Biological risk factors and sleep-related risk factors of type 2 diabetes mellitus

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Chapter 8

General discussion

The aim of this thesis was first, to examine the value of non-modifiable biological factors that could play a role in the development of Type 2 Diabetes Mellitus (T2DM) and could contribute to the improved characterization of people at risk of developing T2DM. Second, we aimed to examine impaired sleep as a possible novel modifiable lifestyle factor associated with T2DM development and progression. In this chapter, first the main findings of this thesis are summarized. Second, some important methodological considerations are discussed, followed by the practical implications of the results. This chapter ends with suggestions for future research and the final conclusions.

Main findings

Non-modifiable biological factors
The results of the first part of this thesis indicated a potential role for the incretin hormones, glucagon and GAD65 antibodies in the development of T2DM. We showed that a low GLP-1 response to an OGTT (chapter 2) and a relative lack of glucagon suppression early after a MMT (chapter 3) were associated with a steeper increase in fasting plasma glucose levels over time in people without T2DM at baseline. These results suggest that an impaired GLP-1 response and glucagon suppression precede glucose deterioration and that these biological factors could indeed play a role in the development of T2DM. This also applies for the presence of GAD65 antibodies, as the results of our meta-analysis showed that diabetes autoimmunity, as reflected by GAD65 antibody positivity, was associated with incident diabetes (chapter 4). However, these results should be interpreted with caution as they were not unambiguous. Namely, we observed an association with change of fasting glucose levels for GLP-1 but not for GIP, for an early relative lack of glucagon suppression after the MTT but not after OGTT and the meta-analysis on GAD65 antibodies was characterized by heterogeneity between studies.

Modifiable lifestyle factor
In the second part of this thesis, we showed that various forms of impaired sleep were associated with T2DM. First, with regard to development of T2DM, we observed that social jetlag of >2h, compared to no social jetlag was significantly associated with an increased prevalence of the metabolic syndrome and (pre)T2DM. Furthermore, napping compared to no napping was associated with an increased prevalence of the metabolic syndrome. In addition, a graded association was observed, such that ≥2 sleep-related characteristics, compared to no short sleep duration, napping, insomnia and medication use, were associated with a 40% higher chance of having the metabolic syndrome. Waist circumference and fasting glucose levels were significantly increased in the participants with larger social jetlag and napping time. In order to confirm any causal associations, prospective studies are needed, but based on our results it was suggested that the association of social jetlag and napping with the metabolic syndrome and (pre)T2DM was

driven by visceral obesity and glucose homeostasis. Second, with regard to T2DM progression, the prevalence of (symptoms of) insomnia in a T2DM population was 39% with a 95%CI of 34% - 44%, which is higher compared to the estimates reported for the general population. In addition, insomnia appeared to be associated with deleterious glycemic control. However, the results of this meta-analysis were limited by the high heterogeneity between the studies and the low methodological quality of the studies examining the association of insomnia with metabolic parameters and glycemic control.

Considerations of observational studies and meta-analyses.

Several aspects should be considered that could have affected the strength of evidence of our results, such as confounding, study design, sample size, the methodological quality of the studies that were included in the meta-analyses and the measurement of sleep-related characteristics.

Confounding

With the exception of the two systematic reviews and meta-analyses that were conducted, the studies described in this thesis had an observational study design. Consequently, our results may be confounded. Although our results were adjusted for several potential confounding variables, it cannot be ruled out that potential confounders were not adequately adjusted for resulting in residual confounding. Depending on how the confounding variable is related to both the exposure variable and outcome variable, this can result in over- or underestimation of the observed association. This limitation mainly concerns the second part of this thesis on sleep, since adjusting for confounding factors is necessary to make firm conclusions about whether impaired sleep is indeed an independent risk factor for T2DM or part of an unhealthy lifestyle or disease and thereby affecting T2DM risk.

