LUDC-IRC Postdoctoral Program 2020 – Project 4

Combined mass spectrometry (MS) and nuclear magnetic resonance (NMR) metabolomics for predicting diabetes-induced dementia

PI: Joao Duarte
Co-PI: Olle Melander

Purpose and aims

Obesity is a multifactorial complex disease that has reached pandemic proportions and is a strong risk factor for T2D, cardiovascular and cerebrovascular disease. In turn, these disorders have been proposed to impact brain function and contribute to dementia. Our overall purpose is to identify circulating biomarkers that can reliably determine future development of dementia. This project has the following specific aims:

1) To develop a combined MS-NMR metabolomics pipeline for blood/plasma samples;
2) To investigate plasma metabolites in the prospective population-based cohort, The Malmö Diet and Cancer Study, including participants who develop vascular dementia (VaD);
3) To investigate plasma metabolites in rodent models of T2D that develop memory impairment.

State-of-the-art/background

Metabolic syndrome and type 2 diabetes (T2D) often progresses from obesity, a pandemic that is favoured by a sedentary lifestyle and the widespread consumption of food products rich in saturated fat and refined carbohydrates (Swinburn et al., 2011). Many factors of the metabolic syndrome impact brain function, such as chronic hyperglycemia, microvascular complications, insulin resistance, dyslipidemia and hypertension (Duarte, 2015; Gaspar et al., 2016). Indeed, there is a growing body of epidemiological evidence suggesting that metabolic syndrome and T2D increase the risk of developing age-related cognitive decline, mild cognitive impairment, vascular dementia, and Alzheimer's disease (AD) (Fizardi et al., 2010; Spauwen et al., 2013).

Processes leading to brain dysfunction might be initiated or aggravated by signals derived from peripheral tissues (skeletal muscle, adipose tissue, liver), which are important nodes for energy utilization. Indeed, dysregulation of systemic energy homeostasis during metabolic disease can change nutrient availability, and promote a hyperglycaemic, hyperinsulinemic, pro-inflammatory environment which will ultimately also affect the brain (Johnson et al., 2012; Maciejczyk et al., 2019). Therefore, metabolic profiling might not only lead to a better understanding of disease mechanisms, but also provide potential biomarkers for improved diagnosis towards precision medicine. In the particular case of this project proposal, alterations of circulating metabolites might provide biomarkers for future development of cerebral derangements leading to dementia.

Metabolomics by NMR

Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the most widely employed methods to analyse metabolites in blood. While MS is a highly sensitive method that detects several hundreds of metabolites in a single measurement, its utilisation for absolute quantification requires isotope-labelled compounds as internal standards (1 standard per metabolite). Therefore, relative peak areas are often reported. Since peak areas also depend on the instrumentation used, they do not directly represent relative concentrations. Although less sensitive, NMR is able to provide absolute metabolite concentrations. Other advantages are of NMR include its high reproducibility and minimal sample preparation (Nagana Gowda et al., 2018). Importantly, NMR is a non-destructive method, and thus the analysed sample is further available for other purposes. MS has been applied at the LUDC for the study of blood metabolomics. We propose to combine MS with NMR spectroscopy, thus widening the metabolic information extracted from each sample.

Significance and scientific novelty

The acknowledgement of multiple subgroups of adult diabetes highlights the need for novel and more personalized approaches characterise the diabetic phenotype and prevent expected diabetes comorbidities. In this project, we will develop a metabolomics pipeline that combines NMR and MS for expanding the panel of metabolites analysed. This will be applied to investigate early biomarkers of cognitive impairment, for which obese and diabetic patients are risk factors.

Preliminary and previous results

Preliminary findings obtained by MS from the Malmö Diet and Cancer Study (MDCS)

Vascular dementia (VaD) is the second most common form of dementia and is attributed to cerebrovascular pathologies. VaD is believed to be related to cardiometabolic risk factors but so far, metabolic alterations preceding VaD have not been studied. A total of 115 plasma metabolites were measured by MS in the prospective population-based cohort, the MDCS (N=3833), including 169 participants who develop vascular dementia during an average follow-up time of 19.3 years. In cox regressions, adjusted for age and sex, eight metabolites were significantly (false discovery rate < 0.05) associated with in increased risk of future VaD. These included seven medium- or long-chain acylcarnitines. Some remained significantly associated with incident VaD when fully adjusted for metabolic syndrome and cardiovascular disease confounders, pointing towards novel associations between long-chain acylcarnitines and future vascular dementia, which were independent of cardiometabolic risk factors. Further studies are needed to test whether altered metabolism of acylcarntines are involved in the pathogenesis of vascular dementia, or if they are biomarkers of early disease progression.

