HIPPOCAMPAL GANGLIOSIDE COMPOSITION IS ALTERED BY METFORMIN AND LIRAGLUTIDE TREATMENT IN A HIGH-FAT HIGH-SUGAR DIET RAT MODEL

Marin Kuharić[1,2], Vedrana Ivić[1], Milorad Zjalić[1], Anita Matić[2,3], Ines Drenjančević[3], Sandor G. Vari[4], Aleksandar Včev[2] and Marija Heffer[1]

[1]Department of Medical Biology and Genetics, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia;
[2]Department of Pathophysiology, Physiology and Immunology, Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia;
[3]Department of Physiology and Immunology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia;
[4]International Research and Innovation in Medicine Program, Cedars-Sinai Medical Center, Los Angeles, California, USA

SUMMARY – Insulin resistance has many deleterious effects on the central nervous system, including the initiation and potentiation of neurodegeneration. While the pathogenesis of Alzheimer's disease has been extensively researched with many insights into the effects of amyloids and neurofibrillary tangles, the connection between the two pathogenic entities has not yet been fully elucidated. Gangliosides are commonly found in neuronal membranes and myelin, specifically in lipid rafts that have been linked to pathological amyloidogenesis. In this study, 64 Sprague Dawley rats with equal sex distribution were separated into four sex-specific groups, as follows: control group on standard diet; group on high-fat, high-sugar diet (HFHSD); group on HFHSD treated with metformin; and group on HFHSD treated with liraglutide. Free-floating immunohistochemistry of the rat hippocampi was performed to analyze group-specific and sex-specific changes in the composition of the four most common gangliosides found in neuronal membranes and myelin sheaths, GM1, GD1a, GD1b and GT1b. The groups on HFHSD showed glucose tolerance impairment and body weight increase at the end of the experiment, whereas the groups treated with pharmacotherapeutics had better insulin sensitivity and decreases in body weight by the end of the experiment. Most changes were observed for GM1 and GD1b. Positive immunoreactivity for GM1 was observed in the male group treated with liraglutide in regions where it is not physiologically found. The changes observed following HFHSD and liraglutide treatment were suggestive of ganglioside restructuring that might have implications on pathological amyloidogenesis. Metformin treatment did not significantly alter the hippocampal ganglioside composition in either sex.

Key words: *Ganglioside; Hippocampus; Insulin resistance; Liraglutide; Metformin; Neurodegeneration*

Introduction

Chronic and progressive neurodegenerative diseases such as Alzheimer's disease still represent a great

challenge for both researchers and clinicians. Some of the most deleterious effects of neurodegeneration include cognitive decline and memory loss. They stem from disruption of hippocampal homeostasis that is based not only on cellular events observed across the central nervous system (neuroinflammation, lipid peroxidation, ferroptosis, disruption of synaptic integrity and transmission, etc.)[1-3], but also on affecting features of the hippocampus that are hallmarks of its unique-

ness, i.e., adult neurogenesis and synaptic plasticity[4,5]. This leads to a pathological shift in hippocampal architecture and creates progressive functional impairments. While Alzheimer's disease has been widely researched, with pathological amyloid deposits and fibrillary tangles identified as some of the key culprits[6], recent investigations have started to implicate insulin resistance into the potential initiation and potentiating neurodegeneration[7]. An insulin resistant state impairs neuronal signaling pathways not only by affecting metabolic regulation, but also by creating a proinflammatory state and even promoting amyloidogenesis[8,9].

Neuronal membranes and myelin sheaths are especially rich in gangliosides. They are glycosphingolipids with a variable number of sialic acid residues. GM1 is the most common ganglioside in both the membrane and myelin, while other common gangliosides include GD1a, GD1b and GT1b[10]. They are often incorporated within lipid rafts, specialized membrane microdomains that carry out a plethora of functions that contribute to the regulation of physiological processes and maintaining structural integrity[11,12]. Lipid rafts are also known to contain the amyloid precursor protein, a membrane protein from which pathological amyloid deposits in Alzheimer's disease are derived[13].

This study aimed to investigate sex-specific changes to ganglioside composition in the hippocampus of a high-fat high-sugar diet rodent model, as well as changes brought on by metformin and liraglutide treatment.

Material and Methods

This study was conducted on hippocampal tissue of Sprague Dawley rats, commonly used in diabetes mellitus research[14]. A total of 64 rats were included in the study, 32 male and 32 female. Each sex was separated into four equal groups (eight rats *per* group), as follows: (1) control group on standard diet (StD); (2) group on high-fat high-sugar diet (HFHSD); (3) group on high-fat high-sugar diet treated with metformin (HFHSD-M); and (4) group on high-fat high-sugar diet treated with liraglutide (HFHSD-L).

