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Pilot study: the comparison of serum N-glycoprofiles of Wistar and SHR rats

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Introduction

Metabolic syndrome (MetS) is a cluster of cardio-metabolic risk factors and comorbidities conveying high risk of both cardiovascular disease [1] and type 2 diabetes mellitus (T2DM). The underlying aetiology of this clustering has been the subject of much debate [2]. Criteria for clinical diagnosis of MetS include elevated waist circumference, blood pressure (BP), levels of serum triglycerides and fasting glucose, and reduced levels of high-density lipoproteins (HDL) cholesterol [3]. Several studies, based on the clinical, patophysiological, genetic, translational or proteomic background of MetS were published [4-6], however, the effect of posttranslational modifications and their role in MetS still raises many questions.

Thus, the basic experimental rat models might offer a reliable alternatives for studying MetS pathologies. Up to this date, little is known regarding the changes in N-glycans during MetS in rodents, but observed similarities between the glycomic profile of rat and human sera provided important selection criteria for choosing an appropriate animal model for pathological and further pharmacological studies [7]. Wistar rats (W) are an albino strain widely used in biological and medical research for their common attributes providing satisfactory scale-up outcomes for pre-clinical predictions [8]. Spontaneously hypertensive rats (SHR) have been developed as animal models for human essential (idiopathic or primary) hypertensions [9-10].

The main goals of our pilot study were: *i)* to describe the glycoprofiles of two different rats strains; *ii)* to evaluate their physiological and pathophysiological liaisons to the possible metabolic distrubances.

Material and Methods

All experimental procedures involving animals were approved by the Ethical Committee of the Institute of Experimental Pharmacology and Toxicology, Animal Health and Animal Welfare Division of the State Veterinary and Food Diet Administration of the Slovak Republic (the number of the permit 3635/14-221) and they conformed to Directive 2010/63/EU on protection of animals used for scientific purposes. Adult male W and SHR rats aged 15 weeks were from the Breeding Station of the Institute of Experimental Pharmacology and Toxicology (Dobra Voda, Slovakia). The rats had free access to water and food and were kept on 12h/12h light/dark cycle and housed 5 animals per cage. Animals were divided into two experimental groups (n = 10 rats/group) and fed standard diet.

The blood was collected from *plexus chorioideus*. ELISA diagnostics kits (Erba Lachema, CR) kits were used to determine the lipid profile from the blood serum. We measured levels of total cholesterol (CHOL), low-density lipoprotein (LDL), HDL, triacylglycerols (TAG) and glucose (Glu). The absorbances of the resulting colored compound was measured spectrophotometrically at 500 nm on LabSystems 352 Multiskan MS Microplate Reader (ThermoFisher Scientific, U.S.).

The level of BP of the animals was measured by non-invasive tail-cuff pletysmografic approach [11].

The analyses of serum N-glycoprofile by MALDI-TOF/MS equipment were done as follows: 10 µl of serum was premixed with 40 µl 10 mM Tris, pH 7.5 + 0.1% SDS and incubated with dithiothreitol (DTT) and iodoacetamide (IAA) according to standard protein reduction and alkylation protocols [12]. To release the N-glycans, serum was incubated with 1 enzyme unit (U) of PNGase F (peptide-N-glycosidase F, Roche) at 37°C overnight. Isolation of N-glycans was performed by PGC SPE (100 mg Supelclean ENVI-Carb, Supelco) as described previously [13] by 60% ACN + 0.1% TFA. To increase the signal intensities and stabilize the sialic acid, N-glycans were subjected to permethylation [14]. Permethylated N-glycans were analyzed by UltrafleXtreme MALDI TOF/TOF mass spectrometer (Bruker Daltonics) in reflectron positive ion mode with 20 mg/ml DHB in 30% ACN + 1 mM NaOH as the matrix solution. Analyzed data were processed by FlexAnalysis (Bruker Daltonics) and GlycoWork Bench [15] software. Obtained MS and representative MS/MS spectra of free and permethylated N-glycans were compared and evaluated with a special focus on their N-glycan type.

The data were statistically evaluated using GraphPad Prism 6 Software (La Jolla, USA). Data were expressed as means ± SEM. One-way analysis of variance (ANOVA) was used to evaluate the difference among all experimental groups (using the Bonferroni multiple comparison test). The level of $p < 0.05$ was considered as statistically significant difference. The asterisks were used to mark significancy as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ when comparing SHR *vs* W rats.