Mouse models for investigating blood metabolomics

We have established models of obesity-associated metabolic syndrome, which are based on exposure to high-fat and/or high sugar diets. We observed a gender dimorphism in glucose intolerance and insulin resistance in mice under a high-fat and high-sucrose diet (HFHSD). Notwithstanding the more severe phenotype in male mice, HFHSD-induced memory dysfunction was observed in both genders (figure 1). Interestingly, dietary reversal improved memory performance, suggesting plasticity of diet-induced brain alterations. We have also obtained data showing the temporal course of cognitive decline induced by ingestion of a high-fat diet (HFD). Short periods of exposure to a 60% HFD, such as 1 week, have already a noticeable tendency for reduced memory performance (figure 2). Robust cognitive deterioration in mice is patent after 4 weeks of HFD exposure. Blood/plasma samples from these models will allow to test how metabolic biomarkers relate to brain structure and function.

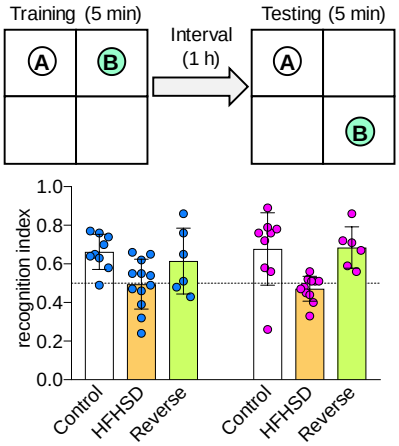

Figure 1. Preliminary results showing impaired memory performance caused by HFHSD exposure for 6 months. Mice have preference for exploring objects moved to novel location, but HFHSD mice do not recognise novelty. Dietary normalisation (reverse) after 4 months of HFHSD leads to memory improvement. Male=blue, female=pink dots.

Pilot NMR spectroscopy experiments

Preliminary NMR spectra were collected from plasma and whole blood samples (figure 3). We tested NMR spectroscopy at 500 MHz (apparatus at the Medical Faculty) in various types of blood, plasma or tissue samples/extracts. Compared to plasma, the extraction of whole blood metabolites gives us access to signals from intracellular cofactors and nucleotides (spectra rich in peaks above 6 ppm), which are not readily observed in plasma. The extraction method will be further optimised to retain the maximum metabolic information from each plasma/blood sample. Sensitivity can further be improved by using the new 600 MHz spectrometer equipped with cryo-coil that will be installed at the Chemistry Department during the summer of 2020.

Figure 2. Development of HFD-induced memory impairment in mice, as detected by the spontaneous alternation in a Y-maze.

