SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Nuclear medical examination of central and peripheral microvascular alterations in metabolic diseases

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1.Introduction

Metabolic diseases, such as type 2 diabetes mellitus (T2DM) and obesity represent a major and escalating public-health problem worldwide. Both T2DM and obesity are clinically important components of today's endemic commonly referred to as Metabolic Syndrome (MetS). Obesity is considered to be responsible for 3.4 million deaths annually. Besides the development of musculoskeletal, vascular and malignant diseases, obesity may ultimately lead to the development of T2DM. Based on recent data the prevalence and incidence of T2DM also continue to increase: in 2017 the global incidence and prevalence were 22.9 million and 476.0 million, while by 2025 these are estimated to reach 26.6 million and 570.9 million, respectively.

Although, the components of MetS lead to wide-ranging consequences, many of which affect the central nervous system and musculoskeletal system, the underlying mechanism is not yet fully elucidated. Given the overwhelming personal, economic and financial burdens as well as the staggering global prevalence and the concomitantly deteriorating quality of life that microvascular changes associated with metabolic diseases force upon societies, there is an urgent need for the introduction of sensitive imaging methods that excel in the early diagnostic assessment of alterations developed on the basis of metabolic disturbances even before apparent clinical signs are present. Such imaging modalities may provide timely detection of vascular changes, enabling the application of preventive steps which aim at both avoiding and decelerating disease progression. Positron emission tomography (PET), single photon emission computed tomography (SPECT) and single photon emission computed tomography/computed tomography (SPECT/CT) are considered to be promising diagnostic tools to investigate subtle metabolic and perfusion impairments related to metabolic diseases in the central nervous system as well as in the musculoskeletal system. These techniques have clinical significance as biological markers derived from functional imaging may be effective early indicators of the appearance of both cerebrovascular and peripheral microvascular diseases. Obesity, and T2DM are associated with brain perfusion abnormalities that may lead to cognitive impairment, neurodegeneration, and dementia. Recent studies also depicted cerebral metabolic abnormalities in T2DM and obesity. In an 2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) PET study García *et al.* found reduced frontotemporal metabolism in participants with T2DM compared to a healthy cohort. Further, Baker *et al.* pointed out decreased metabolism in the posterior cingulate region both in prediabetic and manifest type two diabetic patients.

Another aspect is that both hyperhomocysteinaemia (HHcys) and

methylenetetrahydrofolate reductase (MTHFR) *C677T* polymorphism (rs1801133) are associated with the development of metabolic diseases. MTHFR is one of the key enzymes that takes part in intracellular folate and homocysteine (Hcys) metabolism. There are several known genetic polymorphisms of the *MTHFR* gene, among which the *C677T* polymorphism (rs1801133) is supposed to be the most frequently investigated single nucleotide polymorphism characterised by a cytosine-thymine (C→T) nucleotide switch in the 677th position of the gene. It leads to an alanine-valine amino acid change in the structure of the enzyme, resulting in the production of MTHFR enzyme with reduced activity. The presence of *T allele* (either homozygote T/T or heterozygote C/T variant) associated with decreased enzyme activity results in elevated Hcys and decreased folate levels. Recent studies reported that *T allele* carriers posed a higher risk of suffering from MetS-related metabolic diseases such as T2DM and obesity. Moreover, in the long run, HHcys can result in the onset of neurological disturbances, such as dementia and neurodegeneration. The findings of *Stoccoro A. and co-workers* strengthened that *C677T* (rs1801133) polymorphism is associated with the development of late-onset Alzheimer's disease.

The subsequent isotope diagnostic solutions assessing diabetes-generated peripheral complications are already available in clinical daily routine: [^{67}Ga]citrate SPECT examinations, SPECT studies applying [^{99m}Tc]hexamethylpropylene amine oxime ([^{99m}Tc]HMPAO) labelled white blood cells, [^{99m}Tc]methylene-diphosphonate ([^{99m}Tc]MDP) bone scan, or [^{18}F]FDG PET/CT. However, isotope diagnostic methods suitable for the detection of peripheral microvascular changes are not yet available in the diagnostic workup of diabetes-related peripheral neuropathy (PN). Taking the favourable kinetical characteristic features of [^{99m}Tc]HMPAO into consideration, we assume that microvascular alterations in the lower extremities evoked on the basis of metabolic diseases might be detected based on the [^{99m}Tc]HMPAO uptake of the muscle cells of the legs.

