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A Case-control Study in an Orcadian Population Investigating the Relationship between Human Plasma N-glycans and Metabolic Syndrome

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Keywords: Glycomics; Human plasma glycome; Insulin resistance; Metabolic syndrome; N-glycans

Introduction

Glycosylation refers to the enzymatic post-translational modification in which the addition of complex oligosaccharide molecules (glycans) enriches protein complexity and functional diversity [1]. Glycans have a broad spectrum of biological roles, including their influence on protein folding, cell signaling and immune function [2]. Alongside the genome and proteome, the human glycome is subject to immense variation [3]. This variation arises as the reactions that link individual sugar units together are influenced heavily by several factors, both genetic and environmental, including cell metabolism, genomic enzyme expression and nutrient availability [4]. N-glycosylation, in which the glycan is linked with nitrogen of asparagine or arginine side-chains, is the most common type of glycosidic bond. N-glycan profiles have been found to remain highly stable over periods of time, with even up to one year between measurements [5]. This suggests that alterations to human N-glycan profiles may be due to pathological mechanisms or complex environmental exposures.

Recent advances in high throughput analytical technology, including High Performance Liquid Chromatography (HPLC), have fueled a growing interest in the role of glycans in health and disease [6]. Glycans have already been implicated in the pathology of numerous inflammatory disorders, including inflammatory bowel disease, various cancers and rheumatoid arthritis [3,7].

Metabolic Syndrome (MetS) is defined as a cluster of biochemical and physiological characteristics including hypertension, central obesity, hyperglycemia and dyslipidemia [8]. This syndrome is a global epidemic, which sets the scene for Type 2 Diabetes Mellitus (T2DM) and its microvascular complications, alongside hastening the progression of macrovascular pathologies. On a global scale the prevalence of MetS is rising, hence there is a growing interest in investigating the alterations in metabolism which are involved in pathophysiological processes underlying this syndrome.

Our current understanding of the cardiovascular and metabolic pathways associated with this syndrome presents a complex array of pathological alterations which contribute to endothelial dysfunction, plaque formation and increased thrombotic potential. It has been demonstrated that the increased levels of angiotensin II associated with high blood pressure leads to the activation of many atherogenic processes including the promotion of monocyte adhesion through the increased expression of adhesion molecules [9,10]. Angiotensin II is also known to increase oxidative stress, thrombosis and vasoconstriction [11], whilst several of the adipokines produced in adipose tissue have been found to also have a role in these pathways. The adipokine tumor necrosis factor alpha has been found to contribute to insulin resistance whilst increased circulating levels of PAI-1 have been linked to the heightened thrombotic state found in obesity [12,13].

Abstract

Background: Alterations in glycosylation patterns have long been known to reflect changes in cell metabolism. In this study, we investigated the relationship between human N-glycan profiles and metabolic syndrome.

Method: Between 2005 and 2011, 2,155 individuals from the Orkney Islands (UK) were recruited and biological material, alongside phenotypic measures were collected. Individual N-glycan profiles were measured in plasma using weak anion exchange high performance liquid chromatography and calibrated hydrophilic interaction liquid chromatography. Pre-specified criteria were used to identify 564 cases with metabolic syndrome and 1475 controls. We applied logistic regression to test for association between this binary outcome against measured plasma N-glycans. We also assessed the correlation between N-glycan traits and individual components of metabolic syndrome and compared this to results found in similar analyses based in Chinese and Croatian populations.

Results: 21 N-glycan traits were found to be associated with either an increased or a decreased likelihood of participants having metabolic syndrome, including monosialylated plasma N-glycans (OR of 1.49 (95% CI 1.33, 1.67), q=1.26E-12) and core fucosylated plasma N-glycans (OR of 0.81 (95% CI 0.72-0.90), q=7.75E-4). Notably, consistent results in both sections of this analysis demonstrated the protective association of higher levels of core fucosylated N-glycans.

Conclusion: Our results demonstrate that metabolic syndrome is associated with an alteration in plasma N-glycosylation patterns. The metabolic role of core fucosylated N-glycans is of particular interest for future study.

