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Faculteit Revalidatiewetenschappen

master in de revalidatiewetenschappen en de kinesitherapie

Masterthesis

The correlation between peripheral metabolism and brain metabolism in type 2 diabetes, compared to healthy controls

Teun Claes

Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie, afstudeerrichting revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen

PROMOTOR :

Prof. dr. Koen CUYPERS

BEGELEIDER :

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Situating

Much of the existing research on type 2 diabetes (T2DM) and its impact on the brain has predominantly focused on identifying structural changes through magnetic resonance imaging (MRI). In contrast, comparatively less attention has been directed towards investigating metabolic alterations in the brain, particularly using magnetic resonance spectroscopy (MRS). Given that metabolic alterations may precede structural changes, studying them could provide early insights into cognitive decline. T2DM is also characterised by peripheral metabolic disturbances, including altered glucose and insulin levels. Accordingly, this thesis aims to investigate the correlation between peripheral metabolism and brain metabolism in T2DM, with the goal of advancing a more integrated understanding of the disease's systemic impact on the brain.

The research question was developed and agreed upon in collaboration with my supervisors and promotor. Data collection was carried out by the supervisors as part of the 'Prevention of Heart Failure in Type 2 Diabetes by Exercise Intervention' (PROTECTION) study. The dataset included MRS data from 20 individuals with T2DM and 20 age- and sex-matched healthy controls, as well as peripheral metabolic marker data from overnight fasted blood samples. Although the topic of this thesis diverges from the primary curriculum of rehabilitation sciences, I am grateful to my supervisors, Vandersmissen Jitske, Puustinen Kia, and promotor Prof. Dr. Cuypers Koen, for the opportunity to explore this field and for their invaluable guidance throughout the research process.

Abstract

Background: Type 2 diabetes mellitus (T2DM) continues to pose a major global health issue, currently affecting over 500 million individuals. Its implications extend beyond peripheral metabolism, with growing evidence linking T2DM to alterations in brain structure, metabolism, and cerebral perfusion. Magnetic resonance spectroscopy (MRS) has emerged as a promising, non-invasive technique for investigating early metabolic changes in the brain, offering insight into the underlying pathophysiology of T2DM.

Objectives: This thesis investigates the correlation between peripheral metabolism and brain metabolism in individuals with T2DM, compared to healthy controls, using MRS and fasted blood sample data. It is hypothesised that individuals with T2DM will show lower *N*-acetylaspartate (tNAA), higher myo-inositol (mI), and similar choline (tCho) levels. Additionally, specific peripheral blood markers are expected to correlate with brain metabolites, providing further insight into the systemic impact of T2DM on the brain.

Methods: Participants aged 30-75 were recruited, including individuals with T2DM (n = 20) and age- and sex-matched healthy controls (n = 20). Overnight fasted blood samples were collected to measure HbA1c, glucose, insulin, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and free fatty acids (FFA). Brain metabolite concentrations—tNAA, tCho, and mI—were measured using MRS in four brain regions: sensorimotor cortex (SM1), posterior cingulate cortex (PCC), medial prefrontal cortex (mPFC), and left hippocampus.

Results: Participants with T2DM showed significantly higher levels of HbA1c, glucose, and FFA. Although no significant difference in brain metabolite concentrations was observed between groups, moderate correlations were found between specific peripheral markers and brain metabolites.

Conclusion: Our findings were not fully consistent with previous research, highlighting the need for larger, age-balanced studies to further clarify these relationships.

