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Research Article

Nutriglycomics in Mice Fed High-Fat or Low-Protein Diets

Watanabe N[1]*, Hara Y[1], Kono A[2], Ohashi T[3], Amano M[3] and Nishimura S.I[3]

[1]Graduate School of Human Life Sciences, Showa Women's University, Japan

[2]Department of Human Health and Design, Showa Women's University, Japan

[3]Field of Drug Discovery Research, Faculty of Advanced Life Science, Hokkaido University, Japan

***Corresponding author:** Watanabe N, Graduate School of Human Life Sciences, Showa Women's University, 154-8533 Taishido, Setagaya-ku, Tokyo, Japan

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Abstract

While glycomics describesthe global analysis of carbohydrateswithin a biological system, the neologism, nutriglycomics,defines the combination of nutrition with glycomics.Generally, the glycanprofile of an organism can beaffectedby environmental changes. However, there have been no studies that have investigated the effects of dietary composition on the glycan profiles of blood serum. The aim of this study was to explorethe effects of dietary extremes, specifically high-fat and low-protein diets, on serum glycanprofiles in mice.To this end, 5-week-old, male, C57BL/6J mice were divided into 3 groups and were fed respective experimental diets: control, lowprotein, and highfat. After 3 weeks of feeding on the trial diets,47different glycans were detected in blood serum. The glycan profile of the high-fat diet group was notsignificantly different from that of the control group. In contrast, the low-protein diet group had a significant increase in5 types and a decrease in 13 types of glycans compared to the control group.These results validate our hypothesis that dietary composition, in this case, a low-protein diet, can affect serum glycan profiles in mice.

Keywords: Glycomics; High-fat diet; Low-protein diet; Nutriglycomics; Glycan

Introduction

Glycomics, one of the ever-expanding -omics, refers the global analysis of the glycancomposition of an organism.This detailed study of glycans in biological systems has revealed a complex interplay between glycan structure and function. It is now known that environmental stimuli can lead to changes in the glycan composition of glycoproteins. For instance, serum glycansin Atlantic salmon have been found to be modified due to stress [1]. Similarly, long-term smoking also alters the serum glycan composition in humans [2]. Moreover, the glycan profiles of serum in patients with hepatocellular carcinoma have been reported to differ from those of healthy humans [3].

However, there have been no studies that have directly investigated the effects of dietary composition on serum glycans composition, even though diet is one of the most important factors affecting human health. Notably, Hirose *et al.* hypothesized during an analysis of phylogenic evolution that changes in the glycan profile were due to dietary composition [4]. In an effort todescribe our method of examining this hypothesis of a diet-induced modification of the glycan profile, we neologized the term nutriglycomics, by combining nutrition (nutri-) with glycomics. This study aims to investigate the effects of extreme dietary composition, specifically, high-fat and low-protein diets, on serum glycancompositionin mice.

Materials and Methods

Animal studies

Four-week-old, male, C57BL/6J mice were purchased and fed a commercial, non-purified chow diet (CRF–1, Charles River Laboratories Japan, Inc., Yokohama, Japan) for 1 week. The mice were kept in individual plastic cages at 23±2 °C with a 12-h light-dark cycle (light from 8 a.m. to 8 p.m.). After taming, the mice were divided into 3 experimental groups (n = 6) based on their diet: a control group, a low-protein group, and a high-fat group.Allexperimental diets were prepared from an AIN-93G baseandaltered to include the desired factorsas shown in Table 1. Briefly, the control group was fed an AIN-93G diet, the low-protein group diet contained 5% casein, and the high-fat group diet contained 20% fat.

After distributing the mice into groups, they were fed the experimental diets for 3 weeks. At the end of the experimental period, the mice were dissected for the collection of blood, heart, liver, spleen, kidney, epididymal white adipose tissue, and skeletal muscle (gastrocnemius and soleus)samples. The blood was centrifuged for 10 min at 1,900 ×*g* to obtain serum. Serum samples were stored at -30 °C until analyzed.

All animal studies were performed according to the approved animal research protocol of Showa Women's University.

