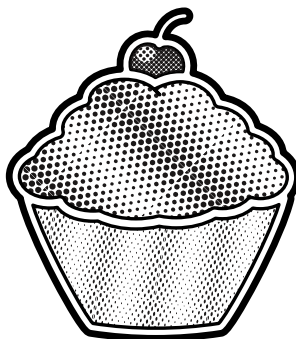


CHAPTER 9

Summary
General discussion &
Future perspectives



CHAPTER 9.1

Summary

Summary of the main findings

In this part of the thesis we review the main results of the presented studies. The aim of the study was to test the hypothesis stating that inasmuch as hyperglycemia-related clinical peripheral microangiopathy is a generalized abnormality in T1DM and also, since in particular proliferative diabetic retinopathy may reflect central or intracerebral microangiopathy, T1DM patients with clinically manifest microangiopathy will have more severe cerebral compromise as compared to patients with uncomplicated T1DM and healthy controls. To this end we addressed the following research questions: 1) What is the effect of T1DM and particularly of hyperglycemia-related clinically manifest microangiopathy on cognitive functions, brain structure and functional and structural cerebral connectivity relative to controls?; 2) What is the role of other risk factors, in particular the presence of subclinical macroangiopathy and the genetic risk marker of cerebral compromise APOE ϵ 4, in T1DM-related brain changes? To this end we included 51 patients with proliferative retinopathy, who could also have additional microvascular complications with a mean age of 45 years and a mean disease duration of 35 years, 53 patients without clinically manifest microangiopathy with a mean age of 38 years and a mean disease duration of 22 years and 51 healthy controls with a mean age of 37 years, who were matched for gender, estimated IQ and education level, and body mass index.

Introduction

In **chapter 2**, a review summarizes the hitherto available literature with regard to T1DM and the brain. This review shows there is compelling evidence that T1DM negatively affects all aspects of the brain, structural, functional, molecular and behavioral. It also indicates that, although chronic hyperglycemia can be considered as the primary cause of these changes, there is little knowledge about the molecular basis of these hyperglycemia related changes. However, it is clear that the cause of cerebral changes is multifactorial, in addition to a substantial role for hyperglycemia-related derangements. In Figure 1 of that chapter a model is presented which takes into consideration the possible multifactorial nature of cerebral compromise.

Microangiopathy and the brain in type 1 diabetes

To test the hypothesis that clinical peripheral microangiopathy is associated with increased prevalence of central or intracerebral microangiopathy, we assessed the prevalence of cerebral microbleeds, white matter hyperintensities and lacunar infarcts in the 3 groups, as described in **chapter 3**. We found the

presence of peripheral microvascular complications to be associated with an increased prevalence of cerebral microbleeds in T1DM. The prevalence and severity of white matter hyperintensities was not further increased by the presence of T1DM or clinical microangiopathy. Lacunar infarcts were not present in this study sample. At the cognitive level, patients with microangiopathy performed worse on measures of general cognitive ability and information processing speed relative to controls and on attention as compared to both other groups. Also, those without microangiopathy performed worse in the information processing speed domain compared to controls. In T1DM patients, the presence of cerebral microbleeds was unrelated to further decrease in cognitive performance, but we found associations with an increase in MEG measured functional connectivity in the upper alpha (10 – 13 Hz) frequency band.

In **chapter 4**, we address MEG-measured functional connectivity during rest in a subgroup of T1DM patients and controls that was included before June 2008. As a measure of functional connectivity, the Synchronization Likelihood was calculated. Patients with microangiopathy showed decreased functional connectivity in the theta (4 – 8 Hz), lower alpha (8 – 10 Hz), upper alpha (10 – 13 Hz) and beta (13 – 30 Hz) frequency bands compared to both their counterparts with uncomplicated T1DM and control subjects. Although it is not possible to exactly determine the location in the brain where the MEG signal had originated from, the decrease in functional connectivity is mainly found on sensors that are located in the central and parietal areas of the brain. Also, connections between these parietal sensors and sensors at occipital and temporal sites are disturbed in these patients. Interestingly, in the lower alpha (8 – 10 Hz) frequency band, T1DM patients without microvascular complications showed an increase in functional connectivity in parieto-occipital connections relative to controls. In both patient groups, functional connectivity was moderately to strongly related to performance within various cognitive domains such as information processing speed, motor speed, attention and executive functions. These findings indicate the importance of adequately functioning connections between multiple cerebral regions for higher order cognitive functions.

