Detrimental links between physical inactivity, metabolic risk and N-glycomic biomarkers of aging

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Title

N-glycome in Older Adults: Importance of Adherence to Physical Activity Guidelines

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INTRODUCTION

Since ageing is accompanied by increased risk of development of chronic diseases including diabetes, depicting cellular and molecular mechanisms underlying disease pathogenesis is key to develop effective measures to combat age-related functional decline.

Recently, N-glycans have been suggested to be susceptible to age-related physiological alterations contributing to disease development (Miura and Endo, 2016). N-Linked enzymatic glycosylation is a complex and common post-translational modification modulating the biological function of proteins, which may affect all physiological functions (Dall'Olio F et al. 2013). Through highly sensitive and quantitatively reliable high-throughput technologies novel disease-specific alterations in N-glycan profile have been uncovered. Notably, alterations in α(1,6)-linked arm monogalactosylated, core-fucosylated diantennary N-glycans (NG1(6)A2F) have been reported in diabetes (Keser et al. 2017) and changes in serum branching α-1,3-fucosylated triantennary glycans (NA3Fb) have been shown in specific cancer forms (Liu et al. 2013). Additionally, agalacto core-α-1,6-fucosylated bisecting diantennary glycans (NGA2FB), triantennary glycan (NA3) and tetra-galactosylated core-α-1,6-fucosylated tetrantennary glycan (NA4) have been linked to metabolic abnormalities (Testa, 2015).

Regular physical activity (PA) is associated with several health benefits including reduced risk of developing chronic diseases and all-cause mortality. Indeed, insufficient amounts of daily time in PA have been linked to abdominal obesity, hypertension, dyslipidemia and hyperglycemia, collectively identified as the metabolic syndrome (MetS) (Edwardson et al. 2012). The substantial age-related rise in prevalence of MetS (Vishram et al. 2014) is mirrored by a concomitant decrease in PA level, where elderly women in particular spend less time in moderate to vigorous PA (MVPA) compared to men (Berkemeyer et al. 2016). Therefore, in order to promote healthy ageing, a weekly amount of 150 min in PA of at least moderate intensity is currently advocated..

While, changes in N-glycome profile may be related to metabolic abnormalities, influence of PA on N-glycans in humans has never been explored. This knowledge may provide novel insights into links between PA behaviors and glycosylation machinery. Whether fulfilling current guidelines on PA for good health in older adults is related to a favorable N-glycan profile, and if such an association is moderated by MetS remains to be elucidated. Therefore, the aim of this study was to examine serum N-glycans profile in a sample of community-dwelling older women with different objectively assessed PA levels and metabolic risk status.

Methods

Participants

A total of 120 elderly community-dwelling women 65-70 yrs of age were recruited from an urban area in Sweden. To be included, participants had to be non-smokers, free of diagnosed pulmonary, cardiovascular, metabolic or rheumatologic diseases, and with no disability with regard to mobility. Intake of prescribed medications was recorded. Written informed consent was obtained from all participants. All clinical investigations were conducted according to the standard set by the Declaration of Helsinki. The study was approved by the regional ethical review board of Uppsala, Sweden.

Metabolic risk outcomes

Height measured to the nearest 0.5 cm and body weight measured to the nearest 0.1 kg were assessed by a portable stadiometer and a digital scale, respectively. The following components of the metabolic syndrome defined by the IDF definition [18] were assessed: waist circumference (WC) was measured to the nearest 0.1 cm with a steel tape at the midpoint between iliac crest and lower costal margin. Systolic and diastolic blood pressures were measured manually after a 15-minute rest in the supine position using a mercury sphygmomanometer. A blood sample was collected after an overnight fast by venipuncture from an antecubital vein. Levels of triglycerides and HDL-cholesterol were determined on a Vitros-5.1 analyser platform using chemistry kits from Ortho-Clinical Diagnostics, Johnson & Johnson. Level of plasma glucose was determined with the Roche Reflotron Plus® system. Information on prescribed antihypertensive and lipid-lowering medications was retrieved. Participants were classified with or without MetS based on the IDF criteria.

Adherence to Physical activity guidelines

PA was assessed by the Actigraph GT3x (Actigraph, Pensacola, Florida) activity monitor during a week as previously described (Nilsson et al. 2017). In brief, at least 4 days with at least 10 hours of wear time per day was required for inclusion. Accelerometer count cut-point for time in MVPA was set to >2019 counts per min in accordance with previous work (Berkemeyer et al. 2016; Troiano et al. 2008). Participants spending a daily average of 30 minutes in MVPA were classified as meeting PA guidelines.