The application of sensitive diagnostic methods capable of detecting subtle functional changes related to T2DM and obesity may provide opportunity to understand the molecular basis of the relationship between MetS and its impact on microvasculature leading to the discovery of new targets for therapeutic intervention and drug development which could be useful tools in both the prevention and deceleration of the progression of metabolic disturbances.

2. Aims of the study

1. We assessed [^{18}F]FDG brain metabolism in metabolic diseases (T2DM, obesity) in three steps

A. Differences between regional [^{18}F]FDG brain metabolism of T2DM and non-DM obese patients were investigated and the brain metabolism of the two groups was compared to normal database
B. [^{18}F]FDG brain metabolic pattern of T2DM and non-DM obese participants was compared, and we evaluated the association of these brain metabolic patterns with different laboratory parameters assessing the metabolic status of the enrolled subjects
C. The relationship between the [^{18}F]FDG brain metabolic pattern of T2DM and non-DM obese people and the presence of methylenetetrahydrofolate reductase *(MTHFR)* gene *C677T* polymorphism as well as serum Hcys levels was estimated

2. We also aimed at comparing the brain perfusion of the two study groups and evaluating factors (age, BMI, presence of metabolic disease) influencing brain perfusion

3. Further, [^{99m}Tc]HMPAO uptake of the lower extremities was assessed in both groups as part of the brain perfusion studies. We also searched for associations between the accumulation of the radiopharmaceutical and the results of neurometer studies designed to detect PN, and we intended to find connections between the tracer accumulation and laboratory parameters characterising the glucose homeostasis of the patients and clinical data (age, BMI) as well

3. Materials and Methods

Our prospective study was carried out within the framework of GINOP tender construction. Besides isotope diagnostic examinations (PET/CT, SPECT, and SECT/CT) and classic radiological imaging methods (brain MRI), enrolled patients underwent comprehensive internal medicine examinations (thorough physical examination with the registration of anthropometric parameters, electrocardiogram (ECG) examinations, blood pressure measurement, laboratory, urinary and genetic tests, echocardiography, and Carotis doppler ultrasound).

3.1. Study participants, inclusion, and exclusion criteria

Fifty-one, fifty-one, fifty-one, forty-three, and fifty-seven patients with T2DM, and forty-five, forty-five, forty-eight, twenty-six, and forty-six non-DM obese participants were involved in the first, second and third part of the brain metabolism study, the brain perfusion, and the lower limb perfusion studies, respectively. Subjects were recruited from the Department of Internal Medicine of the University of Debrecen as well as from a private general medical praxis (Miskolc, Hungary). Patients were selected based on the following inclusion criteria: age between 18 and 70, manifest obesity (BMI >30 kg/m^2) or controlled T2DM, and no history of mental or brain disorders. Exclusion criteria involved gravidity, breastfeeding, acute or chronic inflammatory disease, severe liver disease, ongoing steroid treatment, hyperthyroidism, retinoid intake, history of malignancies except for basal cell carcinoma, carcinoma in situ, crural ulcer, changes in therapy in the previous six months, use of anticoagulant treatment, brain injury or cerebrovascular event in medical history, peripheral microvascular abnormalities, and previously diagnosed or treated PN. In the third part of the brain examination studies participants were sub-classified into two groups depending on the presence of *T allele* of the methylenetetrahydrofolate reductase (MTHFR) gene. The first group was characterised by CC genotype (with no *T allele*), while the second group had either CT or TT genotype (with one or two *T alleles*). Before enrolment, subjects were provided with detailed pieces of information concerning the aims of the study as well as the examinations. All procedures followed were in accordance with the ethical standards of the responsible national committee on human experimentation (OGYEI/2829-4/2017). Informed consent was collected from all patients involved.

3.2. Laboratory parameters

3.2.1. Laboratory parameters assessing metabolic status

With the aim of assessing the glucose homeostasis of the study participants fasting serum glucose levels (reference value: 3.6-6.0 mmol/L) were determined from serum samples containing added sodium fluoride-potassium oxalate (NaF-KOx) spectrophotometrically (Roche Diagnostics), and HbA1c levels were measured by high-performance liquid chromatography (BioRad, Hercules, CA, USA) from K3-EDTA anticoagulated whole blood samples (reference value: 4.2-6.1%). Besides serum glucose and HbA1c levels the following laboratory parameters were also determined: sensitive thyroid-stimulating hormone (sTSH), triglyceride (Tg), cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A-I (ApoAI), and apolipoprotein B (ApoB). Before PET imaging, the patients' actual glucose level was measured (pre-PET glucose) applying a test strip from finger capillary sample right before the start of the [^{18}F]FDG PET/CT examination (ACCU-CHEK ® *Performa,* Roche Diagnostics).

