It is known that individuals respond differently to lifestyle interventions, the most common strategy to prevent and treat T2D, and it has been proposed that it might be due to gene-environment interaction (Franks & Merino, 2018). However, environmental variation can be for example external (such as the amount of daylight), internal (such as the psychological state), behavioral (lifestyle) or other. Thus, the concept of gene-environment interaction is important in the context of this research because the aim is to explore the associations between different T2D risk factors including both genetic variation and environmental factors. Consequently, genetic moderation of both external (the amount of daylight) and internal (depressive symptoms) environmental exposures is explored.

This current research provides further insights into the relationship between depression and T2D and contributes significantly to the scientific literature. The pleiotropy on a genome-wide level between depressive

symptoms and glycemic traits is explored and moreover, the associations are studied on a candidate gene level. Furthermore, environmental factors such as the amount of daylight are taken into account.

3 AIMS OF THE STUDY

Previous studies have identified several risk factors contributing to the genesis of diabetes. In addition to genetic susceptibility, mental health and environmental factors also have an effect in the disease development. Thus, this thesis focuses on four objectives:

1. The first objective of this doctoral thesis is to examine whether there is a common genetic basis of the phenotypic associations between T2D, glycemic indices related to T2D, and depressive symptoms (**Study I**)

2. The second objective is to examine which specific genetic variants are potentially pleiotropic, affecting both glycemic indices and depressive symptoms (**Study I**)

3. The third aim is to study if a common diabetes risk variant rs10830963 in the Melatonin Receptor 1B (*MTNR1B*) gene influences the relationship between depressive symptoms and glycemic indices (**Study II**)

4. The fourth aim of this doctoral thesis is to investigate to what extent the amount of daylight moderates the associations between *MTNR1B* rs10830963 and glycemic indices (**Study III**)

4 MATERIALS AND METHODS

4.1 PARTICIPANTS: STUDY I-III

Study I is based on the summary statistics data from previously published GWA studies, described in detail below. Studies II and III are conducted within the same PPP-Botnia cohort, described in detail below.

4.1.1 STUDY I: SUMMARY STATISTICS OF GWAS FOR T2D, GLYCEMIC INDICES AND DEPRESSIVE SYMPTOMS

The analyses of Study I are based on previously published univariate GWAS summary statistics which did not require generating any new cohort-level results but re-uses consortium-level summary statistic data.

Data on T2D have been contributed by DIAGRAM investigators, and include 34840 cases with T2D and 114981 controls without T2D (Morris et al., 2012). Summary statistics were publicly available and obtained from the www.diagram-consortium.org website (accessed 08.10.2015).

Data on glycaemic traits have been contributed by MAGIC investigators, and include up to 46186 participants without diabetes from up to 21 cohorts (Dupuis et al., 2010). Summary statistics were publicly available and obtained from the www.magicinvestigators.org website (accessed 26.11.2015).

Data on depressive symptoms have been contributed by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) depression working group investigators and include 51258 individuals (Hek et al., 2013). The data and the approval to pursue bivariate studies using the summary statistics on depressive symptoms was obtained from the CHARGE depression working group in March 2014.

4.1.2 STUDY II-III: PPP-BOTNIA STUDY

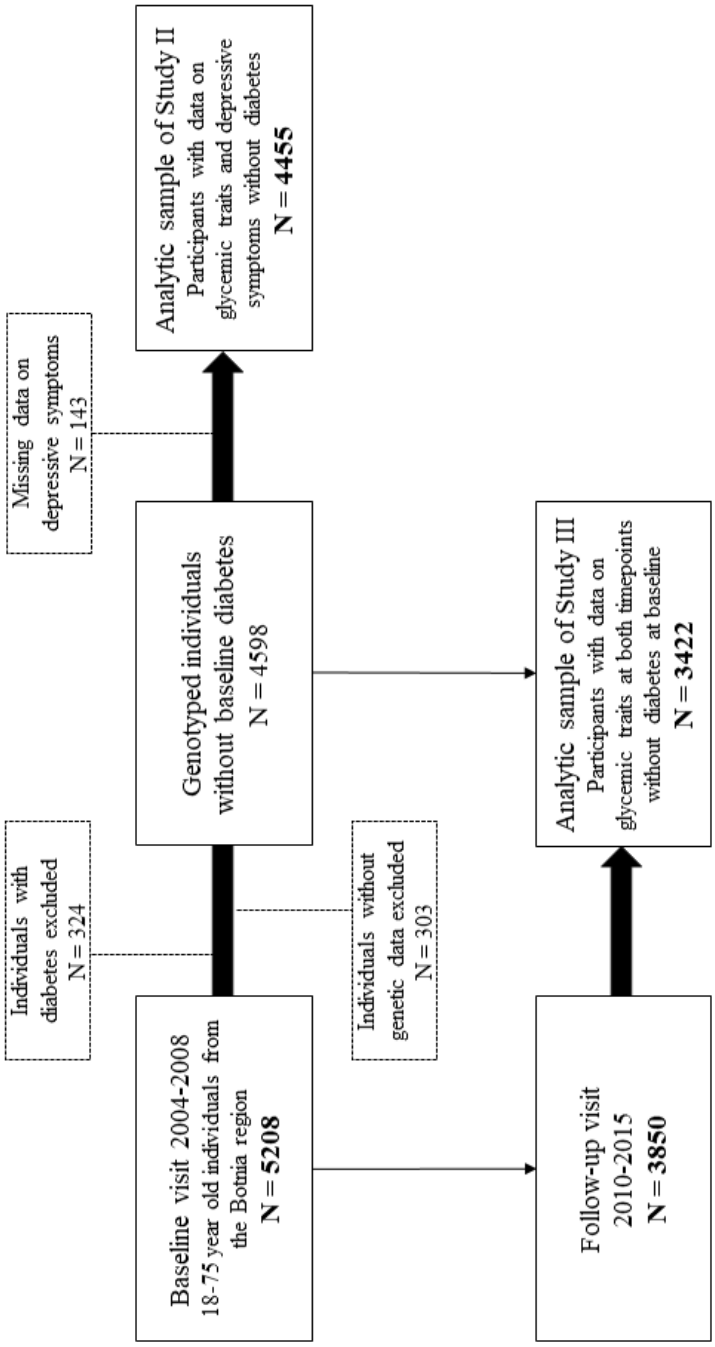
The prospective PPP-Botnia Study is a population-based study in the Botnia region of western Finland. The study is designed to obtain accurate estimates of prevalence and risk factors for diabetes, impaired glucose regulation, and the MetS in the population aged 18 – 75 years and to use this information for prediction and prevention of diabetes. The study is described in further detail elsewhere (Di Camillo et al., 2018) and has the general aims to identify early disturbances in persons with risk of suffering from T2D; to identify gene defects which cause these interferences and which increase the risk of T2D; to investigate which cause these gene defects has for the development of the disease and the outlet of the disease; to try to prevent the development of T2D.

In short, the study is comprised of 5208 individuals participating in the baseline study in 2004-2008. Of those, the analytic sample of Study II comprised of 4455 individuals who were genotyped for rs10830963, did not have T2D and had data on glycemic traits and depressive symptoms. In comparison with the excluded sample (753 individuals), those in the analytic sample were more often women, younger, had lower BMI, more often had higher education, less often smoked, more often used alcohol and reported lower depressive symptoms. They also had lower glucose, insulin, HOMA-IR, HOMA2-IR and higher CIR values (Study II, Table 1). However, the groups did not differ in 30-min insulin, ISI and DI, physical activity, rs10830963 genotype frequencies and in season of the testing date (Study II, Table 1).

The follow-up visits took place in 2010-2015 and 3850 individuals (74% of the baseline study participants) participated in the follow-up. Of those, individuals who did not have T2D at baseline and participated in the follow-up, had genetic data and data on glycemic traits available (3422 individuals) formed the analytic sample of Study III. This analytic sample did not differ from the whole baseline cohort, except that they had lower 120 min and AUC for glucose, smoked less often and used more alcohol (Study III, Table 1).

The flowchart of analytic samples of Study II and Study III is described in Figure 1.

Figure 1. PPP-Botnia flowchart



4.2 MEASURING AND EVALUATING GLYCEMIC TRAITS AND T2D: STUDY I-III

4.2.1 STUDY I: SUMMARY STATISTICS OF GWAS FOR T2D, GLYCEMIC INDICES AND DEPRESSIVE SYMPTOMS

T2D diagnosis in DIAGRAM GWAS was based on several different criteria including self-reported T2D, physician's diagnosis, registry data, and OGTT applying WHO criteria (WHO, 1999).

The quantitative glycemic traits in MAGIC included fasting insulin, fasting glucose, HOMA-β [(20 × fasting plasma insulin)/(fasting plasma glucose – 3.5)] for assessment of β-cell function, and HOMA-IR (fasting plasma insulin × fasting plasma glucose/22.5) for estimation of the degree of insulin resistance (Wallace, Levy, & Matthews, 2004).

