# *N*-glycan profiling of kidney brush border membrane from rats using LC-MS/MS analysis


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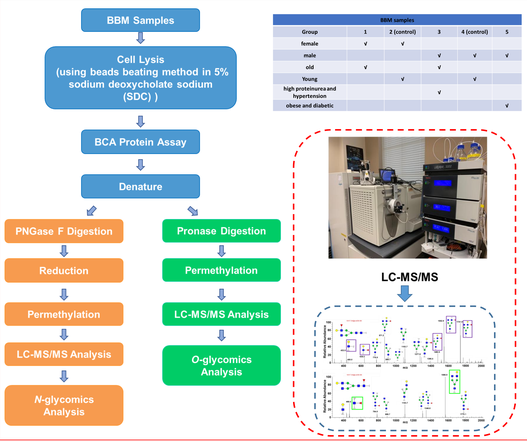
## Introduction

Chronic kidney disease (CKD) is defined as a disease that reduces kidney function and causes kidney damage. Hypertension and diabetes are both the causes and effects of CKD. Proteinuria, which is an important marker of kidney damage, increases with age and is caused by the alternation of glomerular and proximal tubules. To investigate how kidney function is changed by these chronic medical conditions, age-related proximal tubule brush border membrane (BBM) from rats' kidney cortexes are used. The BBMs, which may possess significant biochemical changes, are from 2 disease-free controls and 3 groups with either hypertension, proteinuria, or diabetes. In this study, glycomic analysis was performed to determine if different glycan expressions are associated with these diseases.

## Method

BBMs were isolated using a well-established divalent cation precipitation method. This method enables selective precipitation of other membranes and high-speed pelleting to obtain purified BBM. Cell lysis was performed on BBM by beads beating. Then a BCA protein assay was used to estimate protein amount. Next, BBM pellets were denatured, followed by PNGase F digestion. After 18 hours, formic acid was added to remove SDC and dialysis was used to remove salt. Released glycans were then reduced and permethylated prior to LC-MS/MS analysis. A 75μm×150mm Acclaim C18 column was used at 55˚C with flow rate settings at 350nl/min on a Dionex Ultimate 3000 nano-LC system interfaced to an LTQ Orbitrap Velos mass spectrometer.

## Workflow



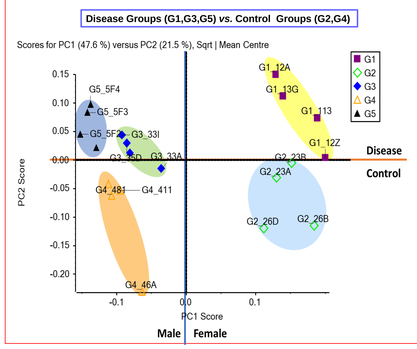

| BBM samples | | | | | |
|---|---|---|---|---|---|
| Group | 1 | 2 (control) | 3 | 4 (control) | 5 |
| female | √ | √ | | | |
| male | | | √ | √ | √ |
| old | √ | | √ | | |
| Young | | √ | | √ | |
| high proteinurea and hypertension | | | √ | | |
| obese and diabetic | | | | | √ |