Keywords: Type 2 diabetes mellitus; Magnetic resonance spectroscopy; Brain metabolites

Introduction

Diabetes mellitus is a chronic metabolic disease that affects insulin levels, which in turn influences blood glucose and multiple bodily functions (World Health Organization, 2024). Globally, the prevalence of diabetes tends to be slightly higher in men than in women, with the vast majority (90–96%) of diabetes patients, particularly older adults, suffering from type 2 (International Diabetes Federation, 2021; Kanyin et al., 2023; Khan et al., 2020; Zhou et al., 2024). The projected rise in diabetes prevalence is alarming, with the International Diabetes Federation (IDF) estimating 643 million cases by 2030, and 783 million by 2045, while the Global Burden of Disease (GBD) study forecasts over 1.31 billion cases by 2050 (International Diabetes Federation, 2021; Kanyin et al., 2023). Consequently, given the public health concern and substantial global burden, this thesis will exclusively focus on type 2 diabetes mellitus (T2DM).

Magnetic resonance imaging (MRI) research has identified structural differences in the brain of individuals with diabetes, including general brain atrophy, microstructural white matter abnormalities, and decreased regional cerebral perfusion compared to controls (Alotaibi et al., 2021; Wang et al., 2021; Zhou et al., 2021). Although structural and perfusion changes in T2DM have been widely studied through MRI, investigations utilizing magnetic resonance spectroscopy (MRS) have received comparatively limited attention (Zhao et al., 2018). Since metabolic alterations in the brain frequently precede structural changes, MRS offers potentially important insights into the early stages of cognitive decline (Alotaibi et al., 2021; Fowler et al., 2022; Wu et al., 2017).

MRS is a safe and non-invasive imaging technique that provides valuable insights into functional and structural changes by measuring in vivo brain metabolite profiles (Alotaibi et al., 2021; Sheikh-Bahaei et al., 2024; Wu et al., 2017; Zhou et al., 2021). Emerging research indicates that brain metabolite concentrations differ significantly in pathological conditions, including T2DM, when compared to healthy controls (Liu et al., 2021; Montes-Gonzalez et al., 2025; Wu et al., 2017; Zhao et al., 2018). Accordingly, this thesis will focus on brain metabolite changes in four distinct regions of the brain: the primary sensorimotor cortex (SM1), posterior cingulate cortex (PCC), medial prefrontal cortex (mPFC) and the left hippocampus (LHIPP). Within these regions, particular attention will be given to the metabolites *N*-acetylaspartate

(tNAA), choline (tCho), and myo-inositol (mI) since these are most often found to be altered in T2DM and best described in the literature (Wu et al., 2017).

Highly abundant in the central nervous system and producing a strong signal with a resonance peak at 2.02 ppm is the metabolite tNAA (Moffett et al., 2007; Soares & Law, 2009). It serves as a biomarker of neuronal integrity and viability and is primarily located in neuronal mitochondria but absent in glial cells (Liu et al., 2021; Sheikh-Bahaei et al., 2024; Soares & Law, 2009). A decrease in tNAA has been linked to early neurodegenerative processes and cognitive dysfunction, primarily affecting memory (Liu et al., 2021; Moffett et al., 2007; Sheikh-Bahaei et al., 2024). Most studies examining tNAA in T2DM report a significant reduction in its concentration compared to healthy controls. These lower concentrations of tNAA were observed in the right frontal and right parieto-occipital regions (Sinha et al., 2014), the frontal lobe (Wu et al., 2017), the bilateral hippocampus (Lu et al., 2019) and the left lenticular nuclei (Lin et al. 2013). However, other studies reported no significant differences in NAA concentrations in the subcortical nuclei region (Ajilore et al., 2007) and the frontal-parietal region (Choi et al. 2023). Total NAA is used due to the current lack of evidence supporting the reliable spectral separation of N-Acetylaspartate (NAA) and N-Acetylaspartylglutamate (NAAG) using either the HERCULES or PRESS sequences (Oeltzschner et al., 2019).