4.2.2 STUDY II-III: PPP-BOTNIA STUDY

PPP-Botnia Study participants underwent a 2-hour OGTT performed in the morning after an overnight fasting. The subjects ingested 75 grams of glucose and one of the participants had worked during the two nights preceding the OGTT.

During the OGTT, venous-samples for glucose and insulin were drawn at fasting and additionally at 30 and 120 min after the glucose load. Plasma glucose was measured with a glucose dehydrogenase method (HemoCue, Ängelholm, Sweden) and serum insulin by a fluoroimmunoassay (Delphia; Perkin-Elmer Finland, Turku, Finland).

The formulas of all indices calculated based on the OGTT used in current thesis are listed in Table 1. We also used online calculator for HOMA2-IR, which is available at https://www.dtu.ox.ac.uk/homacalculator/.

Table 1. Glycemic traits calculated based on the OGTT

$$\text{AUC glucose} = 15 \times \text{fasting glucose}\left[\frac{mmol}{l}\right] + 15 \times 30\text{ min glucose}\left[\frac{mmol}{l}\right] + 45 \times 30\text{ min glucose}\left[\frac{mmol}{l}\right] + 45 \times 120\text{ min glucose}\left[\frac{mmol}{l}\right]$$

$$\text{AUC insulin} = 15 \times \text{fasting insulin}\left[\frac{\text{mU}}{\text{l}}\right] + 15 \times 30\text{ min insulin}\left[\frac{\text{mU}}{\text{l}}\right] + 45 \times 30\text{ min insulin}\left[\frac{\text{mU}}{\text{l}}\right] + 45 \times 120\text{ min insulin}\left[\frac{\text{mU}}{\text{l}}\right]$$

$$\text{HOMA} - \text{IR} = \frac{\text{Fasting insulin}\left[\frac{\text{mU}}{\text{l}}\right] \times \text{fasting glucose}\left[\frac{\text{mmol}}{\text{l}}\right]}{22.5}$$

$$\text{ISI} = \frac{10000}{\sqrt{\begin{matrix}(\text{fasting glucose}\left[\frac{\text{mmol}}{\text{l}}\right] \times \text{fasting insulin}\left[\frac{\text{mU}}{\text{l}}\right] \times \\ \text{mean OGTT glucose}\left[\frac{\text{mmol}}{\text{l}}\right] \times \text{mean OGTT insulin}\left[\frac{\text{mU}}{\text{l}}\right])\end{matrix}}}$$

$$\text{CIR} = \frac{(100 \times 30\text{ min insulin}\left[\frac{mU}{l}\right])}{(30\text{ min glucose}\left[\frac{mmol}{l}\right]) \times (30\text{ min glucose}\left[\frac{mmol}{l}\right] - 3.89\left[\frac{mmol}{l}\right])}$$

$$\text{DI} = \text{CIR} \times \text{ISI}$$

4.3 MEASURING DEPRESSIVE SYMPTOMS: STUDY I-II

Self reported questionnaires on depressive symptoms including Center for Epidemiological Studies Depression Scale (CES-D) and Mental Health Inventory (MHI-5) derived from the 36-item Short-Form Health Survey (SF-36) were used in evaluating depressive symptoms across the studies. Used questionnaires are described in further detail below.

4.3.1 STUDY I: SUMMARY STATISTICS OF GWAS FOR T2D, GLYCEMIC INDICES AND DEPRESSIVE SYMPTOMS

Depressive symptoms in CHARGE GWAS were estimated from the total sum score of the Center for Epidemiological Studies Depression Scale (CES-D) 10, 11 or 20-item versions.

The CES-D is a brief self-report scale designed to measure depressive symptoms experienced in the past week in a general population (Radloff,

1977). The CES-D questionnaire has been established as a reliable and valid measure of depressive symptoms both in the context of various conditions such as cancer, but also in noninstitutionalized (general) populations (Cosco, Prina, Stubbs, & Wu, 2017; Hann, Winter, & Jacobsen, 1999). The questionnaire is divided into scales reflecting major facets of depression including somatic, positive and negative domains. The continuous sum score of the CES-D was used in the study.

4.3.2 STUDY II: PPP-BOTNIA STUDY

Depressive symptoms were reported by the five-item Mental Health Inventory (MHI-5) derived from the 36-item Short-Form Health Survey (SF-36)(Hays, Sherbourne, & Mazel, 1993).

The five-item MHI-5 questionnaire includes following questions: feeling nervous, feeling down in the dumps, feeling downhearted and blue, feeling calm and peaceful (reverse scored) and feeling happy (reverse scored). The questions are rated on a six-point scale ranging from none of the time (1) to all the time (6) during the past 4 weeks.

A sum score of these items is transformed into a scale that ranges from 0 to 100 (Ware & Sherbourne, 1992). A higher value of the sum score of these items reflects higher depressive symptoms.

4.4 GENOTYPING: STUDY I-III

Genotyping of the the cohorts that participated in the genome-wide meta-analyses of T2D, glycemic indices, and depressive symptoms has been described in the original articles (Dupuis et al., 2010; Hek et al., 2013; Morris et al., 2012).

In PPP-Botnia, genotyping of *MTNR1B* rs10830963 was performed either by mass spectrometry or by allelic discrimination method, as described in detail elsewhere (Jonsson et al., 2013).

4.5 THE AMOUNT OF DAYLIGHT: STUDY III

The amount of daylight varies considerably with the season in Finland. The range of day length in western Finland is from 4h 44min to 20h 17min.

Daylight information was provided by the Finnish Meteorological Institute (FMI) Climate Service for Seinäjoki Pelmaa station. Seinäjoki station is the closest meteorological station to the PPP-Botnia study centers in Närpiö, Maalahti, Mustasaari, Vaasa and Pietarsaari. The average distance between

Seinäjoki Pelmaa and the study centers is about 71 km (53 km to Vaasa and 97 km to Pietarsaari.

Daylight (day length in hours) at the testing date from the Seinäjoki station was linked to the baseline and follow-up study dates.

4.6 STATISTICAL ANALYSIS: STUDY I-III

4.6.1 STUDY I

To estimate genetic correlation of depressive symptoms with T2D, fasting glucose, fasting insulin, HOMA-β and HOMA-IR, results from univariate GWAS meta-analyses were combined using LD (linkage disequilibrium) Score Regression tool LDSC (B. K. Bulik-Sullivan et al., 2015). Default options of this command line tool were used for estimating heritability and genetic correlation from GWAS summary statistics. The tool relies on the fact that the GWAS effect size estimate for a given SNP incorporates the effects of all SNPs in LD with that SNP. LDSC is not biased by sample overlap and has been described in detail elsewhere (Bulik-Sullivan et al., 2015).

To identify potential pleiotropic SNPs associated with both depressive symptoms and T2D, fasting glucose, fasting insulin, HOMA-β or HOMA-IR, five independent bivariate GWAS analyses were performed using empirical-weighted linear-combined test statistics (eLC) (Chen & Hsu, 2017) with aggregate data (Z test statistics) from each univariate GWAS meta-analysis. The bivariate analyses were performed only of those SNPs with nominal p-values < 0.05 in univariate GWAS meta-analyses as suggested before (Ligthart et al., 2016). Potential pleiotropic SNPs are reported based on 1) p-value < $5x10^{-8}$ from the bivariate GWAS analyses and 2) the bivariate p-value is at least one order of magnitude smaller than the univariate p-values.

The influence of identified potential pleiotropic SNPs on gene expression were further evaluated in the Brain eQTL Almanac (BRAINEAC, www.braineac.org) database, and GTEx portal V6 (dbGaP Accession phs000424.v6.p1, www.gtexportal.org).

4.6.2 STUDY II-III

IBM SPSS version 23.0 was used for data analysis in Studies II-III (www.ibm.com/spss/). Primary findings are based on multiple linear regression analysis in both studies.

In Study II, multiple linear regression analyses were used to test if the *MNTR1B* rs10830963 genotype influenced the associations between depressive symptoms and glycemic traits by including main effects of rs10830963 and depressive symptoms and their interaction into the

regression equation with indices of glycemic traits as the outcomes. Before proceeding to the interaction tests, we used multiple linear regression analyses to also test if the rs10830963 and depressive symptoms were associated with the glycemic traits and if these effects were independent of each other. In these analyses we used depressive symptoms both as continuous and dichotomized at the clinical cutoff. All associations were tested in the presence of covariates including sex, age, body mass index, education, current smoking status, alcohol consumption, physical activity and season of testing. Skewed variables were log-transformed where appropriate and variables were standardized to the mean of 0 and SD of 1 to facilitate interpretation. To decrease the likelihood of type 1 error, we used the false discovery rate (FDR) procedure to account for multiple testing. The FDR procedure is a method for conceptualizing the rate of type 1 errors in null hypothesis testing when conducting multiple comparisons and is formally described elsewhere (Benjamini & Hochberg, 1995).