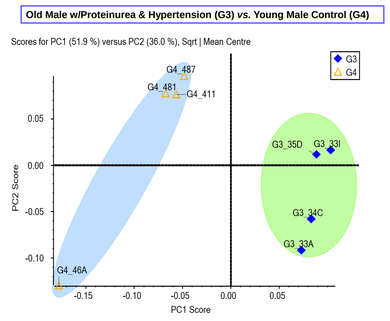


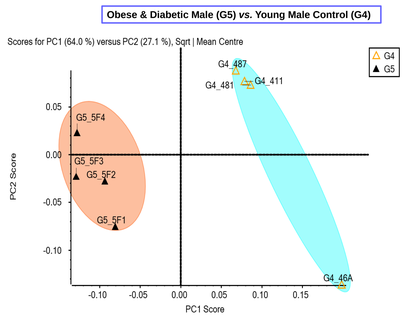
LC-MS/MS



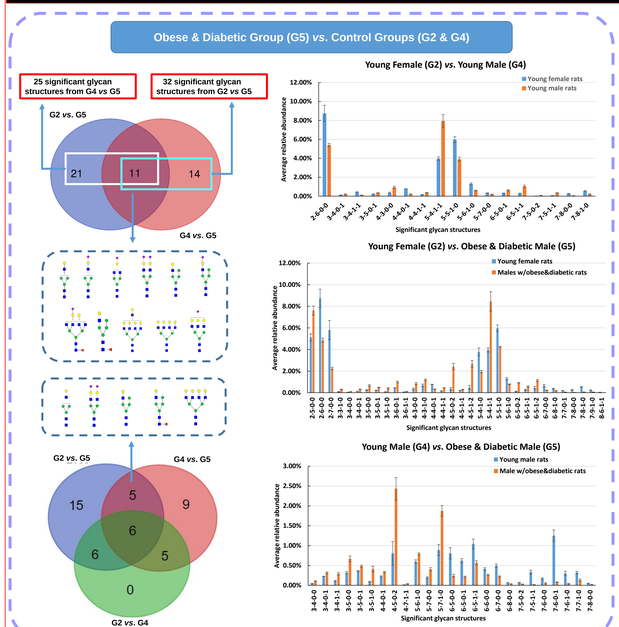
## Principal Component Analysis (PCA ) for *N*-glycomics Analysis