Results and Discussion

First, the basal biochemical parameters of lipid profile and BP levels of two different rats strains from healthy individuals were compared. The corresponding data are registered in **Tab. 1**. The glucose level did not differ among the experimental animals W *vs* SHR, however, in all other

Tab. 1.: The lipid profile and BP levels of W and SHR rats.

	Glu *(mmol/l)*	**TAG** *(mmol/l)*	**CHOL** *(mmol/l)*	**HDL** *(mmol/l)*	**LDL** *(mmol/l)*	**BP** *(mmHg)*
W	6.86±0.25	1.00±0.11	1.56±0.09	1.02±0.03	0.52±0.01	119.3±1.4
SHR	6.78±0.23	0.47±0.05***	1.19±0.03***	0.87±0.01***	0.41±0.03**	191.5± 1.7***

parameters of lipid profile as well as BP level, there were observed significant changes in SHR group compared to W rats. Since, within chronic civilization diseases, e.g. MetS or T2DM, are multifactorial the key role in their development act not only the environment factors (diet, physical activity, stress, life style) but also genetic predispositions. Thus, the use of different rats strains provides the opportunity to elucidate the interrelation between glycosylation changes and the pathophysiological conditions involved that might be reflected also within their glycoprofiles [16,17].

Second, the acquisition and characterization of glycomic profiles derived from blood sera of two different rats strains from healthy individuals were done. N-glycoprofiling of serum samples from two different rat´s strains, led to the identification of more than 100 N-glycan structures. Out of them, accurately 38 relevant and most abundant N-glycan structures were selected. N-glycans were distributed in to the following structural classes according to their glycan type (values expressed as m/z that represents mass-to-charge ratio $[M + Na]^+$): High mannosylated (High – Man) - 1579.8; 1783.8; 1988.1; 2192.2; 2396.4; Complex Bi-antennary (C–Bi) – 1865,9; 1981.9; 2070.1; 2227.1; 2431.2; 2792.4; Complex Bi-antennary fucosylated (C–Bi–Fuc) – 1590.8; 1835.9; 2040.0; 2244.1; 2605.3; 2966.4; Complex Tri-antennary (C–Tri) – 2111.0; 2880.4; 3241.6; 3602.7; Complex Tri-antennary fucosylated (C–Tri–Fuc) – 3415.7; 3776.8; Hybrid - 2186.1; 2390.2; 2472.2; 2635.3; 2676.3; Hybrid fucosylated (Hybrid-Fuc) – 2081.1; 2285.1; 2489.3; 2663.3; 2807.4; 3837.4 and truncated sialylated (High – Sial) N-glycans – 3153.6; 3327.7; 3514.7; 3963.9. The relative intensities of N-glycans within different rat´s strains, sorted into groups according to their glycan type are displayed in **Tab. 2**.

Tab. 2: The relative intensities of N-glycans in W and SHR rats according to their glycan type.

	W	**SHR**
High-Man	38.83 ± 3.52	17.01 ± 2.67 ***
C-Bi	29.46 ± 6.58	32.72 ± 3.48
C-Bi-Fuc	17.08 ± 2.68	26.31 ± 3.22 *
C-Tri	0.22 ± 0.18	2.48 ± 1.04
C-Tri-Fuc	ND	0.91 ± 0.19 ***
Hybrid	8.39 ± 0.50	7.57 ± 0.80
Hybrid-Fuc	6.02 ± 1.14	8.00 ± 0.97
High-Sial	ND	5.00 ± 1.72 *

The comparison of the relative quantitative intensities of N-glycans between W and SHR strains, revealed the most significant changes in the contribution of High-Man and C-Tri-Fuc type of N-glycans. The relative proportion of all High-Man N-glycans in SHR rats was, on average, at half of the level of their presence in W group. Interstingly, there were not detected (ND) any of C-Tri-Fuc or High-Sial N-glycans in W experimental group, while observed in SHR group. The level

C-Bi-Fuc and High-Sial were noticeably higher in SHR compared to W. That implies towards the respectable role of fucosylation and sialylation found in other studies as well [18,19]. The complex molecular bases of the glycomic changes are still under intensive research [20,21].

Our findings support the importance of prospective research into glycosylation alterations in different rats strains [22]. These data of blood sera glycoprofiling in experimental animals might assume as a possible tool for basic research to test therapeutic perspectives within various civilization and metabolic diseases. Further impact on clinical studies tendencies might be considered.

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