In Study III, multiple linear regressions were used to test if rs10830963 and daylight were associated with glycemic traits cross-sectionally both at baseline and at follow-up. To test if daylight moderated the association of rs10830963 and glycemic traits, an interaction term of daylight x rs10830963 was added into the linear regression model following the main effects of these variables. In change analysis, multiple linear regression analyses were used to study if rs10830963, change in the amount of daylight (amount of daylight at the date of baseline testing was subtracted from the date of follow-up testing), or their interaction, were associated with change in glycemic traits between baseline and follow-up (baseline glycemic trait value was subtracted from the respective follow-up value). The analyses of change were adjusted for the baseline value of the outcome variable. The covariates included sex, age, body mass index, smoking, alcohol use, physical activity and educational attainment. Skewed variables were log-transformed where appropriate and daylight, daylight change, glycemic traits, and glycemic trait change variables were standardized to the mean of 0 and SD of 1 to facilitate interpretation.

5 RESULTS

5.1 STUDY I: DEPRESSIVE SYMPTOMS AND GLYCEMIC TRAITS - BIVARIATE GWAS

5.1.1 GENETIC CORRELATION

SNP-based heritability estimates based on the LDSC analysis, were all very low between 0.04 and 0.10. More specifically, the estimates were 0.09 [0.07, 0.12] for T2D, 0.10 [0.06, 0.15] for fasting glucose, 0.07 [0.05, 0.10] for fasting insulin, 0.07 [0.05, 0.09] for HOMA-β, 0.05 [0.03, 0.07] for HOMA-IR, and 0.04 [0.01, 0.07] for depressive symptoms.

Considering the low heritability estimates, we also did not find any significant SNP-based genetic correlations between depressive symptoms and T2D or quantitative glycemic traits. SNP-based genetic correlations between depressive symptoms, T2D or glycemic traits were not significant (p-values > 0.37).

5.1.2 PLEIOTROPIC SNPS

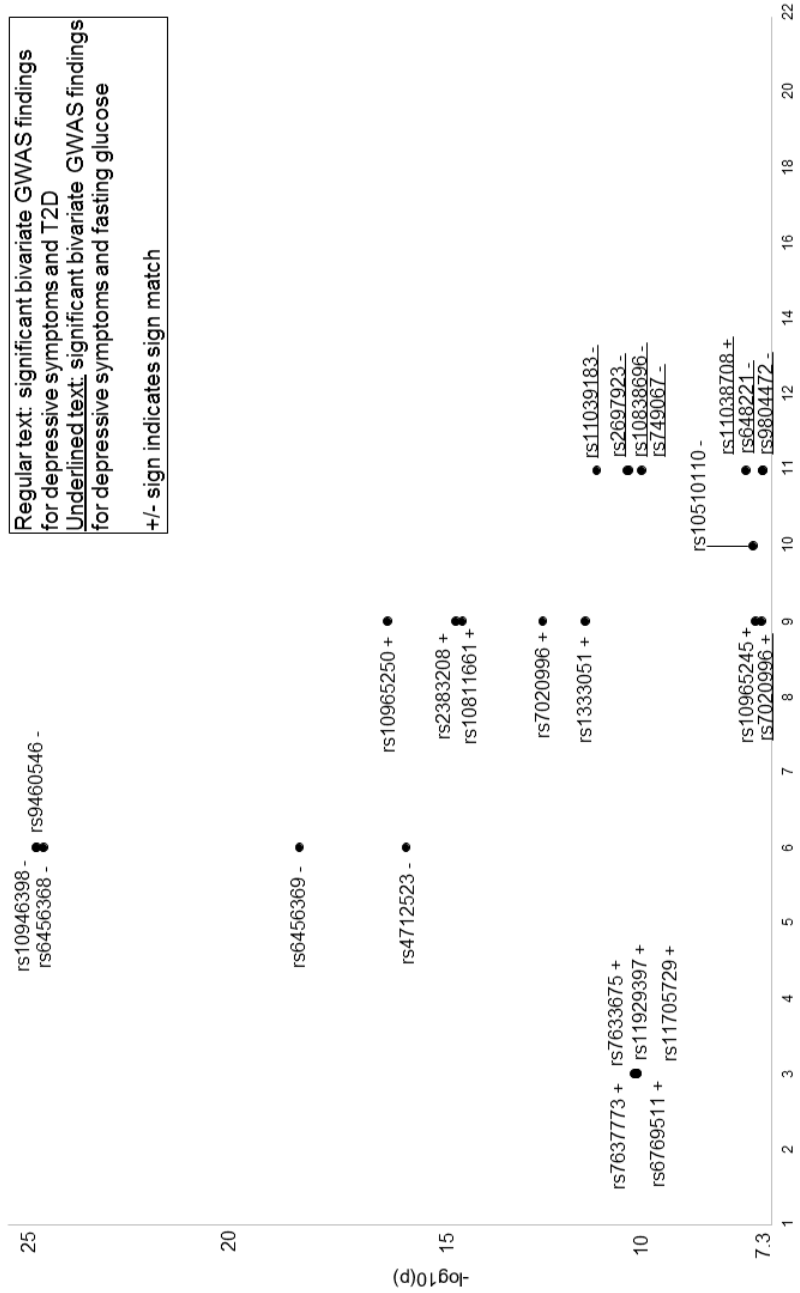
We found several SNPs showing potential pleiotropic effects between depressive symptoms and 1) T2D and 2) fasting glucose (Figure 2). The analyses for depressive symptoms and 1) fasting insulin 2) HOMA-β and 3) HOMA-IR did not result in any significant findings. Significant findings are described in detail below.

Bivariate GWAS analysis for depressive symptoms and T2D resulted in following significant and potentially pleiotropic variants: intronic SNPs in the insulin like growth factor 2 mRNA binding protein 2 gene (IGF2BP2; chr 3) and CDK5 regulatory subunit associated protein 1-like 1 gene (CDKAL1; chr 6), intergenic SNPs close to the CDKN2B antisense RNA 1 gene (CDKN2B-AS; chr 9) and pleckstrin homology domain-containing family A member 1 gene (PLEKHA1; chr 10) (Figure 2). Not all associations were in the same direction with depression and T2D (Figure 2). All these SNPs were associated with significant alterations in expression of several genes in brain tissues in the BRAINEAC database and rs6769511 and rs10510110 as well with expression in other tissues in the GTEx database (Study I, Supplemental Table S4).

Bivariate GWAS analysis for depressive symptoms and fasting glucose resulted in following significant and potentially pleiotropic variants: intronic SNPs in the MAP kinase-activating death domain protein gene (*MADD*; chr11), peroxisomal biogenesis factor 16 gene (*PEX16*; chr 11), intergenic SNPs near

CDKN2B-AS (chr 9) and *MTNR1B* (chr 11) The associations of rs11039183 (*MADD*) and of rs7020996 (*CDKN2B-AS*) with depression and glucose levels were in the same direction whereas for rs11038708 and rs10510110 in *PEX16* and *MTNR1B*, the effect was opposite. Of these four SNPs, rs11039183 was associated with expression of several genes in brain and other tissues in the Braineac and GTEx databases (Study I, Supplemental Table S4).

Figure 2. SNPs showing potential pleiotropic association with depressive symptoms, T2D and fasting glucose



5.2 STUDY II: DEPRESSIVE SYMPTOMS AND GLYCEMIC TRAITS - THE EFFECT OF RS10830963

MTNR1B rs10830963 had a significant main effect on glucose response (fasting and AUC glucose), insulin resistance (ISI) and insulin secretion (DI and CIR). The addition of each copy of the minor G allele was associated with worse glycemic profile: higher fasting and AUC glucose, lower ISI, CIR and DI (Table 2). However, the association with ISI did not survive the FDR correction for multiple testing.