Prior to starting the experiment, the rats were fed a standard diet, had water available *ad libitum*, and were kept in cages with controlled temperature (20-23°C) and humidity (40%-60%). The experiment started once the rats reached 45 weeks of age (week 0) and lasted for 20 weeks. Control group remained on a standard diet and did not receive any pharmacotherapeu-

tic intervention. A change in diet was introduced to the other three groups; the overall energy value of the diet was increased, with increases in the shares of unprocessed fat and carbohydrates. From week 6 to week 20 of the experiment, groups 3 and 4 (male and female) received pharmacotherapeutic interventions. Therapy was administered daily, while control groups were given saline solutions. Groups 3 were treated with metformin (s.c., 50 mg/kg/day, manufacturer: Sigma-Aldrich, St. Louis, MO, USA), while groups 4 were treated with liraglutide (s.c., 0.3 mg/kg/day, manufacturer: Creative Peptides, Shirley, NY, USA). Rats were monitored throughout the experiment, with regular weighing and oral glucose tolerance testing (OGTT) in order to evaluate their body mass, glycemic status and insulin sensitivity. During the experiment, a total of 14 rats expired, with the highest mortality observed in the female HF-HSD-L group, where five rats did not survive until the end of the experiment. The exact cause of death was not identified, but since the experiment was initiated once the rats were at a somewhat advanced age[15] and the fact that they were submitted to strenuous diet and therapy regimen[16], this might have shortened their lifespan. After 20 weeks, once the rats reached 65 weeks of age, they were terminated. Brain tissue was dissected and halved in sagittal plane in order to isolate and analyze the hippocampal tissue.

Free-floating immunohistochemical analysis of four gangliosides was performed, i.e., GM1, GD1a GD1b and GT1b. Primary antibodies for the included gangliosides were manufactured at the Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine in Baltimore, MD, USA. Visualization of the selected antigen-antibody complexes was accomplished using avidin-biotin complex conjugated with horseradish peroxidase (Vector Laboratories, Burlingame, CA, USA, cat. no. PK-6100) and 3,3'-diaminobenzidine (Vector Laboratories, Burlingame, CA, USA, cat. no. SK-4100) as the peroxidase substrate. After staining, the slices were slide-mounted and cover slipped using VectaMount (Vector Laboratories, Burlingame, CA, USA, cat. no. H-5000). The slices were photographed using a digital camera (Olympus D70; Olympus, Hamburg, Germany) set up on a microscope (Zeiss Axioskop 2 MOT; Carl Zeiss Microscopy, Thornwood, NY, USA), under a 40× objective (DP Manager, v.1.2.1.107, DP Controller v.1.2.1.108). Analysis of the micrographs was performed using the Fiji software[17]. Integrated density

values of each sample were calculated for three anatomical regions within the hippocampus, as follows: dentate gyrus (DG), cornu ammonis 1 region (CA1) and cornu ammonis 3 region (CA3).

Body weight and OGTT data showed normal distribution and were expressed as mean and standard deviation. Two-way ANOVA and Tukey's post hoc test were used in the analysis of immunohistochemical data to determine significant differences between the sexes, pharmacotherapy and interaction. Statistical significance was set at $p < 0.05$.

Results

Glycemic status was monitored by OGTT that was performed at three timepoints during the experiment. Changes in glucose levels for every group are shown in Table 1. There were small differences in fasting plasma glucose levels at the start of the experiment, with the female HFHSD-L group showing highest plasma glucose levels. Female HFHSD-L rats did not undergo final OGTT due to their poor physical status.

Changes in body weight throughout the experiment are shown in Figure 1. Weighing was performed

Table 1. Results of oral glucose tolerance testing throughout the experiment

Sex	Group	Week 0		Week 6		Week 20	
		Fasting	2 hours	Fasting	2 hours	Fasting	2 hours
		Plasma glucose concentration [mean (SD)], mmol/L					
Male	StD	5.8 (0.4)	9.1 (5)	6.3 (1.1)	15 (4)	5.2 (0.8)	15.3 (4.3)
	HFHSD	4.3 (0.4)	10.3 (4.6)	6 (0.6)	27 (7.9)	7 (0.8)	12 (4.4)
	HFHSD-M	3.3 (0.4)	8.6 (5.7)	6 (0.9)	20.9 (5.6)	4.5 (0.8)	12 (3.1)
	HFHSD-L	4.2 (1.3)	11.5 (4.4)	5.5 (1.1)	24.5 (7.6)	5.2 (0.4)	14.4 (5.7)
Female	StD	5.1 (0.6)	7.3 (1.2)	4.6 (0.6)	10.1 (5.9)	6.4 (0.4)	14.9 (4.5)
	HFHSD	5 (0.6)	7.1 (1)	5 (0.8)	9.6 (3)	5.6 (1)	15 (9.7)
	HFHSD-M	4.6 (3.9)	6.2 (2)	5.1 (1.6)	16.2 (7.9)	4.8 (0.6)	11.1 (3.6)
	HFHSD-L	6.2 (0.9)	21.9 (11.4)	4.9 (0.6)	18.8 (9.1)	/ *	/ *