Serum analysis

Serum concentrations of glucose and triacylglycerol were measured using the Glucose C-test and Triglyceride E-test kits (Wako Pure Chemical Industries Ltd., Osaka, Japan), respectively. Serum albumin concentrations were measured using the Mouse Albumin ELISA Quantitation Set (BethylLaboratories, Inc., Montgomery, TX, USA).

Glycoblotting

Serum samples were glycoblotted using the previously described method using the automated system, "Sweet Blot" prototype 7 (System Instruments Co., Ltd., Tokyo, Japan) [5]. To summarize, aliquots (10 µL) of serum samples were applied the "Sweet Blot" for pre-treatment and glycoblotting. Enzymatically freed glycans from glycoproteins

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Table 1: Composition and energy ratio of experimental diets.

	Cont	HF	LP
Casein	20	20	5
Corn starch	40.3	32.5	49.8
a - corn starch	13.2	10.5	16.3
Sucrose	10	7.95	12.4
Sybean oil	7	7	7
Lard		13	
Cellrose	5	5	5
Mineral mix (AIN-93G)	3.5	3.5	3.5
Vitamin mix (AIN-93G)	1	1	1
Sum	100	100	100
Energy ratio (%)			
Protein	20	17	5
Fat	16	39	16
Carbohydrate	64	44	79

Cont; Control, HF; High Fat, LP; Low Protein

and unbound carbohydrates were applied to BlotGlyco H beads (Sumitomo Bakelite Co., Ltd, Tokyo, Japan), the beads were washed, and the sialic acids of the bead-bound glycans were methyl-esterified. The processed glycans were then labeled with benzyloxiamine (BOA) and released from the beads. Subsequent detection with MALDI–TOF–MS (Ultraflex 3, Bruker Daltronics, Germany) was performed and the resulting MS spectra analyzed. A representative figure of the mass spectrometry was presented elsewhere [3].

Statistical analysis

The data have been represented as the means ± SE. Significant differences between the control group and experimental groups were evaluated with Dunnett's multiple comparison test. The differences were considered significant at $p< 0.05$.

Results and Discussion

Body weights during the experimental period and the weights of some organs are shown in Table 2. The body weights of the high-fat group were significantly higher than those of the control group at 2 and 3 weeks. Throughout the duration of the experimental term, the body weights of the low-protein group were significantly lower than those of the control group. The weight of the white adipose tissue from the high-fat group was also significantly higher than that of the control group, whereas the weights of the liver, spleen, pancreas, and kidney of the low-protein group were significantly lower than those of the control group. Mean food intake (g/day) and energy intake (kcal/day) are shown in Table 2. The food intake of the high-fat group was significantly lower than that of the control group, but the energy intake of the high-fat group was similar to that of the control group. Food intake and energy intake of the low-protein group were significantly higher than those of the control group.

In general, a high-fat diet is reported to cause an increase of body weight in mice without changing food and energy intake [6,7], whereas low-protein diets cause a decrease of body weight and the weight of some organs [8,9]. Therefore, the results observed can be attributed to the effects of the experimental diets used in this study.

Serum concentrations of glucose, triacylglycerol, total protein, and albumin are shown in Table 3. Serum concentrations of glucose, triacylglycerol, total protein, and albumin in the high-fat group were similar to those of the control group. In contrast, while theserum concentrations of glucose and triacylglycerol in the low-protein group were significantly lower,the total protein and albumin levels were not significantly different from those of the control group. This finding differs from previous reports that a low-protein diet causes a decrease

Table 2: Body weights, food intake and some organs weights.

	Cont	HF	LP	Cont vs HF	Cont vs LP
Body weights (g) 0 week	20.0±0.4	19.7±0.5	20.0±0.3		
1 week	21.4±0.2	22.2±0.4	20.2±0.2		†
2 weeks	23.0±0.2	23.6±0.5	20.4±0.3	*	††
3 weeks	24.2±0.3	25.6±0.6	21.5±0.4	*	††
Food intake (g/day)	6.8±0.8	5.7±0.5	8.9±0.9		
(kcal/day)	25.7±1.2	25.4±0.9	34.8±1.5		††
Organs weights (g)					
Heart	0.129±0.004	0.131±0.004	0.121±0.003		
Liver	0.944±0.016	0.933±0.029	0.737±0.013		††
Pancreas	0.136±0.007	0.139±0.020	0.096±0.005		††
Spleen	0.061±0.002	0.065±0.003	0.051±0.002		††
Kidney	0.361±0.007	0.355±0.020	0.293±0.008		††
White adipose tissue	0.157±0.021	0.332±0.056	0.098±0.018	**	
Brown adipose tissue	0.077±0.004	0.088±0.007	0.067±0.002		
Skeletal muscle	0.229±0.009	0.248±0.007	0.209±0.008		

Cont; Control, HF; High Fat, LP; Low Protein

Data are mean ± SE.