Sleep is suggested to be associated with several lifestyle factors [1], such as physical activity, diet, alcohol consumption and smoking and with other diabetes risk factors such as depressive symptoms [2] and low socioeconomic status [3]. These factors could explain why suboptimal sleep is associated with a higher prevalence of the metabolic syndrome and pre(T2DM). The lifestyle factors may be either confounding or mediating factors. However, due to the cross-sectional design of our studies we cannot disentangle confounding and mediating effects. In our sleep analyses we were able to adjust for several of these factors, namely educational level, job status, smoking, physical activity, depression and BMI. Although adjustment for sex, socioeconomic and lifestyle factors and BMI did alter the estimates, the association remained still significant. It thus seems unlikely that socioeconomic and lifestyle factors are solely responsible for the elevated prevalence of (pre)T2DM and the metabolic syndrome in the current study on social jetlag (chapter 5). Also in chapter 6 on sleep-related characteristics, after maximum adjustment for age, sex,

socioeconomic and lifestyle factors, depression and BMI the association between napping and the metabolic syndrome remained significant. However, in our analyses we were unable to account for other lifestyle factors such as alcohol consumption and dietary intake as those were not measured.

Dietary intake may be a confounder, but also a mediating factor in the association between sleep disturbances and the metabolic syndrome. While impaired sleep has been shown to affect the levels of leptin and ghrelin that stimulate hunger and appetite, impaired sleep may lead to increased and unhealthy food intake and consequently weight gain and thus an increased risk of metabolic syndrome and T2DM [4-7]. Indeed, adjustment for BMI attenuated the association between sleep and pre(T2DM) and the metabolic syndrome (chapter 5 and 6) and is therefore suggested to have a mediating effect on the examined associations. Nevertheless, BMI is a distant proxy for dietary intake, while increased appetite does not necessarily equal increased dietary intake and in addition increased intake does not necessary equal increased weight, underlying the need to measure and to control for dietary intake itself. Dietary intake was not controlled for in our studies and neither in the majority of prospective studies on the association between sleep and risk of T2DM [8-11]. However, another study examining dietary patterns instead [12], showed that a preference for high fat foods, skipping breakfast, snacking, and eating out only partially explained the effects of short sleep duration on the incidence of obesity. This suggests that it is unlikely that poor diet will explain the whole association between sleep and pre(T2DM) and the metabolic syndrome that we observed and that other factors, including physiologic mechanisms such as increased sympathetic activity and decreased brain glucose utilization [7, 13], may play a role.

In addition to impaired sleep being part of an unhealthy lifestyle, other aspects of impaired sleep itself could influence the association. For example, habitual snoring and obstructive sleep apnea are shown to be associated with insulin resistance, the metabolic syndrome and risk of developing T2DM [14-17]. However, sleep itself, independent of snoring and obstructive sleep apnea, also affects glucose and insulin regulation [13]. Consequently, because these sources of impaired sleep don't reflect the adverse processes of sleep itself, the association between sleep and the metabolic syndrome could be overestimated when sleep disturbances like these were highly prevalent in our studies. Unfortunately, no information about habitual snoring, obstructive sleep apnea or other sleep disturbances was available. Since data from the United States and Europe suggest that 14-49% of middle-aged men and 5-23% of the women have clinically significant obstructive sleep apnea [18] this could indeed have affected our results. However, the use of sleep medication, as a marker of sleep disorders, was not associated with the metabolic syndrome (chapter 6), suggesting that the potential presence of sleep disturbances did not affect our results to a large extent. In conclusion, our results suggest that social jetlag and napping had a direct effect on the risk of having pre(T2DM) and the metabolic syndrome. Additional,

prospective, studies that control for dietary intake and account for sleep disturbances are necessary to confirm the role of impaired sleep as in independent risk factor for the metabolic syndrome and (pre)T2DM.

Study design

While the evidence on the role of biological factors in the development of T2DM was based on prospective studies, the studies examining impaired sleep were cross-sectional. The distinguishing feature of a prospective study is that at the time of inclusion of subjects and collection of baseline exposure information, none of the subjects have developed the outcomes of interest. This allows for assessment of the temporality and thereby causality of the studied association. Cross-sectional designs cannot provide strong evidence for temporal relation and thus cause and effect. In addition, prospective designs minimize the chance of reverse causation. In case of reverse causality the exposure is affected by (early stages of) the disease.