A crucial brain metabolite involved in cell membrane phospholipid metabolism and serving as a precursor to acetylcholine, which regulates cholinergic neuron activity, is tCho (Liu et al., 2021; Sheikh-Bahaei et al., 2024; Soares & Law, 2009). However, the literature on choline in T2DM remains inconclusive. Increased concentrations of tCho were observed in both the lenticular nuclei (Lin et al., 2013) and the fronto-parietal region in T2DM (Choi et al., 2024). One study found that individuals with T2DM exhibited a reduced tCho/Cr ratio in the parietal white matter (Sahin et al., 2008), while two other studies found no significant differences in tCho between T2DM and controls (Ajilore et al., 2007; Sinha et al., 2014).

Serving as an osmolyte, mI is a recognized biomarker for glial proliferation and activation (Liu et al., 2021; Sheikh-Bahaei et al., 2024; Tumati et al., 2013). It produces a peak at 3.55 ppm and is involved in second messenger systems (Liu et al., 2021; Tumati et al., 2013). Three studies identified significantly higher mI concentrations in the brain of individuals with T2DM

compared to controls, including the frontal white matter (Ajilore et al., 2007), the occipital and parietal lobe (Wu et al., 2017) and the frontal-parietal region (Choi et al., 2024).

A closer examination of peripheral metabolism in T2DM reveals distinct alterations in both glucose and lipid metabolic pathways, which contribute to glucolipotoxicity, insulin resistance, and pancreatic β -cell dysfunction (Sobczak et al., 2019; Lytrivi et al., 2020; Yan et al., 2024). These metabolic alterations can be evaluated using fasted blood markers such as glucose, insulin, HbA1c, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and free fatty acids (FFA). Among these, HbA1c is a widely recognized marker of long-term glycaemic control over the preceding 8-12 weeks (Sacks et al., 2024; Weykamp, 2013). In parallel, elevated FFA levels have been linked to systemic inflammation, atherosclerosis, and increased cardiovascular risk (Sobczak et al., 2019), whereas higher HDL-C concentrations have been associated with a reduced risk of developing T2DM (Yan et al., 2024).

This thesis aims to investigate the correlation between peripheral metabolism and brain metabolism in individuals with T2DM compared to healthy controls, using brain metabolites and overnight fasted blood sample data. Firstly, it is hypothesised that, in comparison to the control group, participants with T2DM will show a decreased concentration of tNAA, increased levels of mI, and comparable tCho concentrations. Secondly, it is hypothesised that specific peripheral markers obtained from blood samples will show significant correlations with brain metabolite concentrations. By examining these metabolic interactions, we seek to deepen our understanding of the systemic effects of T2DM and their implications on the brain.

Method

Procedure

On the initial testing day, participants received a detailed explanation of the study protocol and signed informed consent forms upon meeting the screening criteria and agreeing to participate. An overnight fasted blood sample (>8 hours) was obtained to assess peripheral metabolism via HbA1c, glucose, insulin, HDL-C, LDL-C, and FFA levels. The samples were processed and stored in collaboration with the University Biobank Limburg (UBILIM) and the BioBank of UZ Leuven. On the second testing day, participants underwent an assessment of brain metabolite levels through magnetic resonance spectroscopy at UZ Leuven. Prior to MRI scanning, participants completed an MRI contraindication questionnaire, and those with any contraindications were excluded.

Magnetic Resonance Imaging

All MRS data were collected using a 3T Philips Achieva dStream scanner with a 32-channel receiver head coil (Philips Healthcare, Netherlands). First, a T1-weighted image was taken to identify the volume of interest. The voxels were positioned based on anatomical landmarks and placed in the following brain regions: PCC (30 x 30 x 30mm³), left SM1 (30 x 30 x 30mm³), mPFC (30 x 30 x 25mm³), and left hippocampus (30 x 15 x 15mm³). For the first three regions, we used the Hadamard Editing Resolves Chemicals Using Linear-Combination Estimation of Spectra (HERCULES) sequence (Oeltzschner et al., 2019). HERCULES employs a four-phase Hadamard encoded editing scheme to analyse an array of low-concentration brain metabolites simultaneously (Oeltzschner et al., 2019). This sequence consists of four sub-experiments, each designed with a distinct editing pulse to enhance the separation of metabolites (Oeltzschner et al., 2019). A newly developed multiplexed linear combination modelling framework is implemented to simultaneously integrate and fit all three Hadamard combinations (Oeltzschner et al., 2019).