Resting-state functional connectivity can also be assessed by fMRI, of which the results are described in **chapter 5**. Functional MRI provides a high spatial resolution and is therefore perfectly suited to localize the cortical regions in which functional connectivity is affected. Comparable to MEG, using fMRI, functional connectivity was decreased in patients with microangiopathy in comparison to the other 2 groups. This was observed in 5 out of the 10

resting-state networks, i.e. the sensorimotor, ventral attention, auditory and language processing, left fronto-parietal and secondary visual network. In these networks, regions that demonstrate reduced functional connectivity include parts of the right pre- and post-central gyri, also known as the motor areas, and parts of the left temporal cortex (temporal pole and the inferior and middle temporal gyri), left parietal cortex (supramarginal gyrus and superior parietal lobule), left superior frontal gyrus and bilateral occipital cortex. In 2 of these networks, the sensorimotor and secondary visual circuits, patients without microangiopathy versus controls showed increased functional connectivity. To examine if the areas of changed functional connectivity were related to cognitive decrements, regression analysis was performed. Weak correlations between better general cognitive ability and information processing speed performance and increased secondary visual network connectivity were indeed found.

Communication between brain regions may be dependent on the interconnecting white matter pathways. The integrity of these white matter pathways in the 2 T1DM groups and controls is described in **chapter 6**. Fractional anisotropy, which reflects the directional dependency of water diffusion and is believed to represent white matter tract integrity, is widely decreased in patients with T1DM, particularly in those with microangiopathy. Lower fractional anisotropy, i.e. decreased white matter tract integrity, was generalized and present throughout many brain regions, although the corpus callosum, corticospinal tracts and inferior fronto-occipital tracts seemed most affected. Interestingly, fractional anisotropy was also lower in patients without microangiopathy when compared to control subjects. This was limited to parts of the corpus callosum and the right corona radiata. These findings indicate that alterations in white matter tract integrity may be an early abnormality in T1DM, and that chronic hyperglycemia may exert a negative effect in white matter tract integrity before microangiopathy becomes clinically manifest. To detail whether this decrease in white matter tract integrity is mainly due to axonal or myelin damage, axial (axonal) and radial (myelin) diffusivity were assessed. The pattern observed was that of a widespread decreased axial diffusivity, more often seen in those patients without microvascular complications than in those with microangiopathy, which might suggest a disturbance in axonal alignment or even axonal damage. Radial diffusivity was increased, but only in patients with microangiopathy. This may suggest that myelin involvement in the disturbance of white matter tract integrity is limited to those who have developed clinically manifest microangiopathy. Measures indicating better white matter integrity of the bilateral inferior fronto-occipital and left corticospinal tract were related to

better performance in cognitive domains, such as information processing and psychomotor speed, general cognitive ability, attention and executive functions. Although correlations were weak after correction for various potentially confounding factors, collectively, these findings suggest that white matter tract integrity is required for the appropriate execution of cognitive functions in this sample of T1DM patients.

The role of other risk factors in diabetes-related brain changes

In **chapter 7** the effect of subclinical carotid artery disease on T1DM-related cerebral compromise was studied in this sample of both patient with and without clinically manifest microvascular complications and healthy controls. The carotid intima media thickness (cIMT) and distensibility (cD) of the right common carotid artery were measured by ultrasound, both of which have been shown to predict future cardiovascular disease and were used as surrogate markers of (subclinical) macrovascular disease. Especially cIMT has previously been found to be related to various aspects of brain structure and functioning in different populations.

Here, we found that cIMT was increased in T1DM versus controls, whereas cD was not altered. However, both measures of subclinical macrovascular disease were independently related to functional and structural connectivity, gray matter volume as well as some cognitive domains. Interestingly, these mostly moderate effects were mainly found in T1DM patients without, and not in the patients with microangiopathy. These findings suggest that the consequences of microangiopathy on the diabetes brain may overrule the more subtle effects of incipient macrovascular disease.

In **chapter 8**, the potential modifying effects of the apolipoprotein E (APOE) genotype on the association of T1DM and cerebral changes is detailed in our cohort. APOE, particularly the $\epsilon 4$ allele, is considered to be a genetic risk factor for cognitive decline in the general population. Approximately 40% of patients with, 26% of patients without microangiopathy and 30% of controls were APOE $\epsilon 4$ carriers in our sample, with at least 1 $\epsilon 4$ allele. T1DM carriers had more frequently an earlier disease onset (age below 7 years) and a lower estimated IQ. In general, T1DM carriers of APOE $\epsilon 4$ showed poorer performance in cognitive domains, especially in information processing speed. Conversely, APOE $\epsilon 4$ in T1DM was related to an increase in gray matter volume, whereas it did not affect functional connectivity. The effect of APOE $\epsilon 4$ was similar in both patient groups. There were, however, differences between APOE $\epsilon 4$ positive male and female T1DM patients. The observed findings for

cognition and gray matter volume were solely attributable to male patients. In all patients, there was a dose-response with regard to these brain parameters. Patients without APOE $\epsilon 4$ had the best cognitive performance but the lowest gray matter volume, followed by APOE $\epsilon 4$ heterozygote patients. Patients that were APOE $\epsilon 4$ homozygous showed the poorest cognitive performance, but had the largest amount of gray matter volume.