While the second part of this thesis is based on cross-sectional studies, reverse causation is an important issue to consider in the association between sleep and the metabolic syndrome and (pre)T2DM. Cross-sectional associations may be driven by reverse causality if T2DM itself leads to impaired sleep or if an underlying unobserved health condition led to both impaired sleep and T2DM simultaneously. For example, T2DM could impair sleep due to pain and nocturia [19]. However, since a large part of the T2DM patients in our studies were screen detected, it is less likely that participants had severe T2DM with physical complications. It is therefore not probable that impaired sleep resulted from pain and nocturia, thereby minimizing the chance of reverse causation. Furthermore, we also observed an association between sleep and early stages of T2DM such as prediabetes and individual features of the metabolic syndrome. This also limits the possibility that our results are driven by reverse causation. Indeed, evidence from prospective cohort studies support the opposite direction of causality, namely that short or poor sleep leads to T2DM over time, with a 40% increased risk of T2DM in those who sleep <6h [20] and a 80% increased risk in those experiencing difficulty maintaining sleep [10, 20].

For diabetes research in general, reverse causality remains an important issue to consider, thus also in prospective studies, due to the interaction of many pathways involved with the development of T2DM. For instance, when examining the association of abnormal glucagon levels and fasting glucose levels in chapter 3, due to the prospective design of our study, we can be more confident that the glucagon levels were measured before the occurrence of the disease and thus that glucagon affects the development of T2DM. However, levels of glucagon might already be affected by the presence of prediabetes since participants with normal glucose tolerance and impaired fasting glucose were studied together. Since we observed no significant effect modification of glucose metabolism status

we think that the effect of reverse causality will be limited. However, due to the limited sample size we may have missed an interaction effect due to a lack of statistical power.

Sample size

The number of participants investigated in the Hoorn Meal Study (chapter 2 and 3) is rather limited and additionally only a small number of participants developed T2DM at follow-up. Due to lack of statistical power as the number of T2DM cases was low (<10), we could only examine the association with changes in fasting glucose levels and not with incident T2DM. Therefore, some considerations: first, the measurement of only fasting glucose levels does not reflect total glucose metabolism and second, we should consider the generalizability of our results to future diabetes risk.

Regarding the first consideration, besides fasting plasma glucose levels, 2-hour post OGTT plasma glucose (2hPG) levels and HbA1c levels are markers of glycemic control as well. Fasting glucose levels are used to define impaired fasting glucose (IFG) levels, while 2hPG levels are used to define impaired glucose tolerance (IGT). Increased fasting glucose levels are closely related to elevated endogenous glucose production by the liver [21] due to increased glucagon levels stimulating gluconeogenesis [22]. However, also the inhibitory effect of insulin on endogenous glucose production becomes defective [23], indicating hepatic insulin resistance. Fasting hyperglycaemia is also characterized by a defective first-phase insulin release [24, 25], potentially related to reduced beta cell volume [26]. In contrast, 2hPG levels reflect severe insulin resistance in skeletal muscle [24, 25]. Beta cell function is also impaired in IGT, but this seems to be secondary to the development of insulin resistance [23]. Recently, HbA1c levels have been included in the diagnosis of prediabetes and T2DM [27, 28]. Increased HbA1c levels reflect a mixture of the pathophysiological defects associated with fasting glucose and 2hPG levels [29-31]. So, by only examining the change of fasting glucose levels as outcome measure in our studies, we mainly assessed the association with changes in insulin release, omitting changes in insulin resistance.

Since glucagon drives gluconeogenesis, thereby mainly affecting fasting glucose levels, we think that only examining changes in fasting glucose levels did not limit our results of chapter 3. However, cross-sectional studies observed that in addition to individuals with IFG also individuals with IGT demonstrate reduced glucagon suppression [25, 32-34]. So although our results add on to these cross-sectional results with regard to prospective changes of fasting glucose levels, we cannot conclude, based on our results, whether this is also applies to the association with 2hPG levels. With regard to the incretin hormones, GLP-1 levels are shown to be positively associated with both insulin release and insulin sensitivity [35], but the ability of GLP-1 to improve beta cell response was more predominant than the ability of GLP-1 to improve insulin sensitivity [35]. This would suggest that our results are not severely limited by examining only the change in fasting glucose levels, which is also