Keywords: Glycomics; Human plasma glycome; Insulin resistance; Metabolic syndrome; N-glycans

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It is thought that insulin resistance also contributes to the classic scenario of endothelial dysfunction and vascular inflammation. Alongside its effect on free fatty acids, insulin insensitivity causes an impairment of the Phosphatidylinositol-3-kinase pathway (PI-3K), which ensures it's anti-inflammatory and vasodilatory effects are ceased. Alongside this, insulin resistance leads to an amplification of the mitogen-activated protein kinase pathway, thus leading to increased production of pro-inflammatory molecules like vascular cellular adhesion molecule-1 and an increased demand for extracellular matrix production [14]. It is thought that such pathways contribute to the classic pathological scenario of endothelial dysfunction and vascular inflammation. Importantly, it is understood that the vascular complications associated with insulin resistance begin before hyperglycaemia is evident in the clinical setting, thus the identification of risk earlier in these patients may be key in providing targeted interventions.

It is evident that the role of glycosylation in the alterations of cell—cell interactions and signaling may be an important area of study. As many of the pathways associated with MetS involve the production of inflammatory cells, glycosylation profiles may prove to be important in providing insight into the metabolic state of productive cells. There is also potential for glycosylation profiles to be useful in identifying those at increased risk of the pathologies associated with MetS, alongside potentially identifying those who have subclinical levels of insulin resistance and atherosclerotic disease. Thus it is evident that the many metabolic changes present in MetS may ensure individuals present with a complex array of N-glycosylation alterations.

The first and only attempt to date at screening for novel glycomic biomarkers for MetS was undertaken by Lu et al. in Chinese and Croatian populations [15]. This study successfully identified several glycan traits which were found to be correlated both in the negative and positive direction with MetS components. Alongside the small population size used in this study (n=212), multiple testing limited the interpretation of the results of this hypothesis generating analysis. In addition, the phenotypic characteristics measured did not cover all components of MetS, lacking measures of lipid levels, waist size and medication use.

The Orkney Complex Disease Study (ORCADES) is a large-scale genetic epidemiology study which completed the recruitment of over 2000 individuals in March 2011 [16]. We utilised this study to investigate the associations between components of the MetS and 46 whole plasma N-glycan components. Thus, it is the aim of this study to identify associations between N-glycans and MetS and to also compare results found in an Orcadian population to results found in Chinese and Croatian populations.

Materials and Methods

Study populations

ORCADES study recruitment procedures and methods: Individuals with ancestry from the North Isles of Orkney were recruited between 2005 and 2011 to participate in the Orkney Complex Disease Study (ORCADES). Means of recruitment included advertisements in local media, posters, talks with local organisations and community leaders, alongside obtaining details from other eligible persons through contact with volunteers. The resultant sample was 40% male and included over 2,000 individuals with an age range of 18-91 years, with a median age of 54 [16]. Participants were interviewed by a trained practitioner regarding demographic characteristics and questionnaires regarding lifestyle, environmental exposures and medical history were completed. Body weight, height and waist measurements were obtained during the physical examination by a trained practitioner. Blood pressure readings were taken twice, minutes apart. Blood samples for glycan, lipid and Fasting Plasma Glucose (FPG) analysis were collected following overnight fasting in test tubes containing Ethylenediaminetetraacetic acid (EDTA) anticoagulant. Samples were then processed immediately and plasma was removed from whole blood and stored at -70°C until further analysis.

Recruitment procedures and methods for study based in Chinese population

As reported in Lu et al. 310 participants were selected for recruitment from a hospital in Beijing (Xuanwu Hospital) whilst undergoing routine health check-ups between April and July 2009. Individuals who had a history of mental or physical illness, alongside those who had taken any medications in the previous two weeks were excluded, thus 212 healthy volunteers were recruited. The resulting sample was 46.7% male, with an age range of 18-89 years and a mean age of 37.8 years [15].