3.2.2. Measurement of serum homocysteine (Hcys)

Hcys levels (reference value: <12.6 μmol/L) were measured from K3-EDTA anticoagulated plasma samples. The samples were transported immediately on ice at 2-8 °C to the Department of Laboratory Medicine, University of Debrecen. Sample preparation with the measurement of Hcys concentration was performed within 30 minutes after sampling. Hcys levels were determined by chemiluminescent microparticle immunoassay (CMIA) on an Architect-i1000SR® analyser (Abbott, Wiesbaden, Germany).

3.2.3. Evaluation of methylenetetrahydrofolate reductase *(MTHFR)* gene *C677T* polymorphism

MTHFR genotyping was performed in 51 T2DM and 48 non-DM obese participants. DNA was extracted from peripheral blood samples obtained into K3-EDTA Vacutainer tubes (Becton Dickinson, San Jose, CA, USA), using QiaAmp DNA Blood Mini Kit (Qiagen GmbH, Germany) according to the manufacturer's recommendations. Genotyping was carried out with the use of the LightCycler 480 Real-Time PCR Instrument (Roche Diagnostics, Mannheim, Germany) with the application of hybridisation probes based on melting point analysis.

LightCycler technology combines rapid-cycle polymerase chain reaction with real-time fluorescent monitoring and melting curve analysis.

3.3. Nerve conducting studies applying neurometer

Measurement of current perception threshold (CPT) applying a neurometer (NM-01/CPT; MSB-MET Ltd., Hungary) was performed to detect peripheral nerve dysfunction. The neurometer is used to quantify both the conduction and the functional integrity of the large and small myelinated as well as the small unmyelinated sensory nerve fibres.

Transcutaneous electric stimulations of the big toes (L5 nerve) were manually applied three times at 2000 Hz, 250 Hz and 5 Hz frequencies through electrodes coated with conductive gel attached to the skin. Patients were asked to press a button when feeling the current stimulus so that CPT values could be automatically generated at each frequency. CPT values were considered to be normal between 200-526, 62-211 and 30-173 at 2000 Hz, 250 Hz and 5 Hz, respectively. Both increased and decreased sensation values refer to the existence of sensory neuropathy. Completion of neurometer studies required high patient compliance.

3.4. Imaging

3.4.1. [^{18}F]FDG-PET/CT acquisition of the brain

All participants had [^{18}F]FDG-PET/CT examinations to evaluate brain metabolism applying AnyScan PET/CT (Mediso, Hungary). PET acquisition was initiated 45 minutes (+/- 5 minutes) after injecting 3.5 MBq/Bw [^{18}F]FDG intravenously using an automated infusion system (MEDRAD Intego, Bayer). A low-dose CT was also performed for attenuation correction. The parameters of static PET acquisition were the following: 10 min/FOV, with voxel size of 2x2x2 mm and matrix size of 160x160x76, while low-dose CT parameters were as follows: 120 kVp and 100 mAs. Before PET imaging, the patients' actual glucose level was measured (pre-PET glucose). Patients with pre-PET glucose >12 mmol/L were rescheduled after glucose control.

3.4.2. Brain MRI imaging

In case of forty-eight T2DM and thirty non-DM obese patients T1-weighted 3D brain resting state functional MRI was obtained for brain mapping by a Philips Achieva 3T scanner with voxel size of 0.5x0.5x1 mm and matrix size of 480x480x175. The T1-weighted 3D turbo field echo protocol was applied for image acquisition with 8 ms repetition time (TR) and 3.7 ms echo time (TE).

3.4.3. Brain SPECT image acquisition

AnyScan S Flex (Mediso, Hungary) dual-head gamma-camera equipped with low-energy high resolution (LEHR) parallel hole collimators was used to image brain perfusion. Patients were not prone to stress or heavy emotional burden before the actual examination. Half an hour before the injection of the radiopharmaceutical subjects were given 1000 mg perchlorate capsule per os to block the unintentional tracer uptake of the thyroid gland. 709±42 MBq [^{99m}Tc]HMPAO (Mediradiopharma, Hungary) was administered into the right cubital vein after a ten-minute rest in the dimly-lit examination room.