supported by our observed prospective association between GLP-1 and change of fasting glucose levels in chapter 2. However, different GIP and GLP-1 responses have been observed between individuals with IFG en IGT, with individuals with IFG showing a reduced GLP-1 secretion and an unaltered GIP secretion and those with IGT showing a reduced or unaltered secretion of GIP and GLP-1 [23]. By only examining the change in fasting glucose levels we lack evidence about the association with changes in insulin resistance, which limits our results, as we may have missed an prospective association of GIP with 2hPG levels as GIP secretion has been shown to be reduced or unaltered in those with IGT in previous cross-sectional studies [23]. In addition, the L cells in the gut may be sensitive to insulin resistance, since weight loss, and thereby reduction of insulin resistance, induces a marked increase in GLP-1 release [36]. This suggest that GLP-1 could be associated with 2hPG levels too. Obviously, this hypothesis needs to be further tested in a prospective setting in studies with larger sample sizes and studying the full spectrum of glucose metabolism.

Regarding the second consideration, by examining associations of glucagon and incretin responses with fasting glucose levels we cannot directly extrapolate to risk of T2DM. However, although our results do not provide direct information on the development of T2DM, it provides more understanding on the underlying mechanisms, since changes in fasting glucose levels, when chronic, lead to T2DM. In addition, our prospective design further suggests a causal association between glucagon and the incretin hormones and fasting glucose levels. Therefore, these results can provide basis for further studies with larger sample sizes to confirm our results and extrapolate these associations to risk of T2DM.

Quality of studies for meta-analysis

The conclusions, value and credibility of a meta-analysis strongly depend on the quality of the included studies to estimate the pooled effect [37]; if you include studies of poor quality, you will also produce a meta-analysis of poor quality. The study quality can be affected by limitations in study design, participant selection, data collection and data analysis. In order to get a clear understanding of the evidence base it is important to include a methodological quality assessment when conducting a systematic review and meta-analysis. In both of our systematic reviews and meta-analyses a considerable part of the studies included was rated as being of low methodological quality. When we excluded the low quality studies in our meta-analysis on GAD65 antibodies (chapter 4) the strong and significant association observed (risk estimate: 3.36 (95%CI 1.9 ; 5.9)) between GAD65 antibody positivity and incident T2DM, became borderline significant. However, since the association was robust to the other sensitivity analyses, we don't think this affected our conclusion to a large extent. Maybe more alarming is the overall level of quality of the studies on metabolic consequences of insomnia in chapter 7. In our systematic literature search, we could identify only 12 studies that adjusted the relation of insomnia with

metabolic parameters and glycemic control for confounders, of which half was of high methodological quality. The four studies that could be pooled in the meta-analysis showed no significant association between poor sleep quality and poor glycemic control, although a trend towards an increased risk was visible, similar to the results of the unadjusted mean difference analyses. This clearly underlines that data from strong methodological studies, correcting for at least age, sex and diabetes duration are necessary to draw firm conclusions regarding the association between insomnia and metabolic parameters and glycemic control.

Measurement of sleep-related characteristics

A factor complicating research on sleep is the lack of standard definitions and thereby the measurement of sleep characteristics. For example, for the term insomnia, there are some liberal definitions [38, 39] that focus solely on the presence of nocturnal sleep disturbances e.g. sleep initiation or maintenance difficulties, whereas other more conservative definitions [40, 41] require additional functional impairment e.g. daytime impairment, sleep dissatisfaction. Our systematic review with meta-analysis on insomnia (chapter 7) illustrated these varied definitions and unequivocal ways to measure insomnia. We chose not to define specific inclusion criteria for the insomnia measure, resulting in included studies that used a wide range of measures to assess insomnia, of which the most frequently one was the Pittsburgh Sleep Quality Index (PSQI). Although the PSQI can identify people who are likely to have a sleep disturbance and poor sleep quality is one of the defining features of chronic insomnia, insomnia and poor sleep quality are not interchangeable terms. As a consequence of the broad range of insomnia measures the reported insomnia prevalence in the individual studies ranged from 6% to 80%. The lowest prevalence was reported by a study [42] that assessed self-reported insomnia diagnosis, while the highest prevalence was reported by a study [43] that used the PSQI, showing that the way insomnia was measured might affect the observed prevalence to a large extent. However, this is of course also determined by the study population in which the prevalence was measured. While in our meta-analysis most of the measures to assess insomnia were not about insomnia but poor sleep quality, this may suggest that our meta-analysis provided a pooled prevalence of 39% with a 95%CI of 34% - 44% for insomnia symptoms rather than insomnia. Regardless of the exact definition of insomnia, the results of our review in chapter 7 indicate that in the T2DM population about a third perceives sleeping problems and that this may result in deleterious glycemic control.