Physical examinations and interviews of participants were carried out by trained nurses and physicians. During these, height, weight and BP were measured (two BP readings, 5 minutes apart). Alongside this, fasting blood samples (5 mL) were obtained after a 12 h overnight fast in a vacuum negative pressure tube containing EDTA, for FPG measure and glycan analysis. Samples were then stored at 4°C, before plasma samples were obtained for glycan analysis by centrifugation at 3000 rpm for 10 min and were then stored at -80°C until analysis.

Recruitment procedures and methods for study based in Croatian population

As part of a large genetic epidemiology program participants were recruited between 2003 and 2004 on the Croatian island of Vis and during 2007 on the island of Knežević [17]. Recruitment procedures included the use of voting registers, contact with local stakeholders and religious organisations. In total, 1991 individuals were recruited, whilst only 520 unrelated participants were used in the analysis.

Trained practitioners measured physical characteristics including many body fat estimation methods such as BMI, bioelectrical impedance, waist measurements and skinfold thickness. Blood pressure was recorded as the average of two independent measurements, whilst blood samples for lipid, FPG, glycan and many more characteristics analysis were taken after overnight fasting in tubes containing anticoagulant. Plasma samples were then immediately separated and stored at -70°C.

Ethics statement

All participants provided written informed consent and the included studies received ethical approval from the approved by their respective ethical committees. This included the NHS Orkney Research Ethics Committee, North of Scotland Research Ethics Committee and the Ethics Committee of the Medical School, University of Split. This project also gained ethical approval from The University of Edinburgh's Centre for Population Health Sciences Research Ethics Subgroup.

Glycan analysis

N-glycans from the plasma samples were released and labelled through exoglycosidase digestion and 2-aminobenzamide respectively, as described previously [6]. A centrifuge was then used to dry glycans before redissolving them in a known volume of water for further analysis. The weak anion exchange high performance liquid chromatography (WAX-HPLC) method was used in order to separate
the plasma N-glycome into 4 groups according to number of sialic acids (monosialylated, disialylated, trisialylated and tetrasialylated).

Hydrophilic interaction High-Performance Chromatography (HILIC), calibrated by an external standard of hydrolyzed and 2-AB-labeled glucose oligomers, was then used to analyse the prepared samples. As displayed above, this chromatographic analysis separated the plasma N-glycome into 16 groups (GP1-GP16). As a quality control measure to check for the effect of the batch process, duplicate analysis of several of the samples was performed and this measure confirmed the reproducibility of the results. See McQuillan et al. [16] for a more detailed description of methods.

After this, in order to improve the precision of analysis, sialidase digestion was performed [18]. This involved drying the samples once more before adding sodium acetate incubation buffer, arthrobacter ureafaciens sialidase enzyme and water to aliquots. After overnight incubation, samples were then passed through a Micropure-EZ enzyme remover enzyme remover. HILIC analysis of these samples separated the plasma N-glycan profiles into 13 groups of desialylated glycans (DG1-DG13). Derived glycan measures, according to structural features were then calculated.

Statistical analysis

Prior to analysis, due to the non-parametric nature of glycan measurement distribution, glycan measurements were rank transformed. Graphs of the distributions of continuous measurements were then assessed for normality before they were compared between cases and controls. The most up to date definition of MetS was used to identify a participant as having MetS and phenotypic characteristics associated with MetS and smoking status and familial relationships. P-values, odds ratios (ORs) and 95% confidence intervals were also reported. The false discovery rate (q) was calculated to adjust for multiple testing and values were generated for each of the correlation coefficients. Results were then compared to that of the analysis conducted by Lu et al.

Results

Characteristics of studied population for case-control analysis

In the case-control study section of this analysis, the Orcadian population analysed consisted of 2,039 individuals, of which 39.6% were male. Table 1 details the distribution of individual MetS components. It is evident that high blood pressure was the most common component of MetS, with 1030 individuals fitting for the criteria for high blood pressure. The second most common component of MetS in this population was a large waist measurement, whilst fewer people were found to have abnormal lipids or FPG. According to pre-specified criteria, 564 participants were found to have MetS. In addition, it was found that 251 and 68 individuals had 4 and all 5 components respectively. As high BP was found to be so common within the population, it was noted that only 299 participants would be considered to have MetS if BP was ignored.