The main characteristics of SPECT examinations were the following: 120 view, 128x128 matrix with 2.36 mm pixel size, 30 sec projection time, with autobody contour.

3.4.4. SPECT/CT imaging of the lower extremities

[^{99m}Tc]HMPAO leg uptake was examined as part of brain perfusion studies without the injection of additional radiopharmaceutical. During this research we intended to assess parallelly the peripheral circulation of patients who were at high risk for developing peripheral microvascular impairments.

AnyScan Trio (Mediso, Hungary) hybrid SPECT/CT camera equipped with LEHR collimators was applied to measure the [^{99m}Tc]HMPAO uptake of the lower extremities. As mentioned previously, 709±42 MBq [^{99m}Tc]HMPAO was injected intravenously into the right cubital vein of the patients through a pre-inserted cannula. Half an hour before the injection of the radiopharmaceutical, the subjects were given 1000 mg perchlorate capsule per os to block the uptake of the thyroid gland. 15 minutes after tracer administration, SPECT/CT of the lower extremities was performed in prone position. The frame included a 40 cm long section proximal

to the region of the feet, including the calf. The duration of SPECT/CT imaging was approximately 20 minutes.

The main settings of leg SPECT examinations were as follows: 128x128 matrix size, 15 sec/frame, 120 views, step and shoot mode with detector configuration of 120 degree, and autobody contour. Low-dose CT was used for attenuation correction with the following parameters: 120 kVp, 50 mAs, 1 pitch, 1 sec rotation time, and 2.5 mm slice thickness. Tera-Tomo Q SPECT reconstruction method (Mediso, Hungary) was applied in attenuation-corrected and non-corrected forms as well.

3.5. Image processing

3.5.1. Assessment of regional brain metabolism with NeuroQ software

FDA-approved NeuroQ application (NeuroQ^{TM} 3.6, Syntermed) was used to analyse regional brain metabolism of each study participant and to statistically compare the individual's scan with an age and sex matched normal whole brain atlas. Participants' [^{18}F]FDG PET files were imported in DICOM format into the NeuroQ software program for automatic quantification, and the activity in 240 standardised regions of interest (ROIs) was calculated. Those regions were considered abnormal which had the uptake values below 1.65 SD of the mean of the normal database.

3.5.2. Comparison of [^{18}F]FDG brain metabolism of T2DM and non-DM obese patients and correlation analyses applying Statistical Parametric Mapping (SPM)

For SPM analysis image pre-processing was performed by an in-house developed pipeline, which involved four main procedures. In the case of the 78 patients who also underwent MR imaging, PET imaging was performed in 71 cases, therefore the image material of these 71 patients was used during image preprocessing. Firstly, T1 weighted images of 71 patients were transformed to the MNI152 template using the ANTS (Avants, 2008) linear and non-linear image registration software, with a 2x2x2 mm voxel size. Then, the PET images of these subjects were registered to their transformed T1 with the FLIRT linear registration tool (Jenkinson, 2001). We used these spatially normalised images to create an FDG template specific to our population by averaging the transformed PET images. Thereafter, we applied linear transformation on every subject's FDG-PET image to create a study-specific FDG

template. This template was used for spatial normalisation of patients' FDG images by using FNIRT software of FSL package. Before the statistical model fitting, we applied the standard „Proportional thresholding SPM protocol" which set the threshold level at the 80 per cent of the mean voxel values. Additionally, the voxel intensity values were linearly scaled to set the mean intensity of the whole brain PET images to 50. Before statistical analyses we smoothed the spatially normalised images with a 16 mm FWHM (full width at half maximum) 3D isotropic Gaussian-kernel, and thresholded them at eighty per cent of average brain voxel values. We used SPM methods both for group comparison (two-sample t-test) and correlation analysis. Statistical images were FWE-corrected (family-wise error, $p<0.05$) and we only considered clusters with an extent of at least 40 voxels.