Practical implications

Non-modifiable biological factors

To date, we can identify those with an increased risk of developing T2DM reasonably accurately based on risk models for the prediction on T2DM [44]. However, this group of individuals at risk is still very large and only a small proportion actually progresses to T2DM. Moreover, the phenotype of people who develop T2DM is highly variable. This heterogeneity might reflect subtypes of T2DM with different pathophysiology and underlines the need to further refine the characterization of people who develop T2DM. In order to differentiate such different subtypes of T2DM several methods for this characterization have been developed.

Recent studies have performed cluster analysis of individuals using serum biomarkers and clinical data to identify T2DM subgroups [45, 46]. Cluster analyses aim to provide such distinct categories by grouping persons in such a way that the persons in the same cluster are more similar to each other than to those in the others clusters. Ahlqvist et al. 2018 [45] identified 5 subtypes of T2DM. The main pathophysiological processes in the subtypes 'severe autoimmune diabetes' and 'severe insulin-deficient diabetes' were similar, except that the first, in addition to insulin deficiency, was characterized by the presence of GAD65 antibodies [45]. The other subtypes were 'severe insulin-resistant diabetes', characterized by insulin resistance and obesity, 'mild obesity-related diabetes', characterized by obesity only and 'mild-age related diabetes' featured by metabolic derangements but the highest age[45]. Comparable clusters were identified by Udler et. al 2018 [47], who clustered genetic loci associated with T2DM, thereby making it more likely that the identified subtypes reflect causal mechanisms of T2DM. Five robust subtypes of T2DM were identified and appeared to represent two different mechanisms causing beta cell dysfunction (different association with proinsulin) and three mechanisms causing insulin resistance (obesity mediated, fat distribution mediated and liver/lipid metabolism mediated) [47]. Other additional major pathophysiological processes, proposed in the literature, include impaired alpha-cell function (elevated glucagon release), impaired incretin activity and increased kidney glucose absorption [34].

Our results of chapter 4 are in agreement with the identified cluster 'severe autoimmune diabetes' by Ahlqvist et al. 2018 [45], as our meta-analysis provides clear evidence that the presence of GAD65 antibodies is associated with incident T2DM. The included studies in the meta-analysis on GAD65 antibodies showed, when pooled, a more than three times increased risk of developing T2DM, but were also characterized by heterogeneity. Nevertheless, the several sensitivity analyses we performed provided broadly consistent results. This autoimmunity pathway seems to be closely aligned with type 1 diabetes mellitus (T1DM) and/or latent autoimmune diabetes in adults (LADA). It should be questioned whether this pathway indeed represents a more severe classification

of T2DM, which presents similar to T1DM or that actually people with LADA or T1DM are identified. Although we excluded studies with specified incident LADA or incident T1DM from our systematic review, there is a possibility there were also LADA and T1DM cases among those now defined as incident T2DM. In a study with adults without T2DM, the age group with the highest frequency of GAD65 antibody positivity was 30-34 years (3.2%) while the lowest frequency was observed in the age group 45-49 years[48]. As the average age in each study included in the review was over 40 years of age, we however believe this to be a very low number. In addition, it has been suggested that the region of antibody binding to GAD65 differs between different GAD65 antibody positive phenotypes [49]. It has been shown that T1DM is associated with restricted GAD65Ab epitope specificity, in comparison with GAD65 antibody positive healthy subjects and first degree relatives of T1DM patients [49]. This could point to different pathophysiological processes associated with autoimmunity and potentially a subtype of GAD65 antibody positivity leading to T2DM. However, as GAD65 antibody positivity is only present in 1-5% of the general population, the clinical importance of GAD65 antibody positivity is debated.