As indicated in Table 2, a higher proportion of females were found to have MetS (30.7% compared to 23%), with the prevalence of MetS in general being 27.7%. Alongside this, the average age of those with MetS was found to be higher. No difference was found between the groups for ‘current’ smoking; however, it was found that those with MetS were more likely to have smoked in the past. Regarding medication use, it was evident that that those with MetS were more than twice as likely

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal/Characteristic absent (%)</th>
<th>Abnormal/Characteristic present (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist measurement (n=2,008)</td>
<td>1109 (55.2%)</td>
<td>899 (44.8%)</td>
</tr>
<tr>
<td>Triglycerides (n=2,081)</td>
<td>1550 (74.5%)</td>
<td>531 (25.5%)</td>
</tr>
<tr>
<td>HDL (n=2,077)</td>
<td>1605 (77.3%)</td>
<td>472 (22.7%)</td>
</tr>
<tr>
<td>BP (n=2,022)</td>
<td>992 (49.1%)</td>
<td>1030 (50.9%)</td>
</tr>
<tr>
<td>Glucose (n=2,079)</td>
<td>1568 (75.4%)</td>
<td>511 (24.6%)</td>
</tr>
<tr>
<td>MetS (At least 3 of the above) (n=2,039)</td>
<td>1475 (72.3%)</td>
<td>564 (27.7%)</td>
</tr>
<tr>
<td>MetS (4/5 components) (n=2,039)</td>
<td>1788 (87.7%)</td>
<td>251 (12.3%)</td>
</tr>
<tr>
<td>MetS (5/5 components) (n=2,039)</td>
<td>1971 (96.7%)</td>
<td>68 (3.3%)</td>
</tr>
</tbody>
</table>

*The variation in examined population size (n) is due to missing data for certain characteristics for individuals

*Characteristics were deemed "abnormal" if they fitted the criteria detailed by Alberti et al. [8]

Table 1: Distribution of MetS components within the ORCADES population.

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The variation in examined population size (n) is due to missing data for certain characteristics for individuals

*Characteristics were deemed "abnormal" if they fitted the criteria detailed by Alberti et al. [8]
to be on an anti-inflammatory medication, whilst those on hormonal medication were less likely to have MetS.

### Analysis of glycan profiles in cases and controls

Table 3 details that 27 out of 46 glycan traits were found to be statistically significantly associated with MetS in the crude model after adjusting for multiple testing. On adjusting for the effect of CRP, age and gender, this number reduced to 24. The additional adjustment for smoking, medication use and familial relationships in the final model ensured that this number was further reduced to 21 significant traits. Of these 21 glycan traits, 6 were derived glycan measures including BADS, BAMS, FUC-C, A2, G0 and G4.

Of these 21 traits, 12 were found to be associated with an increased likelihood of having the MetS (GP1, GP2, GP9, DG1, DG2, DG5, DG10, monosialylated, disialylated, BADS, A2 and G0), whilst 9 were associated with a decreased likelihood of having the MetS (GP5, GP6, GP8, GP13, DG6, DG13, BAMS, FUC-C and G4). The magnitude of effect of the identified positive associations ranged from an odds ratio (OR) of 1.14 (95% CI 1.02, 1.27) for DG5, to an OR of 1.66 (95% CI 1.48, 1.86) for DG10. Regarding negative associations, the magnitude of effect varied from 0.88 (95% CI 0.79, 0.98) for G4 to 0.62 (95% CI 0.55, 0.70) for GP8.

### Analysis of correlation of glycan traits with individual MetS components

Tables 4-7 display a comparison of the results from the ORCADES analysis compared to that found in Lu et al (2011). As displayed in Table 4, five glycan traits were found to be significantly correlated with BMI in all three populations, including G3, TRI (triantennary), FUC-C (core fucose), monosialylated and trisialylated glycans. Of these, monosialylated glycans and FUC-C were found to have negative correlations in each of the three populations, whilst disialylated glycans had a positive correlation coefficient, whilst monosialylated glycans had a negative correlation coefficient, which may reflect a reduced bioavailability of nitric oxide or may also portray the effects of an increased level of angiotensin II.