Significant different of the high-fat group from the control group at *P < 0.05 **P < 0.01.

Significant different of the lowh-protein group from the control group at † P < 0.05 †† P < 0.01.

Table 3: Plasma levels of glucose, triglyceride, and alubumin.

	Control	HF	LP	Cont vs HF	Cont vs LP
Glucose (mg/dL)	117.9±4.4	113.2±5.2	85.1±4.6		††
Triglyceride (mg/dL)	74.2±3.6	77.1±8.0	43.5±4.9		††
Alubumin (g/dL)	3.53±0.05	4.09±0.02	3.51±0.02		

Cont; Control, HF; High Fat, LP; Low Protein
Data are mean ± SE.
Significant different of the low h-protein group from the control group at "†† P< 0.01.

Table 4: Plasma contents of sugar chains (NM).

ActualMW	Structure	Cont	HF	LP	Cont vs HF	Cont vs LP
1362.5	(Hex)2 + (Man)3 (GlcNac)2	1.53±0.04	1.53±0.09	1.62±0.06		
1444.6	(HexNAc)2 + (Man)3 (GlcNac)2	0.00±0.00	0.00±0.00	0.32±0.02		††
1524.6	(Hex)3 + (Man)3 (GlcNac)2	2.19±0.07	2.26±0.13	1.82±0.06		†
1562.6	(HexNAc)1 (NeuGc)1 + (Man)3 (GlcNac)2	0.30±0.06	0.30±0.06	0.33±0.02		
1565.6	(Hex)2 (HexNAc)1 + (Man)3 (GlcNac)2	0.04±0.04	0.08±0.08	0.18±0.08		
1590.6	(HexNAc)2 (Deoxyhexose)1 + (Man)3 (GlcNac)2	0.60±0.02	0.58±0.03	0.66±0.02		
1606.7	(Hex)1 (HexNAc)2 + (Man)3 (GlcNac)2	0.10±0.06	0.06±0.06	0.37±0.03		††
1686.6	(Hex)4 + (Man)3 (GlcNac)2	0.51±0.01	0.54±0.03	0.33±0.07		†
1724.7	(Hex)1 (HexNAc)1 (NeuGc)1 + (Man)3 (GlcNac)2	2.19±0.12	2.20±0.14	2.23±0.10		
1752.7	(Hex)1 (HexNAc)2 (Deoxyhexose)1 + (Man)3 (GlcNac)2	0.27±0.06	0.18±0.06	0.33±0.01		
1768.7	(Hex)2 (HexNAc)2 + (Man)3 (GlcNac)2	0.36±0.12	0.70±0.22	0.54±0.14		
1848.7	(Hex)5 + (Man)3 (GlcNac)2	0.47±0.02	0.52±0.03	0.48±0.02		
1854.6	(Hex)1 (HexNAc)1 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNac)2	0.10±0.10	0.08±0.08	0.33±0.15		
	(HexNAc)1 (Deoxyhexose)2 (NeuGc)1 + (Man)3 (GlcNac)2					
1870.7	(Hex)2 (HexNAc)1 (NeuAc)1 + (Man)3 (GlcNac)2	1.19±0.10	1.34±0.22	1.32±0.16		
	(Hex)1 (HexNAc)1 (Deoxyhexose)1 (NeuGc)1 + (Man)3 (GlcNac)2					
1886.7	(Hex)2 (HexNAc)1 (NeuGc)1 + (Man)3 (GlcNac)2	2.55±0.14	2.39±0.14	1.99±0.08		†
1927.7	(Hex)1 (HexNAc)2 (NeuGc)1 + (Man)3 (GlcNac)2	5.15±0.17	4.68±0.21	7.63±0.25		††
1955.8	(Hex)1 (HexNAc)3 (Deoxyhexose)1 + (Man)3 (GlcNac)2	0.99±0.06	0.93±0.06	0.98±0.04		