HFHSD = high-fat high-sugar diet; HFHSD-L = high-fat high-sugar diet, treated with liraglutide; HFHSD-M = high-fat high-sugar diet, treated with metformin; OGTT = oral glucose tolerance test; SD = standard deviation; StD = standard diet; *female rats in the HF-HSD-L group were not subjected to the third and final OGTT due to poor general physical status.
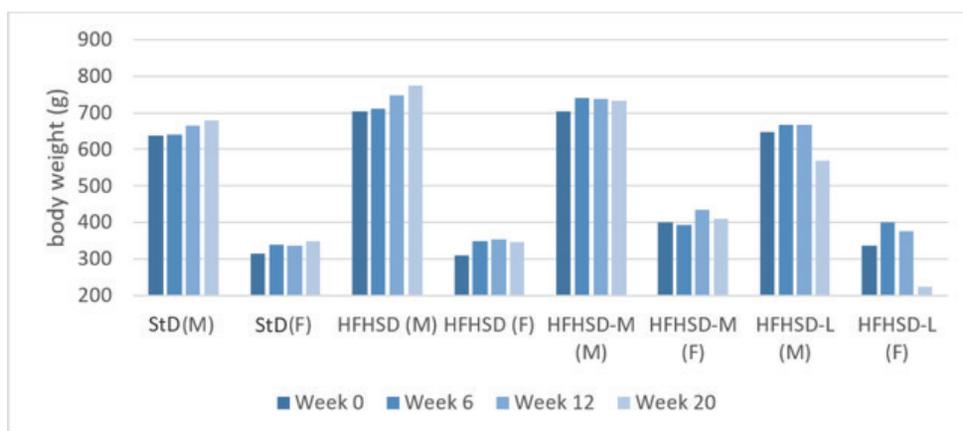

Fig. 1. Changes in rat body weight throughout the experiment.

(M) = male; (F) = female; HFHSD = high-fat high-sugar diet; HFHSD-L = high-fat high-sugar diet, treated with liraglutide; HFHSD-M = high-fat high-sugar diet, treated with metformin; StD = standard diet

at four timepoints during the experiment. Both HF-HSD groups showed weight gain by the end of the experiment, while groups treated with a pharmacotherapeutic showed weight loss by the end of the experiment.

Significant changes in ganglioside composition were observed for all four analyzed gangliosides with certain sex-specific changes. For GM1, there was a significant difference between male and female rats in the StD groups, with immunoreactivity being higher in male rats. GM1 levels were significantly higher in the male HFHSD-L group in comparison with the male HFHSD group. This was observed in all three regions of interest, even in the CA1 region, where the other three groups showed minimal or no immunoreactivity whatsoever. In female rats, GM1 immunore-
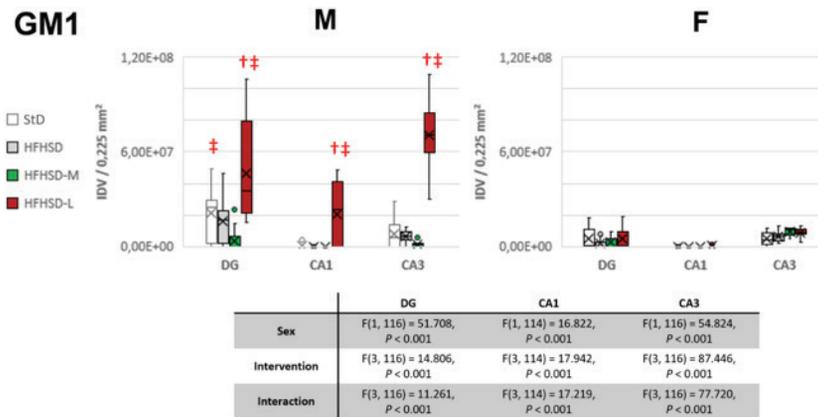

Fig. 2. Immunohistochemical analysis of GM1 ganglioside.