The parameters we used in the HERCULES sequence were the following: NSA = 320, TE = 80 ms, TR = 2000 ms. The SUM spectrum was utilized to quantify brain metabolites using the HERCULES sequence, as it exhibited the lowest coefficient of variation compared to other spectra (Oeltzschner et al., 2019). For analysing the metabolites in the hippocampus, the Point RESolved Spectroscopy (PRESS) acquisition was used. For the PRESS sequence, the following parameters were used: NSA = 128, TE = 35 ms, TR = 2000 ms. The spectral width for all voxels

was set to 2000 Hz in both sequences. Water suppression was achieved using the Multiply Optimized Insensitive Suppression Train (MOIST). Automatic second-order pencil-beam (PB) shimming was applied for optimization. PRESS data was analysed using the LCModel fitting algorithm, while HERCULES spectra were analysed separately, then averaged and aligned using the L1 normalisation, probabilistic spectral registration and the Osprey fitting algorithm with default knot spacing, all within Osprey v2.4.0.

Selection criteria

Participants were recruited through multiple channels, including general practitioners and the endocrinology services of two hospitals, who informed their patients with diabetes about the study. Interested patients were referred to the research team and provided with a flyer containing contact details. Additionally, we collaborated with the Diabetes Liga, which promoted the study on its social media platforms. Finally, study information was disseminated through flyers and social media channels such as Twitter and LinkedIn, as well as via media coverage on TV Limburg.

The inclusion criteria for the type 2 diabetes group were as follows: (1) age between 30 and 75 years, (2) two-hour plasma glucose ≥ 11.1 mmol/L or ≥ 200 mg/dL following a 75g oral glucose load during oral glucose tolerance test, and/or blood HbA1c 6.5-9%.

The exclusion criteria for the type 2 diabetes group and healthy controls were as follows: (1) treatment with exogenous insulin therapy, (2) chronic heart disease (valve insufficiency $\geq$ grade 2), (3) significant arrhythmias, (4) history of recent cardiac events (myocardial infarction, coronary artery bypass graft or percutaneous coronary intervention), (5) clinical heart failure, (6) chronic obstructive pulmonary, (7) cerebrovascular or peripheral vascular disease, (8) severe hypertension ($>160/110$ mmHg), (9) ongoing cancer, (10) severe neuropathy (11), and renal disease ($\text{GFR} < 45/\text{mL}/\text{min}/1.73 \text{ m}^2$).

Healthy controls were deemed to be in good health, according to their medical history reviewed by the researchers, and were matched to T2DM patients according to age and gender.

Statistical analyses

Statistical analyses were conducted using JMP, version 17.2.0 (SAS Institute Inc., Cary, NC, 1989–2025) for Windows. All MRS data are reported in institutional units (i.u.), while insulin is presented in pmol/L, glucose in mg/dL, and HDL-C, LDL-C, and FFA in mmol/L. The normality of all continuous variables was assessed using the Shapiro-Wilk test. As not all peripheral variables met the assumption of normality, their correlations with brain metabolites were analysed using the non-parametric Spearman's rho (ρ) correlation test. Each peripheral marker was individually correlated with each brain metabolite across four brain regions. A p -value of less than 0.05 was deemed statistically significant. Group differences for the same variable were assessed using either a two-tailed two-sample t -test or a Wilcoxon rank-sum test, depending on the distribution and homogeneity of variances.