2010.8	(Hex)6 + (Man)3 (GlcNac)2	1.48±0.05	1.51±0.08	1.60±0.04		
2045.9	(Hex)1 (HexNAc)1 (NeuGc)2 + (Man)3 (GlcNac)2	1.18±0.40	0.45±0.29	0.00±0.00		†
2048.7	(Hex)3 (HexNAc)1 (NeuGc)1 + (Man)3 (GlcNac)2	2.63±0.85	2.99±0.53	0.00±0.00		†
2073.8	(Hex)2 (HexNAc)2 (NeuAc)1 + (Man)3 (GlcNac)2	0.11±0.11	0.10±0.10	0.36±0.16		
	(Hex)1 (HexNAc)2 (Deoxyhexose)1 (NeuGc)1 + (Man)3 (GlcNac)2					
2089.7	(Hex)2 (HexNAc)2 (NeuGc)1 + (Man)3 (GlcNac)2	15.5±1.0	16.0±1.3	14.4±0.93		
2235.8	(Hex)3 (HexNAc)2 (NeuAc)1 + (Man)3 (GlcNac)2	1.88±0.19	1.85±0.19	1.95±0.20		
	(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuGc)1 + (Man)3 (GlcNac)2					
2368.8	(Hex)3 (HexNAc)2 (Deoxyhexose)3 + (Man)3 (GlcNac)2	2.33±0.15	1.97±0.12	2.17±0.13		
2378.7	(Hex)2 (HexNAc)2 (NeuAc)2 + (Man)3 (GlcNac)2	5.14±0.38	5.52±0.42	4.49±0.23		
	(Hex)1 (HexNAc)2 (Deoxyhexose)1 (NeuAc)1 (NeuGc)1 + (Man)3 (GlcNac)2					
2410.9	(Hex)2 (HexNAc)2 (NeuGc)2 + (Man)3 (GlcNac)2	182±8	160±7	176±7		
	(Hex)2 (Deoxyhexose)3 (NeuAc)2 + (Man)3 (GlcNac)2					
2498.9	(Hex)4 (HexNAc)4 + (Man)3 (GlcNac)2	0.10±0.10	0.00±0.00	0.30±0.10		
2516.0	(Hex)3 (HexNAc)1 (Deoxyhexose)1 (NeuGc)2 + (Man)3 (GlcNac)2	1.42±0.12	1.11±0.21	1.30±0.06		
2524.9	(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuAc)2 + (Man)3 (GlcNac)2	0.98±0.08	1.04±0.07	0.76±0.15		
	(Hex)1 (HexNAc)2 (Deoxyhexose)2 (NeuAc)1 (NeuGc)1 + (Man)3 (GlcNac)2					
2540.9	(Hex)3 (HexNAc)2 (NeuAc)2 + (Man)3 (GlcNac)2	0.21±0.14	0.47±0.13	0.58±0.12		
	(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuAc)1 (NeuGc)1 + (Man)3 (GlcNac)2					
	(Hex)1 (HexNAc)2 (Deoxyhexose)2 (NeuGc)2 + (Man)3 (GlcNac)2					
2556.9	(Hex)3 (HexNAc)2 (NeuAc)1 (NeuGc)1 + (Man)3 (GlcNac)2	30.5±1.7	26.0±2.2	28.5±1.1		
	(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuGc)2 + (Man)3 (GlcNac)2					
2731.9	(Hex)2 (HexNAc)2 (NeuGc)3 + (Man)3 (GlcNac)2	16.6±1.4	16.1±0.8	10.0±0.68		††
	(Hex)2 (Deoxyhexose)3 (NeuAc)2 (NeuGc)1 + (Man)3 (GlcNac)2					
2776.0	(Hex)3 (HexNAc)3 (NeuGc)2 + (Man)3 (GlcNac)2	1.02±0.50	0.21±0.21	0.26±0.26		
	(Hex)3 (HexNAc)1 (Deoxyhexose)3 (NeuAc)2 + (Man)3 (GlcNac)2					
2878.0	(Hex)3 (HexNAc)2 (NeuAc)1 (NeuGc)2 + (Man)3 (GlcNac)2	2.51±0.25	2.28±0.24	1.36±0.11		††