Table 2. Associations between *MTNR1B* rs10830963, depressive symptoms and glycemic traits

Panel A: The effect of rs10830963 (CC/CG/GG)							
Outcome (SD units)	β (95% CI)[a]	R^2	P_1	P_2	P_3	P_4	P_5
Glucose							
Fasting	.174 (.142; .206)	.050	<.001	<.001	<.001	<.001	<.001
AUC	.162 (.126; .198)	.119	<.001	<.001	<.001	<.001	<.001
Insulin							
Fasting	.011 (-.030; .051)	.007	.603	.236	.740	.887	.850
AUC	-.020 (-.065; .024)	.015	.368	.480	.658	.724	.744
Insulin resistance							
HOMA-IR	.048 (.007; .089)	.012	.023	.002	.058	.097	.088
HOMA2-IR	.021 (-.027; .068)	.012	.390	.257	.711	.707	.676
ISI	-.047 (-.090; -.004)	.027	.031	.006	.037	.048	.044
Insulin secretion							
DI	-.203 (-.242; -.164)	.094	<.001	<.001	<.001	<.001	<.001
CIR	-.198 (-.240; -.156)	.050	<.001	<.001	<.001	<.001	<.001
Panel B: The effect of depressive symptoms (continuous sum score)							
Outcome (SD units)	β (95% CI)[a]	R^2	P_1	P_2	P_3	P_4	P_5
Glucose							
Fasting	.004 (-.017; .025)	.025	.720	.870	.720	.697	.599
AUC	.033 (.009; .057)	.104	.007	.020	.013	.012	.008
Insulin							
Fasting	.051 (.024; .078)	.009	<.001	.001	.002	.002	.002
AUC	.046 (.017; .076)	.017	.002	.012	.004	.005	.005
Insulin resistance							
HOMA-IR	.051 (.023; .078)	.013	<.001	.002	.003	.003	.003
HOMA2-IR	.050 (.019; .081)	.014	.002	.012	.005	.005	.005
ISI	-.058 (-.087; -.030)	.029	<.001	.001	<.001	<.001	<.001
Insulin secretion							
DI	-.033 (-.059; -.007)	.072	.013	.037	.033	.034	.026
CIR	.012 (-.016; .040)	.031	.395	.610	.594	.600	.645

P_1 – linear regression model 1 is adjusted for sex and age
P_2 – linear regression model 2 is adjusted for sex, age, and body mass index
P_3 – linear regression model 3 is adjusted for sex, age, body mass index, education, current smoking status, alcohol consumption, and physical activity
P_4 – linear regression model 4 is adjusted for sex, age, body mass index, education, current smoking status, alcohol consumption, physical activity and season
P_5 – linear regression model 5 is adjusted for sex, age, body mass index, education, current smoking status, alcohol consumption, physical activity, season and depressive symptoms (in the analysis of rs10830963) or rs10830963 (in the analysis of depressive symptoms)
[a] – β coefficients and R^2 are from the model 1 and show change in standard deviation units in outcome

Higher depressive symptoms were associated with higher glucose response (AUC), higher insulin response (AUC), higher insulin resistance (fasting insulin, HOMA-IR, HOMA2-IR and ISI) and lower insulin secretion (DI) (Table 2). However, the association with DI did not survive the FDR correction for multiple testing. Additionally, we used previously established cut-off of 60 for depressive symptoms and consequently identified individuals with clinically relevant depressive symptoms. We then ran the analyses again using categorical depression as a factor variable which resulted in no changes in the significance of the findings (data not shown).

The furthest right column (P^5) in Table 2 shows that the effects of rs10830963 and depressive symptoms on glycemic traits were independent of each other.

Furthermore, Table 3 indicates that the interactions between rs10830963 and depressive symptoms were not significantly associated with glycemic traits (Table 3).

Table 3. Interaction between depressive symptoms and rs10830963 on glycemic traits

Outcome (SD units)	β (95% CI)[a]	P^1	P^2	P^3	P^4
Glucose					
Fasting	-.004 (-.040; .033)	.321	.327	.654	.572
AUC	-.005 (-.041; .030)	.776	.851	.879	.903
Insulin					
Fasting	.007 (-.033; .047)	.736	.636	.754	.408
AUC	.022 (-.023; .066)	.341	.225	.167	.076
Insulin resistance					
HOMA-IR	.003 (-.038; .044)	.875	.788	.815	.478
ISI	.005 (-.041; .052)	.816	.656	.397	.393
Insulin secretion	-.017 (-.059; .026)	.444	.266	.253	.107
DI					
CIR	.000 (-.039; .039)	.990	.900	.965	.971
Glucose	.008 (-.034; .050)	.714	.670	.592	.423

P^1 – linear regression model 1 is adjusted for sex and age
P^2 – linear regression model 2 is adjusted for sex, age, and body mass index
P^3 – linear regression model 3 is adjusted for sex, age, body mass index, education, current smoking status, alcohol consumption, and physical activity
P^4 – linear regression model 4 is adjusted for sex, age, body mass index, education, current smoking status, alcohol consumption, physical activity, and season
[a] – Beta coefficients are from model 1 and show change in standard deviation units in outcome

5.3 STUDY III: THE AMOUNT OF DAYLIGHT, RS10830963 AND GLYCEMIC TRAITS

Table 4 shows that at baseline and at follow-up the addition of each G allele of the rs10830963, conferring risk for T2D, was associated with higher glucose response (fasting and AUC glucose) and with lower insulin secretion (DI, CIR). At baseline, the addition of each G allele of the rs10830963 was also significantly associated with insulin resistance (higher HOMA-IR and lower ISI), but at the follow-up, rs10830963 was not significantly associated with the HOMA-IR or ISI. Similarly, the addition of each G allele of the rs10830963 was also associated with an increase in fasting glucose and AUC for glucose and with a decrease in DI and CIR values between the baseline and the follow-up but was not associated with change in insulin resistance (HOMA-IR or ISI) between baseline and follow-up (Table 4).

All these associations remained unchanged when adjusted for sex, age, and BMI at the respective visit(s), and when additionally adjusted for baseline education and lifestyle factors at the respective visit(s), except for the associations with HOMA-IR and ISI at baseline, which were rendered non-significant (Table 4).

At baseline, daylight was not associated with any of the glycemic traits (Table 4). However, at the follow-up, individuals studied on days with more daylight had lower fasting glucose, yet higher AUC for glucose, higher insulin resistance (higher fasting insulin, lower ISI), as well as lower insulin response (DI, CIR). Individuals who underwent the OGTT on days with more daylight available at the follow-up than at the baseline, displayed significantly higher increase in AUC for glucose and decrease in CIR between the baseline and the follow-up (Table 4).

MTNR1B rs10830963 and daylight did not interact significantly at the baseline or the follow-up cross-sectional analyses of the glycemic traits (Table 5). However, rs10830963 and change in daylight between the baseline and the follow-up visits interacted significantly with the change between the baseline and the follow-up in fasting insulin and HOMA-IR (Table 5, panel C).

To illustrate these interactions, we divided the participants into three groups according to change in daylight between baseline and follow-up. These tertiles were the following: less daylight at the follow-up than at the baseline (-15h to -2h difference), no or small difference in daylight between the testing days (-1h to 4h difference), and more daylight at the follow-up than at the baseline (5h to 15h difference). These findings show that those with no or one risk allele of the rs10830963 (CC and CG genotypes), change in fasting insulin and HOMA-IR was independent of the change in daylight between the baseline and follow-up testing days (Figure 3). Yet, those homozygous for the risk allele (GG) became more insulin resistant (higher fasting insulin and HOMA-IR) during the follow-up if the amount of daylight was less at the follow-up than at the baseline date compared with individuals who had similar or higher amount of daylight at follow-up than at baseline.

Table 4. Associations between *MTNR1B* rs10830963, daylight and glycemic traits cross-sectionally and over time

Panel A: Baseline						
	rs10830963			**Daylight**		
Outcome	β (95% CI)[a]	*P*[1]	*P*[2]	β (95% CI)[a]	*P*[1]	*P*[2]
Glucose						
Fasting	.221 (.171; .271)	<.001	<.001	.026 (-.007; .060)	.122	.224
AUC	.199 (.151; .247)	<.001	<.001	.030 (-.003; .062)	.071	.086
Insulin						
Fasting	.009 (-.037; .055)	.698	.528	.022 (-.008; .052)	.153	.171
AUC	-.017 (-.067; .032)	.503	.510	.002 (-.030; .035)	.881	.999
Insulin resistance						
HOMA-IR	.046 (.000; .091)	.050	.377	.026 (-.004; .056)	.095	.122
ISI	-.047 (-.093; .000)	.050	.199	-.015 (-.046; .016)	.341	.414
Insulin secretion						
DI	-.248 (-.298; -.199)	<.001	<.001	-.028 (-.062; .005)	.096	.062
CIR	-.224 (-.275; -.173)	<.001	<.001	-.018 (-.052; .017)	.309	.202
Panel B: Follow-up						
	rs10830963			**Daylight**		
Outcome	β (95% CI)[a]	*P*[1]	*P*[2]	β (95% CI)[a]	*P*[1]	*P*[2]
Glucose						
Fasting	.173 (.124; .221)	<.001	<.001	-.069 (-.101; -.037)	<.001	<.001
AUC	.123 (.076; .170)	<.001	<.001	.053 (.022; .084)	.001	<.001
Insulin						
Fasting	.008 (-.036; .051)	.723	.698	.045 (.016; .073)	.002	.002
AUC	-.043 (-.090; .004)	.073	.134	.007 (-.024; .038)	.645	.527
Insulin resistance						
HOMA-IR	.041 (-.002; .084)	.063	.091	.028 (.000; .056)	.052	.043
ISI	-.018 (-.062; .025)	.397	.358	-.023 (-.051; .006)	.124	<.001
Insulin secretion						
DI	-.192 (-.241; -.144)	<.001	<.001	-.048 (-.080; -.015)	.004	.003
CIR	-.213 (-.253; -.152)	<.001	<.001	-.040 (-.073; -.006)	.021	.024
Panel C: Change over time						
	rs10830963			**Daylight change**		
Outcome	β (95% CI)[a]	*P*[1]	*P*[2]	β (95% CI)[a]	*P*[1]	*P*[2]
Glucose						
Fasting	.088 (.043; .134)	<.001	.001	-.017 (-.047; .012)	.253	.094
AUC	.019 (-.029; .067)	.438	.495	.068 (.036; .099)	<.001	<.001
Insulin						
Fasting	-.001 (-.047; .045)	.970	.893	.000 (-.030; .031)	.997	.880
AUC	-.054 (-.103; -.006)	.027	.066	-.010 (-.042; .021)	.519	.500
Insulin resistance						
HOMA-IR	.010 (-.037; .057)	.690	.519	-.002 (-.033; .029)	.897	.818
ISI	-.008 (-.053; .036)	.712	.625	-.024 (-.053; .005)	.104	.185
Insulin secretion						
DI	-.063 (-.114; -.012)	.016	.038	-.030 (-.064; .003)	.071	.077
CIR	-.070 (-.122; -.108)	.008	.042	-.037 (-.071; -.003)	.031	.030