Results

Participants were between 51 and 73 years of age, with a mean age of 61 ± 5.23 years. The diabetic group showed significantly higher levels of HbA1c ($6.66 \pm 0.66\%$, $p < .001$), FFA (471.53 ± 160.19 mmol/L, $p = .023$), and glucose (139.60 ± 32.61 mg/dL, $p < .001$) compared to the control group. No other peripheral metabolic variable showed significant differences. Additionally, there were no significant differences in brain metabolites between the T2DM group and the controls. An overview of these findings is presented in Table 1 and Table 2.

Table 1*Brain Metabolite Concentrations by Region and Group*

Metabolite	Brain region	T2DM (<i>M</i> ± <i>SD</i>)	Control (<i>M</i> ± <i>SD</i>)	<i>p</i> -value
tNAA	mPFC	20.69±4.05	21.44±2.67	<i>p</i> = .735
	SM1	22.85±1.45	21.57±3.91	<i>p</i> = .543
	PCC	22.28±1.55	22.58±1.35	<i>p</i> = .531
	LHIPP	13.78±1.41	13.47±1.24	<i>p</i> = .475
tCho	mPFC	3.45±0.70	3.56±0.54	<i>p</i> = .600
	SM1	2.90±0.35	2.63±0.54	<i>p</i> = .096
	PCC	2.91±0.35	2.85±0.31	<i>p</i> = .610
	LHIPP	3.25±0.55	3.06±0.59	<i>p</i> = .304
ml	mPFC	15.95±3.71	14.68±3.27	<i>p</i> = .257
	SM1	12.39±1.89	11.18±2.60	<i>p</i> = .181
	PCC	16.96±2.18	16.31±1.52	<i>p</i> = .280
	LHIPP	12.88±2.59	11.76±3.24	<i>p</i> = .234

Note. *M* = mean; *SD* = standard deviation; *p* = probability value; tNAA = total *N*-acetylaspartate; tCho = total choline; ml = myo-inositol; mPFC = medial prefrontal cortex; SM1 = primary sensorimotor cortex; PCC = posterior cingulate cortex; LHIPP = left hippocampus; T2DM = type 2 diabetes mellitus.

Table 2*Peripheral Metabolic Markers in T2DM and Control Groups*

Marker	T2DM (<i>M</i> ± <i>SD</i>)	Control (<i>M</i> ± <i>SD</i>)	<i>p</i> -value
Glucose	139.60±32.61	93.7±10.48	<i>p</i> < .001
Insulin	104.50±71.38	72.32±44.49	<i>p</i> = .140
HbA1c	6.66±0.66	5.43±0.25	<i>p</i> < .001
HDL-C	53.05±18.62	56.20±19.34	<i>p</i> = .665
LDL-C	85.15±33.58	105.75±44.89	<i>p</i> = .109
FFA	471.53±160.19	340.11±174.77	<i>p</i> = .026

Note. *M* = mean; *SD* = standard deviation; *p* = probability value; HbA1c = haemoglobin A1c; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; FFA = free fatty acids; T2DM = type 2 diabetes mellitus.

In our analysis, we found moderate ($|r| > .60$) correlations between peripheral metabolism markers and brain metabolites, but no very strong correlations ($|r| > .80$; Chan, 2003). In the T2DM group, HbA1c was significantly negatively correlated with tCho in SM1 ($r = -.59$, $p = .006$), PCC ($r = -.57$, $p = .008$), and the left hippocampus ($r = -.60$, $p = .005$). In comparison, in the control group, ml showed significant positive correlations with HbA1c in SM1 ($r = .62$, $p = .004$) and PCC ($r = .57$, $p = .009$), none of which were observed in the T2DM group.

Regarding HDL-C, the control group showed negative correlations between tCho in SM1 ($\rho = -.53, p = .016$) and ml in the left hippocampus ($\rho = -.49, p = .028$); these correlations were not found in the T2DM group, which showed no significant associations with HDL-C. For LDL-C, the control group showed a negative correlation between tCho in SM1 ($\rho = -.54, p = .013$) and ml in the left hippocampus ($\rho = -.53, p = .016$), which were absent in the T2DM group. In contrast, in T2DM, tNAA in PCC ($\rho = .47, p = .039$), tCho in PCC ($\rho = .47, p = .038$), and ml in the mPFC ($\rho = .55, p = .012$) all showed positive correlations, none of which were observed in the control group.