	(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuGc)3 + (Man)3 (GlcNac)2					
3097.1	(Hex)3 (HexNAc)3 (NeuGc)3 + (Man)3 (GlcNac)2	23.9±1.7	21.0±1.1	13.4±0.68		††
	(Hex)3 (HexNAc)1 (Deoxyhexose)3 (NeuAc)2 (NeuGc)1 + (Man)3 (GlcNac)2					
3243.1	(Hex)4 (HexNAc)3 (NeuAc)1 (NeuGc)2 + (Man)3 (GlcNac)2	2.29±0.21	1.89±0.22	1.00±0.06		††
	(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuGc)3 + (Man)3 (GlcNac)2					
3418.2	(Hex)3 (HexNAc)3 (NeuGc)4 + (Man)3 (GlcNac)2	4.31±0.45	4.17±0.25	1.59±0.13		††
	(Hex)3 (HexNAc)1 (Deoxyhexose)3 (NeuAc)2 (NeuGc)2 + (Man)3 (GlcNac)2					
3564.3	(Hex)4 (HexNAc)3 (NeuAc)1 (NeuGc)3 + (Man)3 (GlcNac)2	0.28±0.03	0.27±0.03	0.06±0.03		††
	(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuGc)4 + (Man)3 (GlcNac)2					
3783.3	(Hex)4 (HexNAc)4 (NeuGc)4 + (Man)3 (GlcNac)2	0.31±0.03	0.28±0.02	0.21±0.01		††
	(Hex)4 (HexNAc)2 (Deoxyhexose)3 (NeuAc)2 (NeuGc)2 + (Man)3 (GlcNac)2					
4104.5	(Hex)4 (HexNAc)4 (NeuGc)5 + (Man)3 (GlcNac)2	0.16±0.01	0.16±0.03	0.07±0.01		†
	(Hex)4 (HexNAc)2 (Deoxyhexose)3 (NeuAc)2 (NeuGc)3 + (Man)3 (GlcNac)2					

Cont; Control, HF; High Fat, LP; Low Protein

Data are mean ± SE.

Significant different of the low h-protein group from the control group at t P < 0.05 tt P< 0.01.

in total protein and albumin levels in serum [8,9]. A possible reason for these conflicting results is the length of the experimental term. The term of this study was 3 weeks, where as the previous reports detailed 8 weeks of observation [8, 9].

The results of glycomic analysis of serum from the experimental mice are shown in Table 4. Forty-seven glycans were detected by MALDI–TOF–MS. The glycan profile of the high-fat group was not significantly different from that of the control group. The low-protein group, on the other hand, had a statistically significant increase in 5 types of glycans and a decrease in 13 types compared to the control group.

Therefore, these results reveal for the first time that dietary composition, specifically a low-protein diet, can affect serum glycan profiles in mice. We acknowledge that the detrimental consequences of an extreme diet may have influenced the observed composition changes to the glycan profiles, as a low-protein diet directly affects the growth of animals. On the other hand, our results indicate that high-fat diets have no effect over short periods, such as 3 weeks.

Furthermore, we propose that nutriglycomics has the potential to be used as a superior assessment of malnutrition over serum albumin levels, which are typically used as an indicator of nutritional status, because glycan profiles were modified without a coinciding decrease in the level of serum albumin in this study. Further research into the effects of various dietary compositions on serum glycan profiles is needed to advance the field of nutriglycomics.

Conclusion

To explore the effects of extreme dietary composition on serum glycans in mice, we performed nutriglycomic analysis using mice fed high-fat or low-protein diets. Our results detail significant changes in the glycan profile of mice fed low-protein diets, whereas no change was observed in mice fed high-fat diets. For the first time, dietary composition has been shown to affect serum glycan profiles in mice, and our findings indicate the potential of nutriglycomics to interpret the complex information derived from glycomic analysis.

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