Impaired alpha cell function and impaired incretin activity are other proposed pathophysiological processes in the literature, but have until now not been indicated as distinct subtypes in cluster analyses. Our results of chapter 2 and 3 provide the first prospective evidence that impaired alpha cell function and impaired incretin activity indeed could be important pathophysiological processes in the development of T2DM. In chapter 3, a high early glucagon response, but not the late and total response, was associated with increased fasting glucose levels 7 years later. In addition, this association was only observed after the mixed meal test, although the results were comparable for the OGTT, but less pronounced and non-significant. Underlying mechanisms suggested for the association between glucagon and T2DM are related to elevated free fatty acids levels, incretin hormones, defective glucagon suppression by glucose and insulin, and insulin resistance in the alpha cells [50]. When alpha cells chronically receive signals of elevated free fatty acids levels, which are normally only received during fasting inducing gluconeogenesis to maintain glucose levels, this could contribute to impaired alpha cell function, defective suppression of glucagon and development of T2DM. In addition, insulin resistance in the alpha cells will make them less responsive to the inhibitory action of insulin [50]. This suggests that defective glucagon suppression is a process related to insulin resistance and may not reflect a single specific mechanism leading to T2DM. However, the observation that defective suppression of glucagon is more evident after an OGTT than after intravenous glucose administration [51] could also suggests the involvement of the incretin hormones [50].

Our results of chapter 2 indicate that reduced GLP-1 secretion precedes glucose deterioration and this reduction might play a role in the etiology of T2DM. However, while we observed an association with change of fasting plasma glucose levels for GLP-1 and

following the OGTT, we did not for the other incretin hormone GIP and neither did we for the mixed meal test. Underlying mechanisms suggested for the association between the incretin hormones and T2DM are related to the broad spectrum of actions of the incretin hormones GLP-1 and GIP. GLP-1 acts on multiple peripheral tissues including the brain, decreasing appetite, and the pancreas where it increases insulin secretion, insulin biosynthesis and beta cell proliferation and reduces glucagon secretion and beta-cell apoptosis [52]. GIP acts on lipid metabolism, stimulating lipolysis in adipose tissue, and has the same effects as GLP-1 on the pancreas except for the control of glucagon [52]. Dysfunction of both incretins could therefore lead to impaired insulin secretion and glucagon suppression, thereby contributing to the development of T2DM, which suggest not one single specific mechanism leading to T2DM. In conclusion, impaired alpha cell function and impaired incretin activity are pathophysiological processes that potentially could further refine the characterization of T2DM but are not likely to reflect a single specific subgroup or pathway, such as perhaps is the case for GAD65 antibody positivity.

A commonly used and popular term for categorization is personalized medicine. Most often the implication of the term personalized medicine is that T2DM will be divided into several distinct categories (type 2A, type 2B etc.), enabling us to categorize each diabetes patient to their specific category. Ultimately this will allow clinicians to say to their patients: 'You have type 2A diabetes, this type of diabetes is caused by a deficiency in B and the best treatment is C'. This kind of personalized medicine indeed works well for subgroups that are defined by an clear underlying pathophysiological process, such as monogenic forms of diabetes like neonatal diabetes and MODY. However, such specific forms of T2DM only account for a small percentage of the cases. Because T2DM is a polygenic condition in which environment as well as genetic predisposition play a large role, it is unlikely that personalized medicine as described above can be applied to other forms of T2DM as well. Although the persons in the core of the clusters will in a way indeed be different from each other, this is less definite for the persons situated at the periphery of that cluster. The persons situated at the periphery of a cluster likely reflect a mixture instead of belonging to a single cluster.