For example, the increased levels of G0, A2 and trisialylated N-glycans were particularly evident regarding core fucosylated glycans and the level of sialylation of glycans. Of these 21 traits, 12 were found to be associated with an increased likelihood of having the MetS (GP1, GP2, GP9, DG1, DG2, DG5, DG10, monosialylated, disialylated, BADS, A2 and G0), whilst 9 were associated with a decreased likelihood of having the MetS (GP5, GP6, GP8, GP13, DG6, DG13, BAMS, FUC-C and G4). The magnitude of effect of the identified positive associations ranged from an odds ratio (OR) of 1.14 (95% CI 1.02, 1.27) for DG5, to an OR of 1.66 (95% CI 1.48, 1.86) for DG10. Regarding negative associations, the magnitude of effect varied from 0.88 (95% CI 0.79, 0.98) for G4 to 0.62 (95% CI 0.55, 0.70) for GP8.

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For example, the increased levels of G0, A2 and trisialylated N-glycans were particularly evident regarding core fucosylated glycans and the level of sialylation of glycans.
leads to a blockade of the anti-inflammatory and vasodilatory effects of TGF-beta [19]. As discussed above, impairment of such pathways has led to a blockade of the anti-inflammatory and vasodilatory effects of insulin, thus contributing to the vascular dysfunction characteristic of T2DM [20]. Our results agreed with this hypothesis, as those with MetS

\[ \text{Glycan trait} \quad \text{Crude OR (95\% CI)} \quad n=1959 \quad p \quad q \quad \text{Baseline adjusted OR (95\% CI)} \quad n=1915 \quad p \quad q \quad \text{Fully adjusted OR (95\% CI)} \quad n=1833 \quad p \quad q \\
\]

Table 3a: Results from regression analysis of glycan components against MetS.

\[ \text{Glycan trait} \quad \text{Crude OR (95\% CI)} \quad n=1959 \quad p \quad q \quad \text{Baseline adjusted OR (95\% CI)} \quad n=1915 \quad p \quad q \quad \text{Fully adjusted OR (95\% CI)} \quad n=1833 \quad p \quad q \\
\]

Table 3b: Results from regression analysis of glycan components against MetS.

\[ \text{Glycan trait} \quad \text{Crude OR (95\% CI)} \quad n=1959 \quad p \quad q \quad \text{Baseline adjusted OR (95\% CI)} \quad n=1915 \quad p \quad q \quad \text{Fully adjusted OR (95\% CI)} \quad n=1833 \quad p \quad q \\
\]

Table 3c: Results from regression analysis of glycan components against MetS.

\[ \text{Glycan trait} \quad \text{Crude OR (95\% CI)} \quad n=1959 \quad p \quad q \quad \text{Baseline adjusted OR (95\% CI)} \quad n=1915 \quad p \quad q \quad \text{Fully adjusted OR (95\% CI)} \quad n=1833 \quad p \quad q \\
\]

q<0.05; statistically significant correlation coefficient in the "Fully adjusted" model

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were more likely to have lower levels of plasma core fucosylated glycans. Thus, in light of the results of this study, level of core fucosylation of plasma N-glycans could be used as a marker for early vascular dysfunction.

Careful consideration was given to the effect of confounding factors in this analysis and the difference in results between the crude and both adjusted models. Tables 3a-3d illustrate the importance of adjusting models in this way. In spite of this, it is clear that there are many other factors, including complex biochemical characteristics that may have had a confounding effect on these results. In addition, although age was adjusted for in the regression models, the relationship between glycans, age and MetS may have been illustrated more effectively had the adjustment been based on genetic data.

The differential medical treatment of cases and controls was evidently an important source of bias in this analysis. The ORCADES only results to be rank transformed prior to analysis. Furthermore, the comparability of their results. Alongside this, when comparing differences between the three included studies, which may have limited results regarding MetS between populations it is important to remember that the qualifying limits for MetS vary between different populations. In order to tackle this, we investigated the correlation of glycan data to be removed prior to analysis (322 removed). Alongside relationships (ASReml) required all participants with any missing values for certain characteristics, thus were removed from the analysis at different stages. This was most evident in the results of the fully adjusted regression model, as the package used to adjust for familial relationships (ASReml) required all participants with any missing glycan data to be removed prior to analysis (322 removed). Alongside reducing the power of this analysis if the missing values were in some way related, this list-wise deletion may have ensured the results of this analysis were biased.