N-glycan analysis

Serum N-glycans were analyzed using DSA-FACE technology as previously described (Tesla, 2015). Briefly, the glycoproteins were first denatured before the sample was heated at 95°C for 5 min and cooled for 15 min in a PCR thermocycler and combined with 3 peptide-

Nglycosidase F. The sample was then transferred to a new PCR plate and evaporated to dryness. N-Glycans were derivatized using a labeling solution (1:1 mixture of 20 mM 8-Amino-1,3,6-PyreneTriSulfonic acid (APTS, Molecular Probes). In order to separate the N-glycans, glycan sialic acids groups containing negative charges are removed by a sialidase-digestion. Labeled N-glycans were analyzed by DSA-FACE technology, using an ABI 3130 sequencer (Applied Biosystems). Data analysis was performed using the Genescan 3.1 software (Applied Biosystems). Ten peaks, each representing a unique N-glycan structure, were detected in all samples. Peak 1 is an agalacto, core-α-1,6-fucosylated diantennary glycan (NGA2F); peak 2 is an agalacto core-α-1,6-fucosylated bisecting diantennary glycan (NGA2FB); peak 3 is the α(1,6)-arm galactosylation of the core-α-1,6-fucosylated diantennary glycan NG1A2F (NG1(6)A2F); peak 4 is the α (1,3)-arm galactosylation of the core-α-1,6-fucosylated diantennary glycan NG1A2F (NG1(3)A2F); peak 5 is a digalacto diantennary glycan (NA2); peak 6 is a digalacto core-α-1,6-fucosylated diantennary glycan (NA2F); peak 7 is a digalacto core α-1,6-fucosylated bisecting diantennary glycan (NA2FB); peak 8 is a triantennary glycan (NA3); peak 9 is a branching α-1,3-fucosylated triantennary glycan (NA3F); peak 10 is a tetra-galactosylated core-α-1,6-fucosylated tetrantennary glycan (NA4) (Liu et al. 2007 and Bunz et al. 2013)

Statistical analysis

Data are presented as means ± SD. Data on N-glycan peaks representing serum levels were checked for normality and log transformed if necessary to fit a normal distribution. Factorial analysis of variance (ANOVA) was used to explore differences in levels of N-glycans between groups based on MetS (with or without MetS) and adherence to PA guidelines (≥ 30 min of MVPA per day or not). Prior to main analysis, we assessed potential influence of age and medication use (yes/no) in all ANOVA models using $p \geq 0.1$ as F-to-remove criteria. Age fulfilled criteria for removal, whereas medication was included when analyzing peak 2 (NGA2FB) only. No interaction effects between groups of MetS and time in MVPA on levels of N-glycans were observed, hence all ANOVA models were based on the full sample. False Discovery Rate (FDR) correction for multiple comparisons was performed using the Benjamini–Hochberg procedure. All statistical analyses were performed using SPSS ver. 23. Based on our sample size with $p<0.05$, small-to-moderate effect sizes (≤ 0.4) are detected with a power of $\geq 80\%$.

Results

A total of 109 women (mean age: 67 ± 1.7 yrs; body mass index: 25.6 ± 4) had complete data on all variables. The activity monitors were worn on average 5.8 ± 0.5 days with a daily wear time of 14.2 ± 1.0 hours. Sixty-two out of 109 women reported intake of medication and 45% were classified with MetS.

Data on N-glycan peaks in all women, and between groups of women with and without MetS are shown in Table 1. Significantly elevated levels of NGA2FB (peak 2) and NA3F (peak 9) were evident in women with MetS, while level of the α(1,6)-arm monogalactosylated (NG1(6)A2F), identified by peak 3, was significantly lower in women with MetS compared to their healthier peers (Table 1). When adjusted by PA grouping, women with MetS had 24% (p = 0.019) and 17% (p = 0.003) higher levels in NGA2FB (peak 2) and NA3F (peak 9), respectively, and 9% (p = 0.022) lower levels in NG1(6)A2F (peak 3) compared to those without MetS. After statistical adjustment for multiple comparisons, difference in NA3F (peak 9) remained significant (adjusted p = 0.005), whereas differences in NGA2FB (peak 2) and NG1(6)A2F (peak 3) became borderline significant (adjusted p = 0.05 for both peaks).