There are alternative views on the categorization of T2DM. Among them is the 'palette model' [53], in which parallel processes are the focus. Instead of distinct diagnostic categories of T2DM this framework focuses on positioning a person based on the major pathophysiological processes ('base colours') that contribute to the development of T2DM. This model anticipates that many individuals with T2DM have multiple parallel defects that affect several of these processes. The persons position for each process, represented by the colour saturation, is determined by relevant inherited genetic variation and the personal history of the non-genetic exposures influencing that particular trait [53]. The overall position with regard to the persons diabetes status is determined by all those individuals traits together. This is represented in the model by a colour that reflects the mixture of the

base colours and saturations. This palette model is potentially more consistent with the current understanding of T2DM.

When we apply our results to the palette model, autoimmunity as reflected by GAD65 antibody positivity, could be a base colour. However, as already mentioned earlier, the clinical importance of GAD65 antibody positivity is debated because of the low prevalence of GAD65 antibody positivity in the general population. In the palette model this is accounted for by the colour saturation; because the development of T2DM due to islet autoimmunity will represent only a small group of people, the colour saturation will be low in most people. In the same way the model takes into account that it could be of great importance in the small group of people who are indeed GAD65 antibody positivity, envisaged by high colour saturation. Next, the palette model assumes that a combination of pathophysiological processes determines the diabetes risk by taking into account the relative importance of each of this processes. Impaired glucagon suppression and reduced GLP-1 could potentially refine the classification of T2DM, but it is unlikely that these processes will completely account for development of T2DM independent of other mechanisms. This may limit the use of these processes for characterization of T2DM. Moreover, larger prospective studies are first needed to confirm our results on fasting glucose levels and extend them to T2DM risk. A refined classification, like this model proposes, could provide a powerful tool to identify people at high risk and provides information about underlying mechanisms, thereby guiding choice of (early) treatment when T2DM is diagnosed.

Sleep-related risk factors

The second part of this thesis elaborated on sleep as a possible novel modifiable lifestyle factor associated with T2DM development and progression. The research in this thesis showed that various forms of impaired sleep including social jetlag, napping and the combination of several sleep-related characteristics were independently associated with having the metabolic syndrome and/or T2DM and that insomnia, in those with T2DM, was associated with adverse effects on several metabolic parameters and glycemic control. Because of the cross-sectional design of these studies, we cannot provide evidence about causality of the association. However, a recent meta-analysis of prospective studies did show that short sleep duration was associated with a higher risk of developing T2DM [54]. Furthermore, a large, longitudinal study with 6-year follow up observed that subjects sleeping on average less than 6 h/night gained over time approximately 60% more visceral fat than subjects sleeping more than 9 h/night [6] and also experimental studies have shown adverse effects of sleep deprivation on insulin resistance [55-57], glucose levels [58-60] and appetite hormone regulation [4, 61].

These physiological pathways provide evidence for the possible ways in which the detrimental effect of impaired sleep on glucose metabolism is mediated. First, poor sleep

quality as well as short sleep duration might cause decreased brain glucose utilization, which leads to hyperglycemia[7]. Second, sleep deprivation can lead to activation of the hypothalamic-pituitary-adrenal axis and an increase of the sympathetic nervous system and systemic inflammatory response, which promotes insulin resistance and hyperglycemia [7]. Third, an alteration in appetite-regulating hormones, including ghrelin and leptin, caused by impaired sleep might also play a role as this increases obesity risk [7].