P[1] – linear regression model is adjusted for sex, age, body mass index

P[2] – linear regression model is adjusted for sex, age, body mass index, smoking, alcohol use, physical activity at respective visit(s), and education at baseline

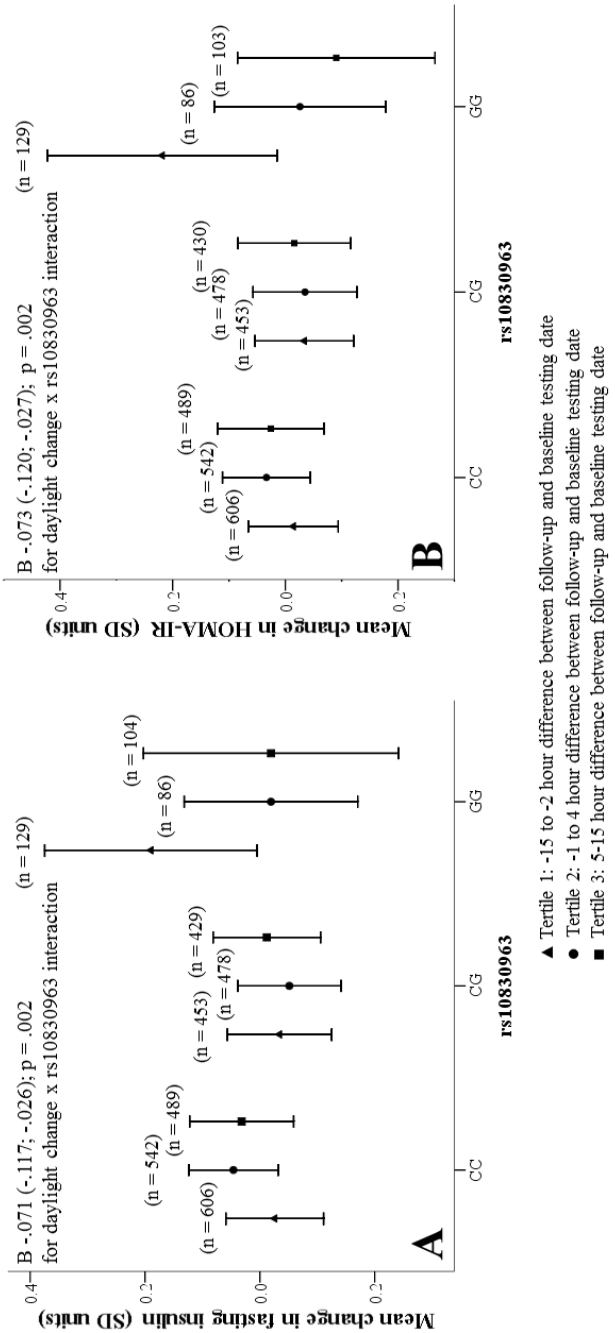
[a] – Beta coefficients are from model 1

Table 5. Interaction between daylight and rs10830963 on glycemic traits

Panel A: Baseline				
Outcome	β (95% CI)	P^1	β (95% CI)	P^2
Glucose				
Fasting	.027 (-.022; .076)	.279	.051 (.000; .102)	.051
AUC	-.015 (-.062; .032)	.531	.001 (-.048; .051)	.960
Insulin				
Fasting	-.009 (-.054; .035)	.680	-.005 (-.051; .042)	.844
AUC	.008 (-.040; .056)	.737	-.002 (-.052; .048)	.940
Insulin resistance				
HOMA-IR	-.004 (-.049; .041)	.862	.005 (-.041; .052)	.830
ISI	-.001 (-.047; .045)	.975	-.005 (-.053; .042)	.833
Insulin secretion				
DI	.022 (-.027; .071)	.375	-.002 (-.053; .049)	.927
CIR	.031 (-.020; .081)	.231	.007 (-.045; .059)	.796
Panel B: Follow-up				
Outcome	β (95% CI)	P^1	β (95% CI)	P^2
Glucose				
Fasting	-.020 (-.068; .028)	.420	-.031 (-.081; .019)	.226
AUC	.017 (-.029; .064)	.465	.008 (-.040; .056)	.746
Insulin				
Fasting	-.020 (-.063; .023)	.372	-.019 (-.064; .025)	.399
AUC	.016 (-.030; .063)	.499	.012 (-.037; .060)	.366
Insulin resistance				
HOMA-IR	-.022 (-.065; .021)	.314	-.024 (-.068; .020)	.290
ISI	.001 (-.043; .044)	.968	.004 (-.042; .049)	.878
Insulin secretion				
DI	-.002 (-.050; .046)	.939	-.001 (-.051; .049)	.978
CIR	.001 (-.049; .052)	.958	.000 (-.052; .052)	.998
Panel C: Change over time				
Outcome	β (95% CI)	P^1	β (95% CI)	P^2
Glucose				
Fasting	-.016 (-.061; .028)	.478	-.027 (-.074; .019)	.252
AUC	-.024 (-.071; .023)	.313	-.032 (-.080; .017)	.201
Insulin				
Fasting	-.071 (-.117; -.026)	.002	-.068 (-.115; -.021)	.005
AUC	-.034 (-.082; .014)	.162	-.034 (-.085; .016)	.181
Insulin resistance				
HOMA-IR	-.073 (-.120; -.027)	.002	-.067 (-.115; -.019)	.006
ISI	-.008 (-.052; .036)	.723	-.019 (-.065; .026)	.407
Insulin secretion				
DI	.006 (-.044; .056)	.817	.009 (-.045; .064)	.731
CIR	.014 (-.037; .064)	.600	.013 (-.040; .066)	.625

P^1 – linear regression model is adjusted for sex, age, body mass index
P^2 – linear regression model is adjusted for sex, age, body mass index, smoking, alcohol use, physical activity at respective visit(s), and education at baseline

Figure 3. The interaction between rs10830963 and change in daylight on fasting insulin and HOMA-IR. The figure is reproduced with permission from the copyright holder (Informa UK Limited, trading as Taylor & Francis Group).



6 DISCUSSION

This thesis consisted of three studies exploring the relationship between depressive symptoms, the amount of daylight and glycemic traits. The central candidate gene in the studies was *MTNR1B* and specifically its variant rs10830963. This study was designed to explore the underlying mechanisms of the common comorbidity of depression and diabetes. Furthermore, one of the aims was to unravel the potential moderating effect of *MTNR1B* in the relationship of daylight and glucose metabolism.

First, it was shown in Study I that there was no common genetic background between depressive symptoms and glycemic traits and T2D in a genome-wide level using previously published GWAS summary statistics. However, several potentially pleiotropic SNPs were identified that were associated to both depressive symptoms and fasting glucose or T2D. Importantly, one of the identified variants was near the *MTNR1B* gene. A variant rs10830963 in the *MTNR1B* gene was extensively studied in the next studies Study II and III.

Second, the relationship between depressive symptoms and glucose metabolism was further explored in a population-based PPP-Botnia Study in Study II. These results indicated that there was no interaction between depressive symptoms and rs10830963 in the analysis of glycemic traits. However, it was shown that the effect of these factors was additive.

Third, it was hypothesized that even though there was no interaction between depressive symptoms and rs10830963, the variant could potentially interact with the amount of daylight. This was assumed against previous research literature as melatonin is directly responsive to environmental light and one's genetic makeup could influence the effect of daylight on glucose metabolism. In Study III it was found in the same PPP-Botnia Study that the amount of daylight was not associated to any glycemic trait at baseline visit but in this study it was also possible to analyze the data from the follow-up. At follow-up, daylight was moderately associated with some of the glycemic traits. Moreover, when exploring the interaction between rs10830963 and change in the amount of daylight on change in glycemic traits, a significant interaction on the change in insulin sensitivity was found.