In the second section of this analysis there were evident procedural differences between the three included studies, which may have limited the comparability of their results. Alongside this, when comparing results regarding MetS between populations it is important to record the qualifying limits for MetS vary between different populations. In order to tackle this, we investigated the correlation of glycans with individual MetS components and identified differences in this between the populations. However, such an analysis does not illustrate the higher susceptibility that certain groups have to the negative pathophysiological consequences of the MetS. Also, it was not possible to directly compare the magnitude of calculated correlation coefficients between these studies, as the ORCADES values were the only results to be rank transformed prior to analysis. Furthermore, the
Glycan trait | ORCADES SBP (n=2,039) r | Chinese SBP (n=212) r | Croatian SBP (n=520) r | ORCADES SBP p | ORCADES SBP q | Chinese SBP p | Croatian SBP p
---|---|---|---|---|---|---|---
G0 | 0.27 | 0.19 | 0.31 | 2.0 E-16 | 1.2 E-15 | 0.0080 | <0.001
G1 | -0.0036 | -0.023 | 0.037 | 0.88 | 0.88 | 0.75 | 0.40
G2 | -0.20 | -0.27 | -0.20 | 2.0 E-16 | 1.2 E-15 | <0.001 | <0.001
G3 | 0.019 | 0.26 | 0.043 | 0.77 | 0.82 | <0.001 | 0.33
G4 | -0.0033 | -0.013 | 0.015 | 0.57 | 0.64 | 0.86 | 0.74
A2 | 0.19 | 0.23 | 0.16 | 1.8 E-14 | 6.1 E-14 | 0.0020 | <0.001
BA | -0.067 | -0.054 | 0.028 | 0.047 | 0.0070 | 0.23
TRI | 0.063 | 0.049 | 0.033 | 0.051 | 0.0040 | 0.27
TA | 0.060 | 0.083 | 0.046 | 0.066 | 0.23 | 0.16
FUC-C | -0.21 | -0.099 | 2.7 E-16 | 1.2 E-15 | <0.001 | 0.026
FUC-A | 0.060 | -0.037 | 0.015 | 0.033 | 0.80 | 0.41
BAMS | -0.21 | -0.075 | 2.7 E-16 | 1.2 E-15 | 0.19 | 0.096
BADS | 0.060 | 0.16 | 0.015 | 0.033 | 0.12 | <0.001
Monosialylated | -0.081 | -0.055 | 0.0058 | 0.016 | <0.001 | 0.21
Disialylated | 0.070 | -0.049 | 0.052 | 0.068 | 0.0010 | 0.27
Trisialylated | 0.066 | 0.090 | 0.028 | 0.047 | <0.001 | 0.042
Tetrasialylated | 0.025 | -0.034 | 0.19 | 0.23 | 0.775 | 0.44

* p<0.05 in the ORCADES population; statistically significant correlation coefficient
f p<0.05 in the Chinese and Croatian populations; statistically significant correlation coefficient

Table 5: Correlation analysis of N-glycan traits with SBP.