3.5.3. Brain SPECT image processing

First, the [^{99m}Tc]HMPAO SPECT image of each subject was registered to his/her T1-weighted MRI image by rigid transformation, using the FLIRT linear registration software. Then we transformed T1 weighted MRI images of the patients by elastic transformation to the MNI152-space (2x2x2 mm voxel size) using the ANTS linear and non-linear image registration tools. The combination of both transformations was applied to the SPECT image to transfer it to the MNI space, so that data of the following standard volumes of interest (VOIs) could be obtained: frontal lobe, parietal lobe, temporal lobe, occipital lobe, limbic region, cingulate, insula, basal ganglia, cerebrum, limbic system, and brain stem.

3.5.4. Lower limb SPECT Image processing

For the quantitative assessment of the tracer uptake of the lower limb muscles we calculated standardised uptake value (SUV_{peak}), measured in fixed (2 cms in diameter) spheric VOIs placed on the sural muscles bilaterally on the attenuation-corrected images.

3.6. Statistical analyses

The SPSS 25 and 27 statistical software package (SPSS Inc.) was used for data analysis. Normality of the distributions was tested using Shapiro-Wilk test. Student's t-test was used to compare parameters with normal distribution while Mann-Whitney U test was applied for the comparison of parameters with non-Gaussian distribution. Two-sample t-test or Mann-Whitney

U-test were applied to perform the genotype-related comparison of laboratory parameters in the whole population and in both groups separately in the third part of the examination. For correlation analyses Spearman's Rank correlation was applied. For voxel-wise group comparison (two-sample t-test) SPM methods were used. In order to investigate the association between the observed variables general linear model was used in the lower limb studies. In the section of brain perfusion, with the aim of identifying influencing factors, general linear regression analysis was performed with Hochberg's correction for multiple regions. For thresholding of the statistical images, we used family-wise error correction (FWE) method *($p<0.05$)*, and we considered region clusters only with an extent of at least 40 voxels (0.32 cm^3, T=4.01).

4. Results

4.1. Results of [^{18}F]FDG brain metabolism in metabolic diseases

4.1.A.1. General

There were no significant differences in age *(t-test, p=0.42)* and gender *(chi-square test, p=0.9)* among the patients enrolled in both groups. Serum glucose, pre-PET glucose and HbA1c levels of the patients were measured. These parameters were significantly higher *(p<0.001)* in the diabetic group. Body weight and BMI were statistically higher in the non-DM obese group. Comparing the two groups, in the diabetic group higher Tg levels *(p=0.01)* were measured, while cholesterol *(p=0.01)*, LDL-C *(p<0.001)* and ApoB *(p=0.04)* levels were significantly higher in the non-DM obese group. In type 2 diabetics HDL-C levels were significantly lower compared to the obese *(p=0.01)*. These differences could be due to the diseases themselves.

4.1.A.2. Evaluation of regional [^{18}F]FDG brain metabolism in metabolic diseases and its comparison with normal database

NeuroQ analysis revealed no statistically significant regional metabolic defect in either group. However, some brain regions were represented with lower cerebral activity, although this was not considered statistically notable either. Among these areas the following could be mentioned: visual cortex (V), left associative visual cortex (lAVC), left primary visual cortex (lPVC), right primary visual cortex (rPVC), right cerebellum (rCbm).

4.1.B. Comparison of [^{18}F]FDG brain metabolic patterns of T2DM and obese patients, and the association between brain metabolism and clinical laboratory parameters

Voxel-based comparison of the two groups revealed markedly decreased metabolism in the region of the precuneus and in the right superior frontal gyrus (rSFG) in the type 2 diabetic group *($p_{FWE}<0.05$)*.

Since the pre-PET glucose levels of the diabetic patients were significantly higher *(p<0.05)* than those of the obese, additional glucose correction was performed during SPM analysis. After pre-PET glucose correction only the region of the rSFG showed hypometabolism in the diabetic group compared to the obese. Additionally, the metabolism in the region of the precuneus was detected to be inversely proportional to pre-PET glucose level, that is, the higher the pre-PET glucose level, the greater the degree of metabolic reduction was.

Further analysis of glucose-sensitive regional metabolic differences was performed in the two study groups separately. In the type 2 diabetic group the following brain regions were detected to show glucose-sensitive hypometabolism:

- precuneus/posterior cingulate gyrus
- left posterior orbital gyrus
- right calcarine cortex
- right orbital part of the inferior frontal gyrus

In the obese group, unlike the diabetic population, we found glucose-dependent reduced metabolism in only one area, namely the region of the right Rolandic (pericentral) operculum *($p_{FWE}<0.05$).*

Regional brain metabolism did not correlate with the other investigated laboratory parameters.