Interestingly, significant differences in N-glycan peaks were indicated when comparing women adhering to the PA guideline to those less active (Table 2). When adjusted by MetS, a 12% (p = 0.006) and a 13% (p = 0.004) lower level of NA3 (peak 8) and NA4 (peak 10), respectively, were still evident among the physically active women compared to those less active. Importantly, both of these between-group differences remained after adjustment for multiple comparisons (Peak 8: adjusted p = 0.020; Peak 10: adjusted p = 0.015). In contrast, the difference in NA3F (peak 9) indicated between PA groups (Table 2) was attenuated after adjustment by MetS (p = 0.15).

Finally, we re-analyzed differences between PA groups after allowing for lighter intensity PA (i.e. below the MVPA threshold, >760 counts per min (Troianio, 2007) to be included into the minimum amount of 30 minutes of daily PA. In contrast to findings based on the MVPA threshold, no differences in N-glycan peaks were observed between PA groups when based on the lower intensity threshold, which may indicate that the influence on N-glycan levels by PA is intensity-sensitive.

Discussion

This study confirms the existence of N-glycan-specific profiles linked to metabolic risk status in older adults. A novel finding is that regardless of metabolic risk status, adherence to PA guidelines is related to a favorable N-glycan profile. This proposed effect on N-glycans only occurs above the moderate PA-intensity threshold.

In accordance with previous reports indicating the occurrence of changes in levels of glycans in adults with metabolic disease (Testa et al. 2015), older women with MetS had increased levels of NGA2FB and NA3F and decreased levels of NG1(6)A2F. Likewise associations between these glycans and elevated waist/hip ratio, levels of triglycerides, glycemia and glycated haemoglobin have also been reported (testa, 2015). While the underlying mechanisms behind changes in serum levels of these specific N-glycans are not fully understood, changes in the activity of specific enzymes including glycosyltransferases, glycosidases may be responsible for the altered N-glycan profile. Alternatively, alterations in serum glycan profile may be due to changes in clearance rate of the glycoproteins (Kobata et al. 2003; Tozawa et al. 2001). A novel finding in the present study was that the MetS-related differences in serum N-glycan levels in older adults seem to occur regardless of whether guidelines for PA are met or not. This finding implies that changes in these specific glycans solely mirror metabolic disease progression and cannot be moderated by differences in physical activity level. Alternatively, the minimum amount of PA currently recommended may be insufficient to influence these specific glycans.

Interestingly, our study revealed that adherence to PA-guidelines is associated to reduced levels of NA3 and NA4 glycans in older women. This novel finding is of particular interest as these glycans have been associated with metabolic abnormalities including increased waist circumference, glycemia, and in patients with tumor (Testa, 2015; Liu et al. 2013). Since the biosynthesis of glycans depends on the concerted action of several enzymes, serum levels can be altered by multiple physiological and pathological conditions. Here we suggest that engagement in PA may have an influence on the machinery responsible for generation of these specific glycans. Moderate to vigorous physical activity is considered as a strong physiological stimulus capable of exerting several acute and chronic structural and metabolic adaptations at the level of several tissues and organs including liver (Uslu et al. 2018). Several serum N-linked glycoproteins are synthesized by the liver and thus, any alterations in N-glycan profile may reflect changes in the physiological state of hepatocytes. Alongside pharmacological therapeutic approaches, our findings support promotion of a physically active lifestyle as a supporting non-pharmacological public health approach.

Given previous studies showing that even low-intensity PA may promote beneficial effects on health biomarkers (Nilsson et al. 2018), we further scrutinized the potential impact of meeting a lower intensity threshold on N-glycan profile. A striking finding was that PA-mediated differences in N-glycan profile were no longer evident when the lighter PA-intensity replaced the higher threshold. Hence PA corresponding to at least brisk walking appears necessary to infer significant changes in N-glycan profile, which is in line with data demonstrating that MVPA is required to reduce the clinically relevant inflammatory biomarker C-Reactive Protein in older adults (Nilsson et al. 2018). A finding of clinical important was that presence of MetS did not alter PA-related differences in NA3 and NA4 glycans, which supports the health-enhancing benefit of having an active lifestyle in the older population across different stages of disease prevention.

To the best of our knowledge, this study provides novel insights into the role of PA in the modulation of N-glycans in older adults with different metabolic risk. Nevertheless, due to the cross-sectional design of this study, experimental trials examining short and long-term effects of PA on N-glycan levels in older men and women with different health status are warranted.

In conclusion, the present study demonstrates novel links between PA and N-glycan profile in older adults. Adherence to PA guidelines appears to infer beneficial effects on specific N-glycans in women with and without the metabolic syndrome. This supports the promotion of a physically active lifestyle at old age across different stages of disease prevention.