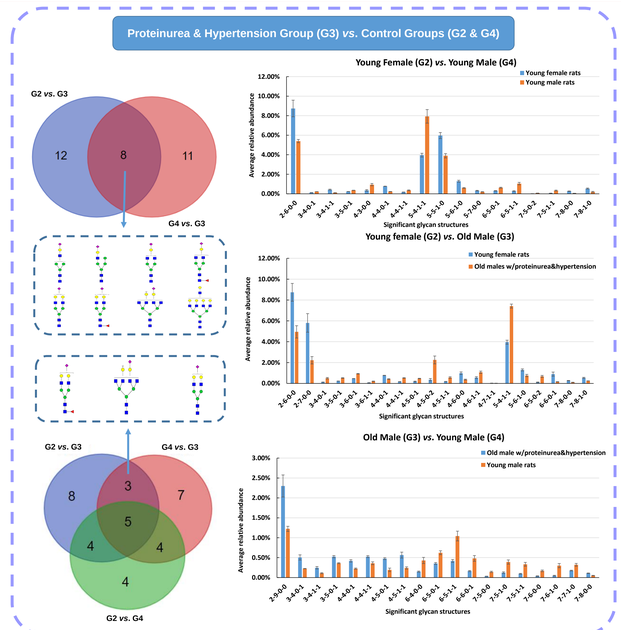


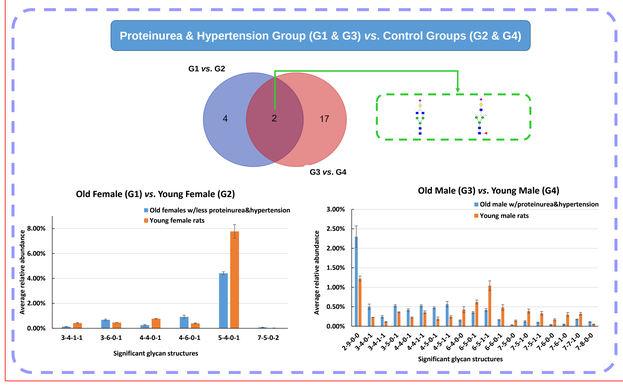



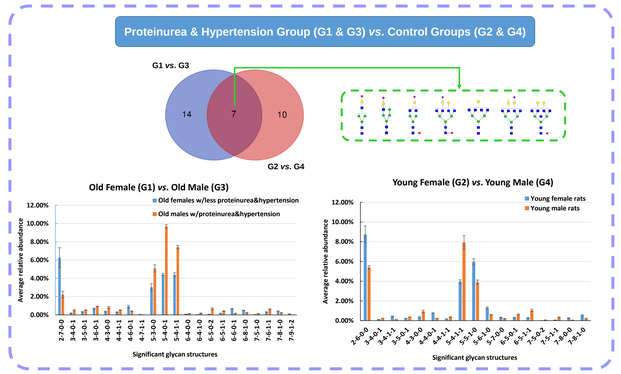




## Comparison of Significant *N*-glycans between Disease-related Groups and Control Groups

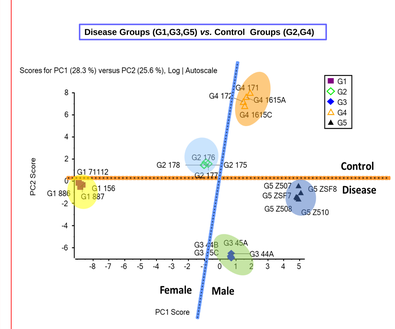


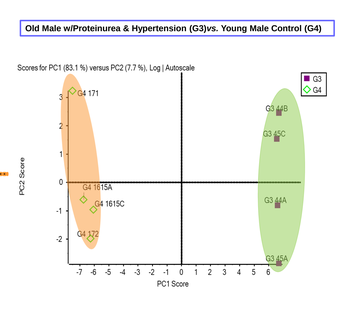



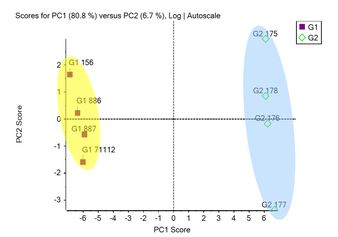




## Principal Component Analysis (PCA ) for *O*-glycomics Analysis



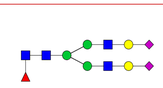








**Nomenclature**

**Example**



| HexNAc | Hex | DeoxyHex | NeuAc |
|---|---|---|---|
| 4 | 5 | 1 | 2 |

Here is an example of N-glycan nomenclature. The model glycan structure contains 4 HexNAc, 5 Hex, 1 Fucose (DeoxyHex), and 2 Neuraminic acid (NeuAc) residue. Hence, the nomenclature is [4512].
Symbols: □ , GalNAc; ■ , GlcNAc; ○ , Galactose; ● , Mannose; ▼ , Fucose; ◆ , N-acetylneuraminic acid.

## Conclusion

- The PCA plots were generated using quantitative results of the 121 *N*-glycans and 69 *O*-glycans that were identified in the 5 groups. When the PCA of the 5 groups was plotted, 5 clusters were distinguishable without any overlap indicating that there were distinct differences between the control and disease groups.
- Quantitative values of the 121 glycans were filtered out by student's t-test with a Bonferroni correction ($p<0.000413$). Bar graphs and Venn diagrams for significant *N*-glycans indicated that *N*-glycans were differently expressed between disease and control groups.
- When compared controls to obese and diabetic group, Venn diagrams showed that 5 out of 11 *N*-glycans might be obese and diabetic related. *N*-glycans including $HexNAc_3Hex_4$, $HexNAc_3Hex_5$, $HexNAc_4Hex_5NeuAc_2$ and $HexNAc_3Hex_5Fuc_1$ were upregulated, while $HexNAc_6Hex_7$ was downregulated in disease group.
- When compared controls to the group with proteinurea and hypertension, Venn diagrams indicated that 3 out of 8 *N*-glycans might be related to proteinurea and hypertension. $HexNAc_4Hex_5Fuc_1NeuAc_1$, $HexNAc_4Hex_5NeuAc_1$ were upregulated, while $HexNAc_6Hex_6Fuc_1$ was downregulated in disease group.
- In the Venn diagram of group 1,2 and 4 comparison, 2 *N*-glycans might be age- and disease-related in overlap area.
- In the Venn diagram of group 1,2,3 and 4 comparison, 7 *N*-glycans might be gender-related in overlap area.

## Acknowledgement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