4.1.C. Association between [^{18}F]FDG brain metabolic pattern and the presence of methylenetetrahydrofolate reductase *(MTHFR)* gene *C677T* polymorphism and serum Hcys levels in T2DM and obese patients

4.1.C.1. Laboratory findings

Our sample was balanced from the point of view of both classifications (disease and genetic alteration). There was no significant difference between the genotype distribution of the study groups *(Fisher's exact test: $p>0.05$).*

The genotype-related comparison of the laboratory parameters (Hcys, serum glucose and HbA1c) showed no significant difference either in the whole cohort or in the two groups separately *(Mann-Whitney U- test or two-sample t-test).*

No difference was observed between the median Hcys levels of the diabetic and the non-DM obese group *(Mann-Whitney U-test, $p>0.1$).* Further, we found no difference in the Hcys values measured in the different genetic subgroups either in the diabetic *(two-sample t-test, $p\geq0.1$)* or in the non-DM obese group of patients *(two-sample t-test, $p>0.1$).* Serum glucose and HbA1c levels of the patients were measured, too. These parameters were significantly higher in the diabetic group. However, these parameters showed no difference among the genetic subtypes within either patient group *($p>0.1$).*

Interestingly, no significant difference was shown between the appearance of elevated Hcys values in case of different genotypes, however Hcys levels above the cut-off value were slightly more frequent in subjects with the presence of *T allele* in both groups, independently of the type of present metabolic disease. However, this elevated frequency statistically was not significant *(Fisher's exact test: p>0.1)*.

4.1.C.2. Brain metabolism

We also examined the effect of abnormal Hcys values on regional [^{18}F]FDG brain metabolism, and its dependence on disease and genotype.

SPM analysis showed that in the region of the right middle temporal gyrus (rMTG) decreased uptake of [^{18}F]FDG was present in participants with Hcys levels above the reference value compared to those who had their Hcys levels in the normal range. SPM also showed reduced tracer uptake in the left calcarine cortex in those diabetics whose Hcys levels exceeded the cut-off value compared to the obese who had their Hcys levels above 12.6 μmol/L. Additionally, in the region of the superior frontal gyrus (SFG) we recognised hypometabolism among the diabetics with *T allele* compared to the diabetics without *T allele.* This region showed no lateralization by SPM, this *T allele*-dependent hypometabolic region appeared bilaterally with similar statistical characteristic features.

4.2. Brain perfusion in T2DM and obesity

We investigated factors that may be associated with cerebral perfusion (normalized to the occipital region) by the general linear model. First, we included the patient group (diabetes mellitus or obese) and gender as factors, and age, blood glucose and BMI as covariates. BMI *(Mann-Whitney: p=0.004)* and glucose level *(Mann-Whitney: p<0.0001)* were integrated in the model since these parameters were significantly different in the two observed groups. However, based on Student's t-test there was no statistically significant difference between the two groups regarding age, because of previous findings in the literature, we also included age in the analysis. Since the effect of blood glucose level on brain perfusion was far from significant both in the multivariate model and in all regions *(p>0.1)*, we eliminated it from the model. From the remaining variables, multivariate analysis with all brain regions as dependent variables identified that BMI and age are significantly *(p<0.0001)* associated with perfusion; and patient group was slightly above threshold *(p=0.0524)*.

Univariate contrast of the group in all but one brain region was negative (normalized perfusion was lower in DM), but after applying Hochberg's correction for multiple comparisons, we found that the presence of DM was an independent significant predictor of normalized regional brain perfusion only in the insula *(p<0.001)*.

Other independent predictors of normalized regional brain perfusion were:

- age in the insula *(p<0.001)* and in the limbic region *(p<0.01)*
- BMI in the brain stem *(p<0.01)*

4.3. Lower limb perfusion in metabolic diseases

4.3.1. General

There were no significant differences in age *(t-test, p>0.1)* and gender *(chi-squared test, p>0.1)* between the groups. To assess the glucose homeostasis of the study participants, HbA1c and fasting serum glucose levels were measured. Both glucose *(Mann-Whitney test: p<0.001)* and HbA1c *(Mann-Whitney test: p<0.001)* levels were significantly higher in the diabetic group compared to the non-DM obese participants. Although diabetic individuals were under strict medical treatment, a decrease in patients' compliance may explain slightly elevated HbA1c levels compared to the reference value. BMI *(Mann-Whitney test: p=0.0013)* and body weight proved to be significantly higher in the non-DM obese group.