The findings are further discussed below, the results emphasize the importance of taking psychological wellbeing and the amount of daylight into account in the context of glucose metabolism. After discussion of the findings, an overview of methodological considerations is given, followed by a general discussion and conclusions with further research directions.

6.1 STUDY I

Study I was conducted combining previously published summary statistics from several sources allowing us to evaluate the common genetic background of depressive symptoms and diabetes using independent and large-scale samples of thousands of individuals. To authors' best knowledge, this was the first bivariate GWAS focusing solely on the possible common genetic background of the known phenotypic association of depressive symptoms and glycemic traits. This study should be considered as the first attempt in focusing the analyses on the common genetic background of depressive symptoms and glycemic traits and T2D.

The findings from the study showed that SNP-based heritability estimates for the traits were low, ranging from 4% to 10%. These low heritability estimates might arise from the study design as GWA studies may not be able to capture relevant genomic variation necessary in the development of complex diseases. Furthermore, in addition to low heritability estimates, the authors were not able to show significant SNP-based genome-wide genetic correlations between the traits of interest (p-values > 0.37). However, these findings were in line with previous findings as another study had also found no genetic overlap between major depressive disorder (MDD) and glycemic traits (Bulik-Sullivan et al., 2015). Furthermore, recent GWAS on MDD found associations between BMI and MDD, they had significant genetic correlation and shared more than 30 independent potentially pleiotropic loci. Obesity is closely associated with T2D, thus these findings are important to discuss, however, the authors concluded that BMI is not either a causal risk factor for MDD or correlated with causal risk factors for MDD (Wray et al., 2018).

On the other hand, not all studies have failed to show genetic correlation between these traits. One population-representative Nordic twin study pointed towards a moderate genetic correlation between depression and diabetes (Kan et al., 2016). However, it needs to be clarified that this study used different methodology as twin study design was used in the research by Kan and colleagues. Nevertheless, the lack of common genetic background as an underlying mechanism in the relationship of depression and diabetes might not hold true based on these findings, suggesting other biological mechanisms to be involved in this association, such as previously proposed cytokine-mediated inflammatory response or HPA axis dysfunction (Moulton et al., 2015).

To continue, although a potential common genetic co-predisposition was expected, the authors were not able to show significant genetic correlation between depressive symptoms and glycemic traits and T2D. Nevertheless, current study identified novel SNPs which may contribute to the correlation of depressive symptoms and glycemic traits. Several pleiotropic SNPs were identified between depressive symptoms and fasting glucose and T2D but not between other glycemic traits. The authors propose that these variants need to receive further research attention.

In the analyses of depressive symptoms and T2D, potentially pleiotropic variants in the *IGF2BP2* and *CDKAL1* genes and near the *CDKN2B-AS* and *PLEKHA1* genes were found. Not all the associations were in the same direction between the traits which means that these results address the topic that biological processes underlying phenotypic correlation might operate in different directions or possibly emphasize the role of chance findings. Those identified variants had been associated with some cardiometabolic traits previously (Dorajoo, Liu, & Boehm, 2015; Prasad & Groop, 2015) but not with depressive symptoms in any of the previous studies. Those variants were novel in relation to depressive symptoms and the effect should be further explored because all of the potentially pleiotropic variants altered an expression of genes in various regions of the brain. Thus, future research should focus on the biological relevance of those variants on depression and its subclinical symptoms.

In the analyses of depressive symptoms and fasting glucose, potential pleiotropic loci in the *MADD* and in the *PEX16* genes and near the *CDKN2B-AS* and the *MTNR1B* genes were identified. Once again, all of these associations were novel in relation to depressive symptoms but had been identified in relation to metabolic traits (Cornes et al., 2014; Dupuis et al., 2010). The variant rs6483221 near the *MTNR1B* gene that showed potential pleiotropy between depressive symptoms and fasting glucose was of the most interest to the authors in the context of current research as another variant rs10830963 in *MTNR1B* was extensively studied in relation to depressive symptoms and the amount of daylight on glucose metabolism in other two studies presented in current thesis.

Taken together, the bivariate GWAS study showed that the *IGF2BP2*, *CDKAL1*, *CDKN2B-AS, PLEKHA1, MADD, PEX16* and *MTNR1B* may be associated with both metabolic traits and depressive symptoms and their biological plausibility should be further investigated. However, it needs to be pointed out that in the recent GWAS on MDD, variants in or near these genes were not identified to be associated with depression (Wray et al., 2018). Wray and colleagues (2018) identified 44 variants to be associated with MDD using data on 135,458 cases and 344,901 controls, none of those overlapping with the variant identified in current study. Nonetheless, this study was based on depression diagnosis and not its symptomatology, thus on an endophenotype level, further investigation of those variants is justified.

However, these few potentially pleiotropic variants were identified from around 2.4 million variants, which raises the question of potentially sporadic findings and rather support the view that depression and diabetes might be independently acquired due to the effect of lifestyle or other factors rather than sharing common genetic background. Previous studies show that there are several behavioral factors contributing to the bidirectional relationship. Depressed patients are often displaying poorer health behavior and smoking and obesity are two of the main factors driving the associations (Katon et al., 2004). These associations can also be seen on a brain level, one additional

plausible mechanism is through functioning of the dopaminergic reward circuitry, that modulates food reward and is linked with obesity (Karatsoreos et al., 2013) and that show alterations in depression; this circuit includes projections from ventral tegmental area to nucleus accumbens that are regulated by input from the habenula, hippocampus and prefrontal cortex.

Despite the limitations of the study that are discussed under the chapter of methodological considerations, no overall SNP-based genome-wide genetic relation was shown between depressive symptoms and glycemic traits. Understanding these findings allow researchers to explore other avenues in finding the common underlying mechanisms of commonly co-occurring depression and diabetes.

6.2 STUDY II

In Study II, the relationship between depressive symptoms and glycemic traits was explored further and the potential moderating effect of *MTNR1B* rs10830963 in these associations was studied.

It was shown in a large PPP-Botnia cohort from Finland that contrary to expected, the known diabetes risk variant rs10830963 does not influence the associations between depressive symptoms and glycemic traits. Rather, it was shown that the effects of depressive symptoms and rs10830963 are independent and additive. Thus, the main interesting findings arise from the main effects of depressive symptoms and rs10830963. The study showed that the addition of each copy of the risk allele G was associated with poorer glycemic profile that was evaluated based on higher fasting plasma glucose concentrations and also glucose response to OGTT, higher insulin resistance and lower insulin secretion. Higher depressive symptoms were also associated with poorer glycemic profile. It was shown that more depressive symptoms were associated with higher glucose response to OGTT, higher fasting insulin and insulin response to OGTT, higher insulin resistance and lower insulin secretion.

These findings are highly relevant for the research literature. Earlier GWAS studies have identified promising candidate genes for glycemic traits (Dupuis et al., 2010; Manning et al., 2012; Scott et al., 2007) and for depression (Hek et al., 2013; Ripke et al., 2013) and our own previous research indicated potential pleiotropic effect of one variant near the *MTNR1B* gene on depressive symptoms and fasting glucose. Thus, exploring the potential moderating effect of one candidate gene *MTNR1B* on the known relationship of depression and diabetes was crucial.

The findings indicate that depressive symptoms and rs10830963 have independent effects on glucose metabolism, furthermore, these effects are independent of the covariates including sex, age, BMI, smoking, alcohol consumption, physical activity and educational attainment. Thus, these findings do not support the role of *MTNR1B* as a contributing factor in the

relationship between depression and T2D, yet are in line with previous findings on the level of main effects (Moulton et al., 2015; Prasad & Groop, 2015).

The findings are important as molecular studies have shown increased expression of MTNR1B in β-cells in pancreatic islets of individuals carrying the *MTNR1B* risk variant (Lyssenko et al., 2009). Based on that, this variant is not only associated with glycemic traits in GWA studies but also seems to be functional. It has been suggested that the pathogenic effects on melatonin are likely mediated through inhibitory effects on β-cell function, which results in worsening of glucose metabolism (Jonsson et al., 2013; Peschke, 2008).

From the perspective of the relationship between depressive symptoms and glycemic traits, current study emphasizes the same conclusion as in the Study I – most probably behavioral factors play the biggest role in underlying this relationship as proposed also previously (Moulton et al., 2015).

Despite the limitations, current study did not unravel the biological underpinnings of the common comorbidity between depressive symptoms and T2D in relation to *MTNR1B* rs10830963 variant. Taken together, first two studies of current thesis show that the relationship between depression and diabetes is independent of one's genetic makeup.