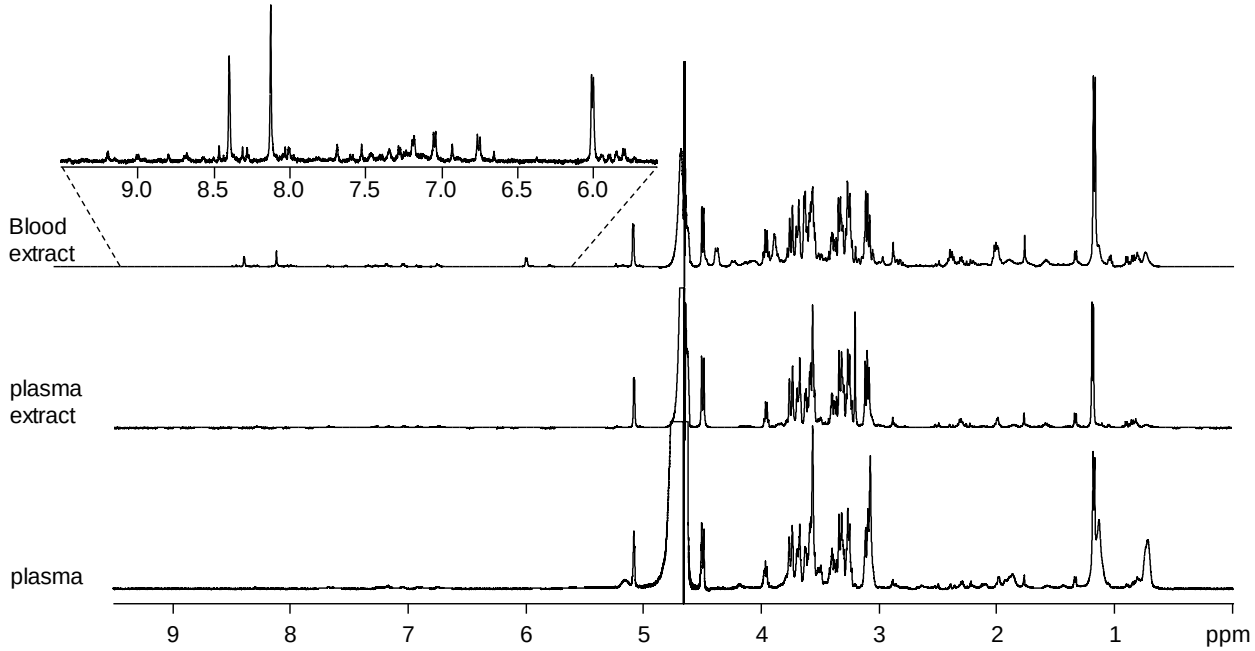

Figure 3. Typical NMR spectra obtained in a pilot study on the 500 MHz spectrometer at the Faculty of Medicine. Top: whole blood metabolite extract with a chloroform-methanol mixture. Middle: plasma extract obtained after protein precipitation with methanol. Bottom: spectrum acquired from plasma sample directly dissolved in D2O. All spectra were obtained with a CPMG sequence modified to include pre-saturation of the water peak.

Research plan

All analyses will be conducted on blood, which is the most widely studied specimen in metabolomics because of easy collection. The fact that blood flows through every organ, renders it a particularly attractive biological sample for investigating disease-associated pathways, as well as identifying peripheral metabolites that impact brain function.

Aim 1) To develop a combined MS-NMR metabolomics pipeline for blood/plasma samples

For developing robust analytical capabilities, this aim will encompass the 3 following tasks:

1. Optimisation of sample preparation and NMR spectroscopy protocols;
2. Implementation of data processing pipeline that includes data reduction and multivariate pattern recognition analysis in NMR spectra, as well as determination of metabolite concentrations.
3. Development of statistical integrative analysis of metabolomics data from both NMR spectroscopy and MS.

Aim 2) To investigate plasma metabolites in the MDCS

We will conduct NMR spectroscopy on samples from the MDCS, including from participants who develop vascular dementia. Obtained metabolomics data will be integrated with that obtained by MS. All data will be analysed together, including multivariate data analyses and the selection of potential biomarkers that predict the risk of developing dementia.

Aim 3) To investigate plasma metabolites in rodent models

We will perform NMR spectroscopy and MS on blood samples from mice exposed to obesogenic diets, which readily develop metabolic syndrome.

1. Mice will be exposed to HFHSD for 6 months, HFHSD for 4 months followed by reversal to control diet for other 2 months, or regular control diet for 6 months (as in figure 1).
2. Mice will be exposed to diets containing either 10% (control) or 60% fat content (HFD, as in figure 2). Blood samples will be collected at after 1 and 4 weeks of exposure to these diets.

Data will be analysed with the tools developed above (aim 1) to validate potential biomarkers from human blood (aim 2).

Time plan:

Year 1				Year 2			
Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Aim 1							
		Aim 2					
			Aim 3				

Implementation/organisation:

Melander's lab has access to samples from the MDCS and expertise on MS metabolomics. Duarte's lab has expertise on mouse behavior testing (Duarte is manager of the Mouse Behavior Platform at BMC), and on NMR spectroscopy (in vivo and on tissue extracts). A research engineer from Duarte's lab (Sara Larsson) will conduct metabolic characterization of mouse models, cognitive function assessments, and sample collection.

References

Duarte JMN (2015) Metabolic alterations associated to brain dysfunction in diabetes. *Aging Dis* 6(5): 304-321.

Frisardi V, Solfrizzi V, Seripa D, Capurso C, Santamato A, Sancarlo D, Vendemiale G, Pilotto A, Panza F (2010) Metabolic-cognitive syndrome: a cross-talk between metabolic syndrome and Alzheimer's disease. *Ageing Res Rev* 9(4): 399-417.

Gaspar JM, Baptista FI, Macedo MP, Ambrósio AF (2016) Inside the Diabetic Brain: Role of Different Players Involved in Cognitive Decline. *ACS Chem Neurosci* 7(2): 131-142.

Johnson AR, Milner JJ, Makowski L (2012) The Inflammation Highway: Metabolism Accelerates Inflammatory Traffic in Obesity. *Immunol Rev* 249(1): 218-238.

Maciejczyk M, Żebrowska E, Chabowski A (2019) Insulin Resistance and Oxidative Stress in the Brain: What's New? *Int J Mol Sci* 20(4) pii:E874.

Nagana Gowda GA, Djukovic D, Bettcher LF, Gu H, Raftery D (2018) NMR-Guided Mass Spectrometry for Absolute Quantitation of Human Blood Metabolites. *Anal Chem* 90(3): 2001-2009.

Spauwen PJ, Köhler S, Verhey FR, Stehouwer CD, van Boxtel MP (2013) Effects of type 2 diabetes on 12-year cognitive change: results from the Maastricht Aging Study. *Diabetes Care* 36(6): 1554-1561.

Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, Gortmaker SL. (2011). The global obesity pandemic: shaped by global drivers and local environments. *Lancet* 378: 804-814.