4.3.2. Evaluation of [^{99m}Tc]HMPAO uptake in the lower extremities

While in the diabetic group no significant difference was detected between the SUV_{peak} values of the right and left legs *(Wilcoxon signed rank test: p=0.092)*, the [^{99m}Tc]HMPAO uptake of the right leg of the non-DM obese participants was significantly lower than that of the left leg (median difference: 0.033; *Wilcoxon signed rank test: p=0.0015)*. When comparing the two groups, a notable difference was detected between the SUV values of the lower limbs of the study participants (112 vs. 88 legs, median SUV_{peak}: 0.523 vs. 0.598; for the DM and non-DM obese group, respectively, *Mann-Whitney test: p<0.0001)*. In the diabetic group significantly lower average SUV values of the legs were measured compared to the non-DM obese group (56 vs. 44 patients, medians: 0.517 vs. 0.607; *two-sample t-test for the log(average SUV) values: p<0.001*).

Correlation analyses were performed to evaluate the connection between the [^{99m}Tc]HMPAO uptake of the legs and the nerve conducting studies. No statistically significant

correlation was established between the SUV_{peak}-values measured on a given limb and the CPT values registered on the same extremity at any of the three frequencies either in the whole population (*p>0.1)* or in the T2DM and non-DM obese groups separately *(Spearman's rank correlation: p>0.1)*.

Applying the general linear model, we investigated the association between [^{99m}Tc]HMPAO muscle uptake with the laboratory parameters and clinical data. Four significant predictors of muscle tracer uptake were detected: BMI *(p<0.0001)*, age *(p=0.0283)*, HbA1c *(p=0.0068)* and glucose *(p=0.0044)*. Since SUV_{peak} proved to be log-normal, the logarithm of SUV_{peak} was used as dependent variable in the general linear model.

4.3.3. Neurometer studies

Neurometer studies did not reveal any statistically significant differences between the two study groups *(Mann-Whitney test: p>0.1*). The incidence of abnormal CPT values was not significantly different in the diabetic and obese group *(Fischer's exact test: p>0.1)*.

In the diabetic group, nerve conducting test showed a positive correlation with HbA1c levels *(Spearman's rank correlation: p=0.0002*). However, among the non-DM obese group of patients, HbA1c and CPT values did not show correlation.

5. Summary

Background: Metabolic syndrome and its individual components lead to wide-ranging consequences, many of which affect the central nervous system and the musculoskeletal system. Since rising evidence suggests that metabolic diseases lead to significant cerebral and peripheral microvascular impairments there is an urgent need for the introduction of sensitive imaging methods that excel in the early diagnostic assessment of alterations developed based on metabolic disturbances even before apparent clinical signs are present.

Aims: In this study, we first examined and compared the [^{18}F]FDG regional brain metabolic pattern of participants with T2DM and non-DM obese individuals, then the relationship between these metabolic patterns and the presence of MTHFR gene *C677T* polymorphism as well as serum Hcys levels was estimated as well. Further, we also aimed at identifying independent factors that contribute to the appearance of regional brain perfusion changes besides those that are already known. Finally, [^{99m}Tc]HMPAO uptake of the lower extremities of the subjects was assessed to evaluate peripheral microcirculation.

Methods: Fifty-one, fifty-one, fifty-one, forty-three, and fifty-seven patients with T2DM, and forty-five, forty-five, forty-eight, twenty-six, and forty-six non-DM obese participants were involved in the first, second and third part of the brain metabolism study, the brain perfusion, and the lower limb perfusion studies, respectively. Fasting serum glucose, pre-PET glucose levels, different laboratory parameters characterising metabolic status and Hcys levels were determined. MTHFR genotyping was also performed. Measurement of CPT values applying neurometer (NM-01/CPT) was used to evaluate peripheral nerve dysfunction. [^{18}F]FDG PET/CT (AnyScan PC, Mediso) scans, estimating brain metabolism, were transformed to MNI152 brain map after T1 registration and used for SPM-based group comparison of brain metabolism corrected for pre-PET glucose, for the investigation of Hcys and MTHFR *C677T* polymorphism-related brain metabolism and correlation analysis with laboratory parameters. Then, patients underwent [^{99m}Tc]HMPAO brain perfusion SPECT imaging applying AnyScan S Flex dual-head gamma camera (Mediso, Hungary). Since the SPECT and MRI images were co-registered and transformed to the MNI152 atlas space, data of the following standard VOIs could be obtained: frontal lobe, parietal lobe, temporal lobe, occipital lobe, limbic region, cingulate, insula, basal ganglia, cerebrum, limbic system and brain stem. As part of brain perfusion studies, [^{99m}Tc]HMPAO SPECT/CT examinations of the legs were performed to evaluate the radiopharmaceutical accumulation of the lower limbs.