Glycan trait | ORCADES DBP r | Chinese DBP r | Croatian DBP r | ORCADES DBP p | ORCADES DBP q | Chinese DBP p | Croatian DBP p
---|---|---|---|---|---|---|---
G0 | 0.11 | 0.037 | 0.27 | 3.7 E-06 | 2.1 E-05 | 0.61 | <0.001
G1 | -0.037 | -0.096 | 0.084 | 0.11 | 0.16 | 0.19 | 0.057
G2 | -0.095 | -0.13 | 3.2 E-05 | 0.94 | 0.95 | 0.004 | 0.94
G3 | -0.0017 | -0.14 | 0.03 | 0.32 | 0.36 | 0.85 | 0.28
G4 | -0.023 | -0.048 | 0.32 | 0.36 | 0.85 | 0.28
A2 | 0.081 | 0.053 | 0.14 | 3.5 E-4 | 0.0012 | 0.47 | 0.0020
BA | -0.053 | -0.19 | 0.018 | 0.020 | 0.049 | 0.0000 | 0.68
TRI | 0.064 | 0.21 | -0.012 | 4.8 E-3 | 0.014 | 0.0050 | 0.79
TA | 0.026 | 0.060 | -0.025 | 0.26 | 0.31 | 0.42 | 0.57
FUC-C | -0.11 | -0.18 | -0.023 | 2.7 E-16 | 4.7 E-15 | 0.013 | 0.61
FUC-A | 0.041 | -0.068 | -0.070 | 0.075 | 0.13 | 0.30 | 0.12
BAMS | -0.11 | -0.12 | 3.5 E-06 | 2.1 E-05 | 0.10 | 0.056
BADS | 0.041 | 0.12 | 0.12 | 0.075 | 0.13 | 0.092 | 0.010
Monosialylated | -0.040 | -0.28 | -0.060 | 0.079 | 0.13 | <0.001 | 0.17
Disialylated | 0.033 | 0.19 | 0.025 | 0.14 | 0.18 | 0.0080 | 0.56
Trisialylated | 0.033 | 0.076 | 0.14 | 0.18 | 0.0020 | 0.063
Tetrasialylated | 0.0014 | -0.10 | -0.15 | 0.95 | 0.95 | 0.17 | 0.0010

* p<0.05 in the ORCADES population; statistically significant correlation coefficient
f p<0.05 in the Chinese and Croatian populations; statistically significant correlation coefficient

Table 6: Correlation analysis of N-glycan traits with DB.

Analysis of only 3 components of MetS ensured that the extrapolation of these results to consider MetS as a whole is limited. It is also important to note that it is not possible to ascertain the direction of causality of these observed associations and it is possible that these findings are a consequence of altered metabolism in the MetS. Further study with the use of large cohort populations will be essential in order to investigate the causal relationship of these associations. Once this has been determined, the clinical utility of glycans as biomarkers for risk stratification can be determined.

In addition to these limitations, it is important to recognize that the glycan analysis technique used relies on relative quantification, it is not possible to determine whether an identified increase in one glycan trait is not solely due to a decrease in others, or an increase in the plasma level of the protein to which it is bound. Thus limiting the conclusions we can draw from such an analysis. In order to overcome this, future glycomic studies can instead isolate and examine the N-glycan profile of specific proteins.

**Conclusion**

Our results demonstrate that SBP, BMI, FPG and MetS as a whole are associated with an alteration in human N-glycosylation patterns. Our results show that such alterations vary by component, thus it is likely that such alterations reflect a complex array of the metabolic changes found in this syndrome. Such information may prove to be...
Table 7: Correlation analysis of N-glycan traits with FPG.

