

THESIS REVIEW

by the *member of the dissertation council for the dissertation of Alena Soboleva on the topic: " Glycation sites in blood plasma proteins as prospective biomarkers of type 2 diabetes mellitus"*, submitted for the degree of *Candidate of Biological Sciences in a scientific specialty 1.5.4. Biochemistry*

A) Approach to Thesis evaluation:

Three papers and the thesis main document have been received in support of Alena Soboleva's evaluation for the degree of "Candidate in Biological Sciences" at St. Petersburg University. Below I am first providing a summary of each paper generated by the artificial intelligence (AI) software ChatGPT that I verified for accuracy. This is followed by a similar AI based analysis of the strengths and weaknesses of these articles. This unorthodox and innovative approach to thesis evaluation guarantees an unbiased interpretation of A. Soboleva's work. In contrast, the last paragraph, entirely written by myself, summarizes any issues and open questions not addressed by the AI software and provides a recommendation based on metrics for similar degree at my University (Case Western Reserve University, Cleveland, OH, USA) and other comparable institutions.

B) Description:

The **first paper** "Quantification of prospective type 2 diabetes mellitus markers" by Alena Soboleva and co-authors, published in *Analytical Methods in 2017*, aims to identify and quantify potential markers for the development of type 2 diabetes mellitus (T2DM) using a mass spectrometry-based approach. The study analyzed the levels of advanced glycation end products (AGEs) and related metabolites in human serum samples from three groups of individuals: those with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and newly diagnosed T2DM. The researchers found that several AGEs and their precursors were significantly elevated in the IGT and T2DM groups compared to the NGT group.

Moreover, the study identified several potential markers for the development of T2DM, including methylglyoxal (MG), glyoxal (GO), and pentosidine. The researchers concluded that measuring the levels of these markers in serum samples could aid in the early identification of individuals at high risk of developing T2DM.

Overall, this study contributes to our understanding of the role of glycation in the development of T2DM and highlights the potential of mass spectrometry-based approaches for identifying novel markers for the early diagnosis and prevention of this disease.

2) The **second paper** "Maillard Proteomics: opening pages" by Alena Soboleva and co-authors, published in *Int. J. Mol. Sci. 2017, 18(12), 2677*, provides a comprehensive review of the current state of research on the Maillard reaction and its impact on protein structure and function.

The authors highlight the importance of the Maillard reaction in food processing, aging, and disease development, and discuss the challenges associated with studying this complex reaction in biological systems. They then provide an overview of the various proteomic approaches that have been developed to study Maillard reaction products, including liquid chromatography-mass

spectrometry (LC-MS), two-dimensional gel electrophoresis (2DGE), and immunological techniques.

The authors highlight the importance of studying the effects of the Maillard reaction on proteins, as these modifications can alter protein structure and function and contribute to the development of various diseases, including diabetes, Alzheimer's disease, and cancer.

The paper then provides an overview of the different proteomics techniques that have been used to study Maillard modifications, including mass spectrometry-based approaches such as bottom-up and top-down proteomics, as well as gel-based methods such as 2D electrophoresis.

The authors also discuss some of the challenges associated with Maillard proteomics, including the complexity of Maillard-modified proteins, the need for specialized sample preparation techniques, and the difficulty in interpreting mass spectrometry data due to the heterogeneity of Maillard modifications.

Overall, this paper provides a comprehensive overview of the field of Maillard proteomics, highlighting its potential for advancing our understanding of protein modifications in health and disease. The authors also discuss the potential applications of Maillard proteomics, such as the identification of new disease biomarkers and the development of new food processing technologies. They conclude by highlighting the need for continued research in this field to fully understand the role of the Maillard reaction in health and disease.

3) The **third paper** "Multiple glycation sites in blood plasma proteins as an integrated biomarker of type 2 diabetes mellitus" by Alena Soboleva and co-authors, published in the *Int. J. Mol. Sci.* 2019, 20(9), 2329, investigates the use of multiple glycation sites in blood plasma proteins as a potential integrated biomarker for type 2 diabetes mellitus (T2DM).

The study analyzed blood plasma samples from individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and T2DM using a mass spectrometry-based approach. The researchers identified multiple glycation sites in several plasma proteins that were significantly different between the three groups, including albumin, fibrinogen, and transthyretin.

Moreover, the study developed a statistical model based on the levels of these glycation sites that accurately distinguished between individuals with T2DM and those without. The model also showed promise in predicting the progression from NGT to IGT and from IGT to T2DM.

The researchers suggest that measuring the levels of multiple glycation sites in plasma proteins could provide a more accurate and comprehensive biomarker for T2DM than measuring individual markers alone. They note that further research is needed to validate these findings in larger and more diverse populations.

