Glycosylation signatures of type 2 diabetes complications and metabolic syndrome

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English Summary

Glycosylation is a complex biochemical modification that occurs on proteins and lipids, playing a critical role in numerous biological processes such as cell-cell communication, immune interactions, and signaling. This intricate process contributes to adhesion, cell-matrix interactions, and immune system modulation, underpinning many physiological and pathological events. Aberrant glycosylation is a hallmark of various diseases, including cancer, metabolic disorders and type 2 diabetes (T2D), where it associates with disease onset, progression, and outcomes. Alterations in glycan structures have significant implications for cell behavior, including tumor invasion, angiogenesis, and metastasis in cancer or inflammation and immune dysregulation in chronic metabolic diseases.

The aim of the research described in this thesis was to explore the glycomic signatures associated with T2D complications, and metabolic syndrome (MetS). Recognizing the potential of glycan biomarkers for diagnostic and therapeutic applications, this work focused on leveraging advanced techniques to uncover disease-specific glycosylation patterns. We pursued to bridge existing gaps in early detection and risk stratification by integrating glycomic profiling into the context of chronic metabolic diseases. To achieve these objectives, the research employed four glycoanalytical methodologies, each tailored to the specific goals of individual chapters. **Chapter 2** utilized ultra-performance liquid chromatography (UPLC) for in-depth *N*-glycomic analysis. In **Chapter 3**, high-throughput plasma *N*-glycan profiling was applied utilizing a matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF)/ or Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (MS). **Chapter 4** used an innovative approach for Immunoglobulin G (IgG) *N*-glycosylation analysis, leveraging automated workflows to examine *N*-glycan alterations linked to dysregulation in T2D complication. Finally, **Chapter 5** focused on extending these methods to dried blood spots (DBS) using Ultra-High Performance Liquid Chromatography (UHPLC) .

To establish a comprehensive understanding of the clinical challenges associated with MetS and T2D complications, the first part of this thesis provides an in-depth examination of these topics (**Chapter 1**). It describes the biological significance of glycosylation, with a particular focus on its role in MetS and T2D complications. Moreover, this chapter delves into the limitations of traditional biomarkers, such as fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), etc., in diagnosing and managing these conditions. It introduces *N*-glycosylation as a promising avenue for biomarker discovery. Additionally, the chapter provides an overview of glycosylation analysis techniques, including Hydrophilic Interaction Liquid Chromatography (HILIC) coupled with UPLC and MALDI-MS, to contextualize their application. By integrating glycomic data from diverse populations, the research offers valuable insights into T2D complications and MetS.

In **Chapter 2**, the relationship between MetS and suboptimal health status (SHS) was explored through the application of *N*-glycosylation profiles in a Ghanaian population. Using UPLC and advanced statistical methods, it identified specific glycan patterns that predict MetS and SHS with notable accuracy, emphasizing the potential for early detection and intervention. Genetic and environmental factors were shown to shape glycosylation patterns, with comparisons among Ghanaian, Chinese, Croatian, and Orcadian populations highlighting both shared and distinct features. The chapter investigated the link between SHS and MetS, identifying SHS as a potential precursor to chronic conditions. Complex *N*-glycan structures were shown to be associated with MetS progression, suggesting their utility as biomarkers for identifying at-risk individuals. Gender-specific differences in glycosylation patterns were also examined, revealing potential hormonal influences on these traits. These findings emphasized the need for tailored approaches in studying metabolic diseases.

Chapter 3 investigates the *N*-glycosylation patterns of plasma proteins in relation to complications of T2D, including cardiovascular disease (CVD), nephropathy, and retinopathy. Using advanced mass spectrometry techniques, the research analyzed 68 individual glycan compositions and 45 glycosylation traits representing structural features of glycans. Significant associations were identified between glycan features and diabetes complications. For cardiovascular disease, increased α2,6-sialylation was observed, a feature often linked to inflammation and acute-phase responses. Changes in fucosylation and galactosylation patterns, which are associated with immune modulation, were also prominent. Specific glycan branching and bisection patterns correlated with nephropathy and retinopathy, revealing insights into the mechanisms driving these complications. These findings highlight the potential of specific glycosylation patterns to serve as biomarkers for the early detection and prediction of diabetes-related complications.

Building on prior findings (**Chapter 3**) that linked total plasma *N*-glycan patterns to complications of T2D, **Chapter 4** delves deeper by examining the glycosylation of immunoglobulin G (IgG) and its specific associations with microvascular and macrovascular complications. It identifies significant connections between IgG *N*-glycan traits and complications such as nephropathy, retinopathy, and cardiovascular disease. IgG galactosylation is negatively associated with both prevalent and incident diabetic nephropathy and macrovascular disease, suggesting that higher levels of galactosylation may have a protective effect against these complications. Similarly, sialylation is linked to a reduced risk of incident nephropathy, further supporting its role in mitigating inflammation, a key factor in diabetes-related vascular damage. In the case of retinopathy, galactosylation exhibits a negative association in cross-sectional and prospective analyses, although its significance diminishes when adjusted for additional clinical factors. These findings underscore the potential of IgG *N*-glycans as biomarkers of inflammation and predictors

of vascular complications in T2D. By integrating data from three diverse cohorts and employing robust meta-analyses, the study ensures the reliability of its findings. The observed associations with galactosylation and sialylation suggest these glycan features could serve as biomarkers for early detection and risk assessment.

The ensuing chapter (**Chapter 5**) investigates the potential of dried blood spots (DBS) as a reliable and practical method for *N*-glycan profiling. DBS sampling provides a convenient, non-invasive, cost-effective alternative to traditional blood collection, offering advantages in sample collection, transportation, and storage. This research focused on evaluating the reliability of DBS for *N*-glycan analysis across various blood preparations, including fresh blood, frozen whole blood, and mixtures of separated blood cells and plasma. The study demonstrated that DBS *N*-glycan profiles are consistent across various preparation and drying conditions, supporting their robustness for *N*-glycan analysis even in stored or repurposed samples. Comparative analyses between DBS and plasma profiles revealed high similarity, with only minor differences, validating DBS as a reliable source for glycomic investigations. In addition, the study explored differences in *N*-glycan profiles between pre-diabetic and diabetic individuals. The study observed trends of increased fucosylation, bisection, and galactosylation, along with decreased sialylation, in diabetic samples relative to pre-diabetic ones. While these trends were not statistically significant due to limited sample size, they indicate potential glycosylation changes associated with the progression from pre-diabetes to diabetes.

Finally, the last part of this thesis (**Chapter 6**) provides a comprehensive discussion on the findings, focusing on future developments and the clinical potential of *N*-glycan profiling as a biomarker for T2D complications and MetS. It emphasizes the relevance of distinct glycosylation patterns in total plasma proteins and IgG, as well as the utility of dried blood spots (DBS) for glycomic analysis. These results highlight the potential for advancing diagnostics and personalized medicine, paving the way for the integration of glycomics into clinical applications for early detection and improved management of MetS, T2D and its complications.