Glucose and insulin showed no significant correlations with any brain metabolites in either group. Finally, FFA in the T2DM group showed no significant correlations with brain metabolites, while the control group showed a positive correlation with tNAA in SM1 ($\rho = .63, p = .005$).

Discussion

While several previous studies have identified significant differences in brain metabolites between T2DM patients and healthy controls, this thesis did not observe statistically significant group-level differences in tNAA, tCho, or ml. However, moderate correlations were identified between the selected peripheral metabolic markers and brain metabolites, offering important insights into the early brain metabolic changes associated with systemic metabolic dysfunction in T2DM.

The absence of significant differences in tNAA, tCho, and ml between T2DM participants and healthy controls diverges from much of the existing literature, which often reports reduced tNAA and elevated ml in individuals with T2DM (Wu et al., 2017; Zhao et al., 2018; Liu et al., 2021). Several plausible hypotheses may account for this discrepancy. Firstly, the participants were older than those in prior investigations on this topic (Zhao et al., 2018). As brain metabolite levels naturally change with age, the distinction between healthy and pathological metabolic alterations may be less prominent in older adults (Cleeland et al., 2019; Grachev & Apkarian, 2001). Secondly, with 75% of the sample comprising male participants, the gender imbalance may have influenced the results. Lastly, it is also noteworthy that the control group contained elevated glucose outliers, which may be indicative of a pre-diabetic condition. Existing literature has explored the associations between peripheral blood markers and brain metabolite concentrations. Contrary to our findings, Lin et al. (2013) reported a significant

correlation between fasted blood glucose levels and brain metabolites, specifically, a negative correlation with NAA/Cr and a positive correlation with Cho/Cr (Lin et al., 2013). Similarly, Choi et al. (2024) identified correlations between plasma glucose levels and both Cho and ml concentrations in the fronto-parietal regions (Choi et al., 2024). Lin et al. (2013) also found a significant negative correlation between NAA/Cr and HbA1c, as well as a significant positive correlation between Cho/Cr and HbA1c in the lenticular nuclei, but not in the thalamus. In contrast, Ajilore et al. (2007) observed no significant correlations between HbA1c and either ml or Glx in the dorsolateral white matter and subcortical nuclei regions. Paradoxically, we found a significant negative correlation between tCho and HbA1c in the SM1, PCC, and left hippocampus. This finding diverges from the generally reported positive associations in the literature. While the lack of significant group-level metabolite differences may appear inconclusive, the observed correlations have practical value. Clinically, the findings suggest that peripheral markers, particularly HbA1c may offer early insight into brain metabolic vulnerability, even before clear neurodegeneration is apparent on MRI.

This thesis has several limitations. Although we found some correlations between the peripheral markers and brain metabolites, this does not imply a causal relationship between them. The limited sample size of this thesis may also account for the lack of significant differences observed in brain metabolite levels between the groups. Additionally, potential confounding factors such as caffeine intake, use of non-insulin medications, and alcohol consumption were not assessed, which may have influenced the measurement of brain metabolites.

Conclusion

This thesis explored how peripheral metabolism correlates with in vivo brain metabolism in T2DM patients, employing magnetic resonance spectroscopy alongside markers obtained from overnight fasted blood samples. Contrary to the first hypothesis, no significant difference in brain metabolite concentrations was found between individuals with T2DM and healthy controls. Consistent with the second hypothesis, we observed moderate correlations between certain peripheral markers and brain metabolites. However, these results did not entirely align with the existing literature. Future research with larger sample sizes and a more balanced age distribution is needed to clarify this relationship and improve our understanding of the systemic effects of T2DM on the brain.

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