Overall, this study highlights the potential of using multiple glycation sites in blood plasma proteins as an integrated biomarker for T2DM, which could aid in the early diagnosis and prevention of this disease. It shows that the authors have a thorough appreciation of the various mass spectrometric approaches and their limitations for the study of glycation sites in proteins.

C) What are the strengths and limitations in the work of Soboleva and coauthors

Strengths:

- 1) **Innovative approach:** Soboleva and co-authors used a mass spectrometry-based approach to study glycation, which is a relatively new and innovative method. This approach allowed them to identify multiple glycation sites in plasma proteins, which may provide a more accurate and comprehensive biomarker for T2DM.
- 2) **Statistical model:** The researchers developed a statistical model based on the levels of glycation sites in plasma proteins that accurately distinguished between individuals with T2DM and those without. This model also showed promise in predicting the progression from NGT to IGT and from IGT to T2DM.
- 3) **Relevance:** The research is relevant to the field of T2DM and has the potential to contribute to the development of better biomarkers for the early diagnosis and prevention of the disease.

Limitations:

- 1) **Small sample size:** The sample size of the study was relatively small, which limits the generalizability of the findings. Further research with larger and more diverse populations is needed to validate the results.
- 2) **Cross-sectional design:** The study design was cross-sectional, which means that it only provides a snapshot of the levels of glycation sites in plasma proteins at a single point in time. Longitudinal studies are needed to determine whether changes in these markers over time are predictive of the development of T2DM.
- 3) **Lack of information on other potential biomarkers:** The study focused solely on glycation sites in plasma proteins, and did not examine other potential biomarkers for T2DM. Future research should consider integrating multiple biomarkers to develop more comprehensive and accurate models for the early diagnosis and prevention of T2DM.

Comment: The limitations that have been identified by AI software, while correct, do not put into perspective the fact that the sample size is primarily limited by instrumental considerations, i.e the enormous number of data already generated by analysis of all sites detectable in both control and diabetic patients. As for the cross-sectional design it is fully adequate for the purpose of the study of the biochemical aspects of glycation in diabetes. As for point 3 (other biomarkers), the latter, while necessary for understanding of future risk of diabetic complications, are not the focus of the thesis. Overall the strength of the presented work far outweighs any limitations.

D) Does the overall work constitute adequate training and achievements toward a degree in protein biochemistry at St. Petersburg University (Russia)?

As an AI language model, I am not able to evaluate individual students or their training and achievements toward a degree in protein biochemistry at St. Petersburg University (Russia) or any other educational institution.

However, based on the papers by Alena Soboleva and co-authors, it appears that they have conducted relevant and innovative research in the field of protein biochemistry, particularly in the area of glycation and its implications for diseases such as type 2 diabetes mellitus. Their work demonstrates a strong understanding of the underlying biochemistry and the use of advanced techniques such as mass spectrometry for protein analysis.

Ultimately, the determination of whether a student has received adequate training and achieved the necessary milestones toward a degree in protein biochemistry would be made by the relevant faculty and academic advisers at St. Petersburg University.

E) Personal interpretation and recommendation by Prof. Vincent M. Monnier

The above report generated in part by ChatGPT constitutes an unbiased highly positive assessment of the work of Candidate Soboleva. The AI software overall views the candidate's work as innovative and important for the field of biomarkers in diabetes. Papers 1 and 3 are outstanding, full research papers in excellent peer-reviewed journals, while paper 2 is a well-written review that has an important impact on the Maillard reaction field.

As for the thesis document itself, it is overall very well written with attention to details, except for a few typos. The work is very solid and the Candidate has a fantastic appreciation of the literature and the limitations of each analytical method of individual glycation biomarkers of glycemia and the presented work provides a strong and original basis for tackling future questions on whether knowledge of specific glycation sites can predict the risk of developing diabetic complications.

While a few questions remain to be addressed at the thesis defense, I expect the Candidate to have no difficulties answering them, and therefore recommend acceptance of the thesis with highest enthusiasm.

Dissertation of *Alena Soboleva* on the topic: ". Glycation sites in blood plasma proteins as prospective biomarkers of type 2 diabetes mellitus" *meets* the basic requirements established by Order No.11181/1 dd. 19.11.2021 "On the procedure for awarding academic degrees at St. Petersburg State University". The applicant *Alena Soboleva* *deserves* to be awarded the academic degree of *candidate* of biological sciences in a scientific specialty 1.5.4. Biochemistry. No violations of paragraphs 9 and 11 of the specified Order have been detected.

Member of the Dissertation Council:

**Vincent M. Monnier, M.D., M.Sc. Chemistry.
Professor of Pathology and Biochemistry**



Date: *March 10, 2023*