<table>
<thead>
<tr>
<th>Glycan trait</th>
<th>BMI r (q) n=2,011</th>
<th>SBP r (q) n=2,006</th>
<th>DBP r (q) n=2,004</th>
<th>FPG r (q) n=2,078</th>
</tr>
</thead>
<tbody>
<tr>
<td>G0</td>
<td>0.083 (0.0052)</td>
<td>0.27 (1.2E-15)</td>
<td>0.11 (2.1E-05)</td>
<td>0.18 (1.7E-05)</td>
</tr>
<tr>
<td>G1</td>
<td>-0.11 (5.6E-07)</td>
<td>-0.035 (0.88)</td>
<td>-0.037 (0.16)</td>
<td>0.032 (0.058)</td>
</tr>
<tr>
<td>G2</td>
<td>-0.082 (0.0013)</td>
<td>-0.20 (1.2E-15)</td>
<td>-0.095 (1.4E-04)</td>
<td>-0.020 (0.75)</td>
</tr>
<tr>
<td>G3</td>
<td>0.053 (0.0048)</td>
<td>0.019 (0.82)</td>
<td>-0.0017 (0.95)</td>
<td>0.0081 (0.75)</td>
</tr>
<tr>
<td>G4</td>
<td>-0.014 (0.88)</td>
<td>-0.0033 (0.64)</td>
<td>-0.023 (0.36)</td>
<td>-0.025 (0.44)</td>
</tr>
<tr>
<td>A2</td>
<td>0.047 (0.37)</td>
<td>0.19 (6.1E-14)</td>
<td>0.081 (0.0012)</td>
<td>0.16 (2.3E-03)</td>
</tr>
<tr>
<td>BA</td>
<td>-0.092 (5.7E-05)</td>
<td>-0.067 (0.047)</td>
<td>-0.053 (0.049)</td>
<td>0.065 (0.75)</td>
</tr>
<tr>
<td>TRI</td>
<td>0.13 (1.0E-07)</td>
<td>0.063 (0.051)</td>
<td>0.064 (0.014)</td>
<td>-0.040 (0.72)</td>
</tr>
<tr>
<td>TA</td>
<td>0.0044 (0.53)</td>
<td>0.060 (0.066)</td>
<td>0.026 (0.31)</td>
<td>-0.092 (0.12)</td>
</tr>
<tr>
<td>FUC-C</td>
<td>-0.15 (1.2E-08)</td>
<td>-0.21 (1.2E-15)</td>
<td>-0.11 (4.7E-15)</td>
<td>-0.16 (2.2E-08)</td>
</tr>
<tr>
<td>FUC-A</td>
<td>-0.074 (0.0031)</td>
<td>0.060 (0.033)</td>
<td>0.041 (0.13)</td>
<td>-0.00093 (0.17)</td>
</tr>
<tr>
<td>BAMS</td>
<td>-0.15 (1.2E-08)</td>
<td>-0.21 (1.2E-15)</td>
<td>-0.11 (2.1E-05)</td>
<td>-0.16 (2.2E-08)</td>
</tr>
<tr>
<td>BADS</td>
<td>-0.074 (0.0031)</td>
<td>0.060 (0.033)</td>
<td>0.041 (0.13)</td>
<td>-0.00093 (0.17)</td>
</tr>
<tr>
<td>Monosialo</td>
<td>-0.16 (1.8E-10)</td>
<td>-0.081 (0.016)</td>
<td>-0.040 (0.13)</td>
<td>-0.021 (0.12)</td>
</tr>
<tr>
<td>Disialo</td>
<td>0.15 (1.8E-09)</td>
<td>0.070 (0.068)</td>
<td>0.033 (0.18)</td>
<td>0.10 (2.3E-04)</td>
</tr>
<tr>
<td>Trisialo</td>
<td>0.12 (1.5E-06)</td>
<td>0.066 (0.047)</td>
<td>0.033 (0.18)</td>
<td>-0.043 (0.45)</td>
</tr>
<tr>
<td>Tetrasialo</td>
<td>-0.040 (0.068)</td>
<td>0.025 (0.23)</td>
<td>0.0014 (0.95)</td>
<td>-0.038 (0.32)</td>
</tr>
</tbody>
</table>

Presented numerical results are that of the ORCADES study population

<table>
<thead>
<tr>
<th></th>
<th>Indicates the result was replicated across all three studies</th>
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<tr>
<td></td>
<td>Indicates the result was significant in ORCADES and Chinese studies only</td>
</tr>
<tr>
<td></td>
<td>Indicates the result was significant in ORCADES and Croatian studies only</td>
</tr>
<tr>
<td></td>
<td>Indicates the result was significant in Chinese and Croatian studies only</td>
</tr>
</tbody>
</table>

useful as a risk stratifying biomarker, perhaps identifying those for whom metabolic alterations are more advanced. Such information may prove useful in the clinical setting, ensuring that those with subclinical levels of inflammation and insulin resistance can be identified and targeted for preventative treatments. Alongside this, these results highlight that further investigation of the importance of the core fucosylation of proteins in the state of insulin resistance is required. Future research in this area should involve the N-glycan analysis of individual glycoproteins (e.g. IgG) in cohort study populations.
References