Results: Pre-PET glucose level dependent hypometabolism was detected in the precuneus in type 2 diabetic individuals compared to the non-DM obese participants. Voxel-based correlation analysis showed significant negative correlation of the metabolism in the following brain regions with pre-PET glucose in diabetes: precuneus, left posterior orbital gyrus, right calcarine cortex and right orbital part of the inferior frontal gyrus; whilst in the obese group only the right Rolandic (pericentral) operculum proved to be sensitive to pre-PET glucose level. Brain regions with decreased metabolism associated with Hcys and MTHFR *C677T* polymorphism were also detected. We pointed out that the presence of diabetes was an independent significant predictor of normalized regional brain perfusion only in the insula. Independent predictors of normalized regional brain perfusion were also depicted: age in the insula and in the limbic region, and BMI in the brain stem. Finally, in the diabetic group significantly lower leg SUV values were detected compared to the non-DM obese group. BMI, age, HbA1c, and glucose level proved to be significant predictors of sural muscle tracer uptake.

Conclusions: To our knowledge, this is the first study to perform pre-PET glucose level corrected comparative analysis of brain metabolism in T2DM and obesity. We also examined the pre-PET glucose level dependency of regional cerebral metabolism in the two groups separately. BMI appeared to be a novel factor affecting brain perfusion. In one specific region, the insula, we detected a difference between the obese and the diabetic group. These findings may be significant in the understanding of the development of cognitive impairment in metabolic diseases. We assume that [^{99m}Tc]HMPAO uptake of leg muscles is associated with microcirculation, so quantitative [^{99m}Tc]HMPAO SPECT/CT might be a sensitive method for the evaluation of lower limb microvascular alterations.

6. Main findings and conclusions

1. Pre-PET glucose level dependent hypometabolism was detected in the precuneus in type 2 diabetic individuals compared to non-DM obese participants. To our knowledge, this is the first study to perform pre-PET glucose level corrected comparative analysis of brain metabolism in T2DM and obesity.
2. Disease dependent glucose sensitive regional brain hypometabolic alterations were detected in the following brain regions: precuneus/posterior cingulate gyrus, left posterior orbital gyrus, right calcarine cortex, right orbital part of the inferior frontal gyrus and in the region of the right Rolandic (pericentral) operculum.
3. Decreased brain metabolism associated with elevated Hcys levels was depicted in the right middle temporal gyrus.
4. Further, disease dependent hypometabolism was found in the region of the left calcarine cortex related to increased Hcys levels in T2DM.
5. To our knowledge this is the first study to detect brain stem hypoperfusion associated with BMI.
6. Hypoperfusion was experienced in the insula in T2DM.
7. In the diabetic group significantly decreased lower limb [^{99m}Tc]HMPAO uptake was found, that may be in connection with peripheral microvascular alterations.
8. Four significant predictors of sural muscle tracer uptake were detected: BMI, age, HbA1c and glucose.

7. Publication

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Subject: PhD Publication List

Candidate: Zita Képes
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Képes, Z.**, Nagy, F., Budai, Á., Barna, S., Esze, R., Somodi, S., Káplár, M., Garai, I., Varga, J.: Age, BMI and diabetes as independent predictors of brain hypoperfusion.
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2. **Képes, Z.**, Aranyi, S. C., Forgács, A., Nagy, F., Kukuts, K., Hascsi, Z., Esze, R., Somodi, S., Káplár, M., Varga, J., Emri, M., Garai, I.: Glucose-level dependent brain hypometabolism in type 2 diabetes mellitus and obesity.
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3. **Képes, Z.**, Aranyi, S. C., Forgács, A., Nagy, F., Kukuts, K., Esze, R., Somodi, S., Káplár, M., Varga, J., Emri, M., Garai, I.: Homocysteine-related alterations of [18F]FDG brain pattern in metabolic diseases.
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