6.3 STUDY III

The final study III investigated in the PPP-Botnia cohort the relationship between rs10830963 and glycemic traits in a longitudinal study setting while studying the potential moderating effect of the amount of daylight in these associations. Daily changes in daylight and seasonality are associated with physiological processes and the geographical location of Finland allowed to study the extensive variation in daylight in relation to insulin and glucose metabolism over time in a prospective study. To the author's best knowledge, this study was the first to investigate the amount of daylight as a continuous variable allowing us to capture the variability in the increase versus decrease in day length in the analyses of glycemic traits. The rationale of current study was associated with the hypothesis that disturbances in hormonal profiles might arise from major changes in daylight potentially causing circadian misalignment.

The main findings of Study III, that was conducted in a large, prospective population-based sample of individuals residing in Western Finland, consist of three main parts.

First, it was found in the cross-sectional analysis of daylight on glucose metabolism that individuals who got tested during the lighter days of the year, had lower fasting glucose but higher fasting insulin levels and worse glucose response to OGTT (higher glucose AUC, lower DI and CIR) at follow-up but not at baseline visit. Furthermore, the longitudinal analyses showed that

individuals who got tested on lighter days at the follow-up than at the baseline showed worsening of glucose response to OGTT across the follow-up.

Second, the study replicates findings from our research group in relation to *MTNR1B* rs10830963 and glycemic traits with additional analysis completed at the follow-up and considering the change in glucose metabolism. It was shown that each risk G allele of the *MTNR1B* rs10830963 was associated with higher fasting glucose and additionally with worse glucose response to OGTT. In the previous study (Study II), the focus was on the baseline visit, yet in current study, it was possible to evaluate changes over time. Thus, longitudinally, each G allele was additively associated with worsening of glucose response to OGTT across the follow-up (higher fasting glucose, lower DI and CIR). Finally, the findings showed that risk genotype GG carriers of *MTNR1B* rs10830963, who underwent the OGTT on darker days at follow-up than at the baseline, showed deterioration of insulin sensitivity across the follow-up. These findings were independent of the covariates; thus the findings thus suggest that individual glycemic profiles may be modulated by daylight, the *MTNR1B* genotype and their interaction.

The findings were in line with previous findings from the Swedish GLACIER cohort. Similarly to the GLACIER study, we showed that more daylight is associated with lower fasting glucose (Renström et al., 2015). It is important to note that GLACIER cohort is from the Västerbotten region in Sweden which means that the latitude of PPP-Botnia and GLACIER are very similar. Furthermore, the GLACIER study has not been the only study reporting better glycemic profiles during seasons with more daylight (Shore-Lorenti et al., 2014). For example lower BMI and lower fasting glucose has been reported during summer when there is more daylight available (Chen et al., 2008; Mavri et al., 2001; Suarez & Barrett-Connor, 1982). In our study, we showed associations between daylight and glucose metabolism only at follow-up and longitudinally but not at baseline visit. Not all previous studies consistently show the associations between the amount of daylight and glucose metabolism. For example Waldhauser and Dietzel (1985) showed no seasonal differences in fasting glucose values (Waldhauser & Dietzel, 1985). Furthermore, previous research has found conflicting results in the relationship of seasonality and insulin values. Both higher and lower insulin values have been reported in lighter season (Berglund et al., 2012; Isken et al., 2011).

The authors had the opportunity to study the effect of daylight also in a longitudinal study setting and we showed that more daylight is associated with worse glucose response to OGTT and worsening of the glycemic profile across the follow-up. Apart from lower fasting glucose, our cross-sectional and longitudinal findings suggest that more daylight is associated with worse glycemic profiles. However, previous research has found mixed findings (please see above), thus future studies should further explore the relationship using more detailed measures on the daylight exposure, which the amount of daylight or season do not directly capture.

The authors propose that one of the potential mechanisms through which daylight has an effect on glucose metabolism is related to reduced periodic melatonin exposure that occurs during the light season. Shorter nocturnal melatonin peak caused by the known inhibitory effects of light from an early sunrise on melatonin secretion gives an insight into the potential mechanism through which light exposure influences metabolic profile. Based on the findings from Luboshitzky and colleagues (1998), it is known that there is a seasonal variation in the concentration of melatonin in the human pineal gland. Long photoperiod from April to September is associated with higher melatonin concentrations at night; short photoperiod is shown to be possibly associated with phase-delay in melatonin secretion (Luboshitzky et al., 1998).

Furthermore, the study showed that the interaction between change in daylight and rs10830963 was associated with glycemic profiles. It was found that rs10830963 GG carriers became more insulin resistant when less daylight was available at the follow-up than at the baseline visit. In the context of previous studies, this is in contrast with findings from the GLACIER study. Renström and colleagues (2015) showed the lowest 2h glucose concentrations during the dark season in individuals with the GG genotype (Renström et al., 2015). However, this interaction finding was not replicated in the GLACIER study follow-up conducted a decade later (Renström et al., 2015). This once again confirms, that further research is needed in order to explore this relationship further because current literature shows inconsistent findings.

However, both biological and behavioral factors may influence these associations. It is well known that environmental influences are important in human behavior but furthermore, seasonal effects can als arise from other environmental factors in addition to the amount of daylight such as the changes in temperature. These factors collectively could have effect on human behavior including caloric intake and physical activity (Ishii et al., 2001). In addition, some other behavioral factors such as changes in eating patterns, hunger, alcohol use and alertness can also be associated with light exposure. Additionally, the feeding-fasting cycle itself could play a critical role in the circadian rhythm of metabolic processes as the dominant environmental cue (Dibner & Schibler, 2015).

Taken together, this study shows that individual glycemic profiles can vary according to the amount of daylight, the *MTNR1B* rs10830963 genotype and their interaction.

6.4 METHODOLOGICAL CONSIDERATIONS

6.4.1 LIMITATIONS

The studies discussed in current thesis had several limitations. Study I used previously published GWAS summary statistics and this meta-analysis

methodology might not capture all relevant genetic variation as only common genetic variation is explored. Furthermore, even though previously published summary statistics were used, the sample might have still been underpowered to detect genetic effects typical for complex traits. Importantly, the authors did not find replication cohorts for Study I, thus further research should focus on the replication of these findings. The study has methodological restraints as twin studies show higher heritability estimates for these traits. However, it is typical that SNP-based heritabilities are considerably lower than heritabilities based on twin studies. Furthermore, rare mutations that are not explored in GWAS have higher effect compared to common variants which penetrance is low or indeed zero. Additionally, in further studies, the importance of family history has to be noted.

Study II and III also had several limitations with the main one being the fact that we did not measure melatonin concentrations or its circadian variation. Furthermore, sleep has been shown to play an important role in glucose metabolism but were not evaluated in current studies more deeply than adding it as a covariate (which did not change the findings). Also, while the variant rs10830963 in the *MTNR1B* is also common in other ethnic groups, these associations may be exaggerated in Finns living at latitudes with large seasonal variations in light and darkness. The applicability to other ethnic groups remains to be seen. In Study II, self-reported MHI-5 instrument was used for measuring depressive symptoms that is derived from the 36-item short form health survey SF-36. Thus, Study II did not utilize more commonly used instruments such as Beck Depression Inventory-II (BDI II) or CES-D (Smarr & Keefer, 2011) and therefore lacked the the more clinically relevant measurement of depression.

Regarding the limitations of Study III, it needs to be pointed out that we were not able to explore other proposed alternative mechanisms through which light exposure might influence glycemic traits. For example, due to the lack of data on vitamin D levels, we were not able to test if the vitamin D hypothesis holds true in the relationship between the amount of daylight and glucose metabolism. Furthermore, several other advanced data analysis methods such as the repeated measures mixed modeling or path analysis could have been used to address the research questions, however, linear regression was chosen as the analysis method, especially since we only had only two datapoints available in Study III.

Finally, we do not know if missingness would have influenced the findings as imputation of missing data was not used in Study II-III.

6.4.2 STRENGTHS

The main strength of Study I was related to the summary statistics that were the most comprehensive to date and allowed the exploration of the genetic basis of the known phenotypic relationship of depression and diabetes on a

genome-wide level among independent samples. The state-of-the-art, newly developed, truly novel methodology at the time was used, allowing to address if the potential underlying genomic associations are 1) pleiotropic, and 2) if the associations between depression and diabetes are modified by genomic loci.

One of the main strengths of Study II is related to the state-of-the-art measures on glycemic traits that allowed the researchers to compute several indices based on the measurements after fasting, 30 minutes after the glucose load and 120 minutes after the load. In general, regarding both Study II and III that used PPP-Botnia cohort benefitted from the detailed clinical examination that was carried out at two timepoints, on average 6.8 years apart.

A strength of Study III is its population-based longitudinal design and data on the amount of daylight available both at baseline and follow-up derived from a meteorological station in Pelmaa, Seinäjoki near the PPP-Botnia study centers. The geographic location of PPP-Botnia study centers allowed to explore the large variation in the amount of daylight available at these latitudes.