Evidence from the earlier mentioned experimental and prospective studies imply that the relationship between sleep impairment and T2DM is indeed causal. Therefore, interventions that increase the amount and the quality of sleep could potentially serve as treatments and as primary preventative measures for the metabolic syndrome and T2DM, alternatively to the use of diabetes medication. This would implicate prescribing one their pillow rather than pills. We now need intervention studies to establish whether sleep interventions can indeed extend and/or improve sleep and consequently will result in improved markers of T2DM. Our results imply that changing the lifestyle factors adjusted for in our sleep analyses, such as physical activity, may not be enough to prevent the metabolic syndrome or pre(T2DM) among individuals who have impaired sleep. In contrast, interventions targeted directly on sleep are needed. Examples of behavioral interventions include helping patients to modify maladaptive sleep habits and educating them about healthier sleep hygiene practices. For example, results of a randomized controlled trial (RCT) showed that the group that received a behavioral consultation session targeting sleep hygiene significantly increased sleep duration, compared to the control group [62]. Furthermore, sleep extension led to behavioral changes namely reduced free sugar intakes, compared to habitual short sleep but no differences in hormone levels were observed as appetite regulating hormones were similar [62]. In addition, interventions systematically manipulating sleep, such as light therapy, are proposed as effective methods to advance the circadian rhythm and sleep timing. For example, results of a placebo-controlled home study showed that blue light therapy in the morning had an effect on melatonin production at night and that voluntary sleep timing can be advanced[63]. However, the effect of these interventions on glucose metabolism has to be studied. Additionally, the sleep extension intervention did not achieve the recommended 7–9 hours of sleep [62], so an integrated approach combining sleep with other lifestyle factors such as physical activity and alcohol consumption, and perhaps medication might be needed to reach optimal sleep and thus optimal metabolic changes.

In conclusion, sleep interventions that have been tested on a larger scale will have to tell whether sleep interventions like these are really useful for improving sleep. Additionally, it will be interesting to see whether this also translates into improved glycemic and metabolic outcomes, such as glucose levels and parameters of the metabolic syndrome, and perhaps also in a reduced risk to develop diabetes complications in those with T2DM.

Future directions and final conclusion

Our results described in chapter 2 and 3 provide the first prospective evidence that, impaired alpha cell function and impaired incretin activity could be important pathophysiological processes in the development of T2DM. However, in order to determine whether impaired glucagon suppression and reduced GLP-1 can play a role in the refined characterization of T2DM, larger prospective studies are needed to confirm our results on fasting glucose levels and extend them to other T2DM parameters and T2DM risk. The results of chapter 4 suggest a T2DM subtype that represent autoimmunity as pathophysiological process for T2DM. However, the clinical importance of GAD65 antibody positivity is possibly limited because of the low prevalence of GAD65 antibody positivity in the general population. Nevertheless, it adds to our knowledge on the pathophysiological processes of T2DM and in a framework, such as the palette model, GAD65 antibodies could still be of great importance in the small group of people who are indeed GAD65 antibody positive. In general, the access to ever larger biobanks and health care data via electronic medical records, makes it possible to further refine the characterization of T2DM, using a combination of clinical, anthropometric, biochemical, genetic and immunological measures. However, we should be careful and avoid putting a label on everything, just because we can. The fact that individuals with T2DM respond, although to different degrees, to most treatments irrespective of the specific underlying pathophysiological process through which they act, fits the notion that T2DM is represented by multiple parallel pathophysiological processes and underlines that we may have to move the focus from defining subgroups based on etiology to an approach that defines subgroups based on the differences in response to treatment and drugs[64], while this is the ultimate goal of personalized medicine.

The second part of this thesis showed that various forms of impaired sleep were independently associated with the prevalence of the metabolic syndrome and (pre)T2DM. In addition, insomnia in those with T2DM appeared to be associated with adverse metabolic effects. Therefore, sleep seems to be an important modifiable factor related to T2DM. Further research is needed to explore whether sleep interventions for the prevention and treatment of T2DM are efficient and feasible on a large scale. For instance, it would be relevant to examine whether an intervention that improves sleep also results in improved glycemic and metabolic outcomes and perhaps also in a reduced risk to develop diabetes complications. Because would it not be great to prescribe people their pillow instead of pills. Furthermore, the relative importance of targeting sleep with regard to T2DM risk, in comparison with other lifestyle factors such as physical activity and dietary intake needs to be examined. Irrespective of all this, many people and also health care providers are not aware of the importance of sleep. There is a lot to be gained in promoting awareness. Advice on factors that promote good sleep and the risk factors for poor sleep should be a first step. With regard to personalized prevention and medicine, sleep could potentially be an

additional modifiable lifestyle factor for the prevention of T2DM and is likely to be a factor that can contribute to reducing T2DM risk in many people. For those experiencing severe sleeping problems, such as insomnia, awareness of sleep problems and specifically targeting sleep can be essential in preventing and treating T2DM.

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