7 GENERAL DISCUSSION

Diabetes prevalence estimates have increased 281% between 2000 and 2017 (Cho et al., 2018) making it one of the fastest growing burdens of disease. It has been estimated that the rapid rise in diabetes prevalence is associated with socioeconomic changes, such as changes towards sedentary lifestyle causing weight gain among other issues (Cho et al., 2018). However, in addition to the known effect of lifestyle on the development of diabetes, it is also important to emphasize the effect of mental health in relation to diabetes. Knol and colleagues (2006) have concluded in their meta-analysis that the effect of depression on diabetes is comparable to known lifestyle factors contributing to the development of diabetes (Knol et al., 2006). In current studies, the authors also explored the relationship between psychological factors and diabetes that has been shown to be affected by a number of potential mechanisms including individual's biological vulnerability – also one of the topics to explore in current thesis.

The authors were able to replicate previous consistent findings showing the association between depression and diabetes (Pan et al., 2012; Renn et al., 2011) on a level of depressive symptoms and glycemic traits (Study II). Yet, these phenotypic associations were not shown to have common genetic background on a whole genome level, which was one of our hypotheses (Study I). Nevertheless, several common genetic variants were found to be associated with both depressive symptoms and glycemic traits that might potentially be pleiotropic and require further research to evaluate the causality of these novel associations.

The authors did not identify diabetes risk variant *MTNR1B* rs10830963 to be associated with both depressive symptoms and glycemic traits in our study, yet melatonin pathway has been indicated to be involved with both depression and diabetes (de Bodinat et al., 2010; Peschke, 2008) and another variant rs6483221 near *MTNR1B* was identified as being potentially pleiotropic between depressive symptoms and fasting glucose. Thus, in Study II the authors explored the interaction between depressive symptoms and *MTNR1B* rs10830963, one of the most consistently identified diabetes risk variant, on glycemic traits. Instead of confirming an interaction, we identified the relationship to be additive – both depressive symptoms and *MTNR1B* have an effect on glucose metabolism but the effect is independent. This gives new knowledge to the research literature as the associations of *MTNR1B* and depressive symptoms on glucose metabolism have been confirmed independently (Tuomi et al., 2016; Yu, Zhang, Lu, & Fang, 2015) but it was additionally shown that when exploring the potential interaction, the effect remains independent.

Taken together, the findings from current research indicate that the known phenotypic association between depression and diabetes is not significantly

explained by genetic factors on genome-wide level and on candidate gene level. There might be other mechanisms causing this common comorbidity of depression and diabetes. For example, it has been proposed that psychosocial pressure, stress, contributes to HPA axis dysfunctions which could manifest in fat accumulation and insulin resistance (Björntorp, 2001). Cytokine-induced HPA axis dysregulation has also been shown (Pace & Miller, 2009). Inflammatory mechanisms are indeed associated with both conditions (Stuart & Baune, 2012). Mechanistically, cytokines may interact with the serotonergic neurotransmitter system and induce tryptophan depletion (Fujigaki et al., 2006). Furthermore, it has been suggested that obesity might be an amplifying factor in this relationship given the existence of inflammatory pathways in all three conditions with the inter-relational potential (Stuart & Baune, 2012). Abdominal obesity in patients with T2D is indeed associated with the severity of depressive symptoms (Labad et al., 2010).

We also should not exclude the possibility of epigenetic changes as underlying this relationship because even though our genome contains all the information, many of the traits are determined by gene regulation. And even though we were able to show the additive effect of depressive symptoms and *MTNR1B,* but not gene-environment interaction, epigenetic changes might be seen on a molecular level as humans are adjusting to the environment across the lifespan for adaptivity (Kanherkar, Bhatia-Dey, & Csoka, 2014).

Furthermore, behavioral factors might play a significant role in this relationship as depression is related to poorer health behavior and adherence to treatment (Carter & Swardfager, 2016). Unhealthy lifestyle (including alcohol consumption, smoking, unhealthy diet and sedentary behavior) is more common among patients with depression but also with T2D compared to the general population (Bellou et al., 2018; Cabello et al., 2017).

Our behavior is also affected by our environment. Importantly, the amount of daylight is one of the key environmental cues that influence our behavior and physiologic processes (Duffy & Czeisler, 2009). Thus, the authors wanted to further explore the associations between *MTNR1B* and the amount of daylight on glucose metabolism. It was expected that there is an interaction between the amount of daylight and *MTNR1B* on glycemic traits as melatonin is directly responsive to daylight and its effect on glucose metabolism is known (Tuomi et al., 2016). The authors were able to explore these associations in a longitudinal study design and we showed some support to the interaction between daylight and *MTNR1B* on glucose metabolism, but these associations require further research attention. Currently, one of the leading hypothesis linking the amount of daylight with diabetes has been the vitamin D hypothesis suggesting that vitamin D has a direct effect on insulin action (Parker et al., 2010; Pittas, Lau, Hu, & Dawson-Hughes, 2007). The deficiency of vitamin D has been shown to impair insulin secretion of the pancreatic β-cells and increase the insulin resistance. However, vitamin D supplementation has not resulted in consistent effects on glucose metabolism (Jamka et al., 2015).

Furthermore, as discussed earlier, epigenetic regulation might play a role in this relationship as well because it provides a potential mechanism for cells in our body to integrate genetic programs with environmental signals in order to achieve adaptivity. DNA methylation appears to act as a critical mediator of the complex interactions between genetic, environmental and developmental systems in mammals (Powell & LaSalle, 2015).

Last, in addition to the feasible role of biological and molecular mechanisms underlying the relationship between the factors explored in this thesis, behavioral components should be also taken into account. As people are responsive to their environment, it can be estimated that one's behavioral pattern is also influenced by the extensive seasonal changes taking place in Finland, affecting both the lifestyle factors, such as eating habits and physical activity, but also mental wellbeing. Combined with the stress from the burden of dealing with a chronic illness, this is in favour of the disease progression theory from the dynamic model viewpoint (Cramer et al., 2016), making those with already sensitive underlying networks more responsive to environment and potentially making the chronic disease management more burdening to them. Consequently, behavioral factors that are affected by the seasonal pattern such as unhealthy lifestyle that is frequently seen in both depression and T2D development and maintenance collectively contribute to the disease burden.

8 CONCLUSIONS

It is known that T2D is a complex disease and glucose metabolism is affected by various factors including mental health, environmental factors and genetics. The known multifactorial origin of T2D implicates that the disease could be triggered in genetically susceptible individuals in the presence of relevant environmental risk factors (Prasad & Groop, 2015). The disease development is associated with modern lifestyle, going hand in hand with the rapid increase in obesity in the society (Rössner, 2002). But also individuals' mental wellbeing plays a significant role in glucose metabolism and in the development of diabetes.

Although human genome has not changed markedly during the last few decades, we are seeing a dramatic rise in lifestyle associated diseases including T2D. This, together with the findings of current study, emphasize the effect of environmental factors in the disease development.

The findings from current study explored the relationship between depressive symptoms and glycemic traits on the whole-genome level but also on the candidate gene level studying the effect of known diabetes risk variant rs10830963. The results show that the known phenotypic relationship between diabetes and depression is not significantly explained by one's genetic makeup. Rather, the effect of mental health and genetics are both important in relation to glucose metabolism and we suggest that these effects could be additive. Furthermore, we emphasized the effect of daylight on glucose metabolism, showing that also these associations were independent of the known diabetes risk variant rs10830963.

These results are clinically relevant with implications for T2D prevention drawing more attention to the effect of daylight, depression and one's genetic profile that all have an additive effect on the disease development.

8.1 FUTURE DIRECTIONS

This thesis suggests that depressive symptoms, the amount of daylight and genetics go hand in hand in relation to glucose metabolism. Still, the causality needs to be identified in further research. Even more, future studies in larger sample sizes than ours are needed to either confirm or refute our findings.

Additionally, more molecular and experimental data is needed to explain these associations and the underlying mechanisms. We do not know if sleep-wake rhythm might affect the relationship between daylight, depression and metabolic outcomes and future studies should focus on this relationship.

Moreover, future studies should investigate the effect of chronotype on these associations as chronotype reflects how individual circadian clocks are entrained within the 24-hour day. Additionally, very long and very short days

might challenge the network within the circadian pacemaker as it is known that the principal circadian clock entrains to the sun light (Paul, Saafir, & Tosini, 2009). Furthermore, future studies should investigate whether these associations are through vitamin D or independent of it.

Additionally, other photoperiod-related environmental variables and meteorological parameters such as daily temperature should be further investigated. Finally, epigenetic changes should be further explored on a molecular level.

Even further emphasis, both from the perspective of research and developing intervention strategies, should be given to behavioral factors contributing to the development of both depression and diabetes.

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