*Significant change compared with the StD group; †significant change compared with the HFHSD group; ‡significant change between the same groups of different sexes; comparisons between the HFHSD-M and HFHSD-L groups of the same sex were excluded. The table within the image shows results of the two-way ANOVA test; CA1 = cornu ammonis 1; CA3 = cornu ammonis 3; DG = dentate gyrus; HFHSD = high-fat high-sugar diet; HFHSD-L = high-fat high-sugar diet, treated with liraglutide; HFHSD-M = high-fat high-sugar diet, treated with metformin; IDV = integrated density value; StD = standard diet
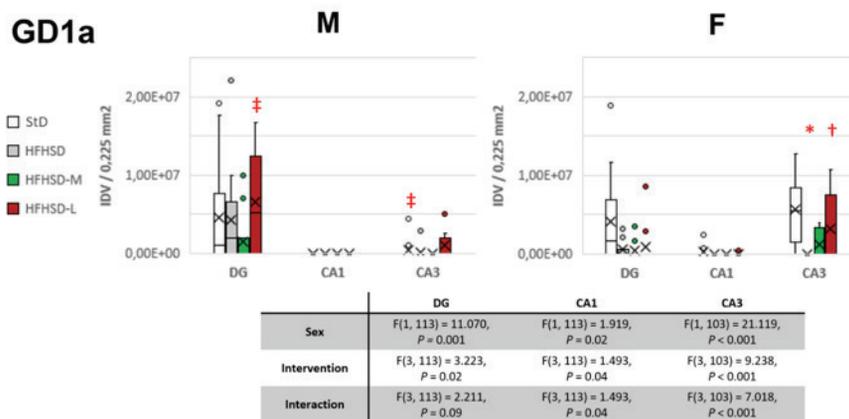

Fig. 3. Immunohistochemical analysis of GD1a expression.

*Significant change compared with the StD group; †significant change compared with the HFHSD group; ‡significant change between the same groups of different sexes; comparisons between the HFHSD-M and HFHSD-L groups of the same sex were excluded. The table within the image shows results of the two-way ANOVA test; CA1 = cornu ammonis 1; CA3 = cornu ammonis 3; DG = dentate gyrus; HFHSD = high-fat high-sugar diet; HFHSD-L = high-fat high-sugar diet, treated with liraglutide; HFHSD-M = high-fat high-sugar diet, treated with metformin; IDV = integrated density value; StD = standard diet

activity remained low, with pharmacotherapy showing little to no effect. Changes in GM1 immunoreactivity are shown in Figure 2.

The levels of GD1a were similar between the male and female StD groups in the DG, with little to no immunoreactivity detected in the CA1 region. CA3 immunoreactivity was observed in female StD rats. Liraglutide treatment showed alterations to GD1a levels in both sexes, i.e., increase in immunoreactivity in the CA3 region in female rats where statistical significance was reached and increase in immunoreactivity in the DG in male rats, but this change did not reach statistical significance. Changes in GD1a immunoreactivity are shown in Figure 3.

Concerning D1b immunoreactivity, it showed most differences between groups and between sexes.
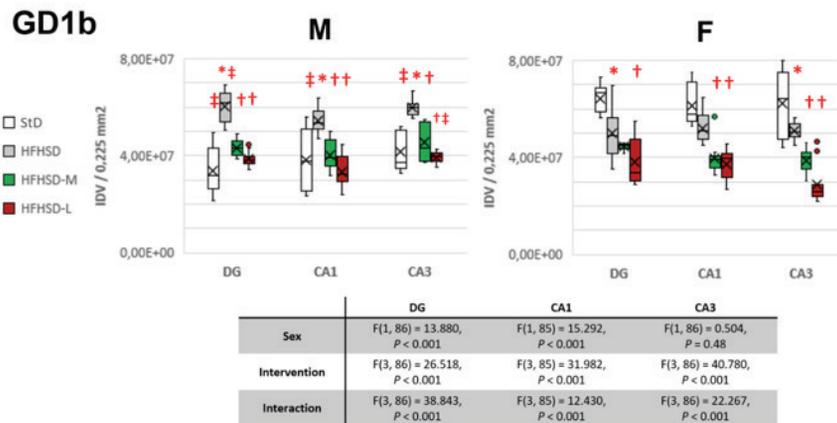

Fig. 4. Immunohistochemical analysis of GD1b expression.

*Significant change compared with the StD group; †significant change compared with the HFHSD group; ‡significant change between the same groups of different sexes; comparisons between the HFHSD-M and HFHSD-L groups of the same sex were excluded. The table within the image shows results of the two-way ANOVA test; CA1 = cornu ammonis 1; CA3 = cornu ammonis 3; DG = dentate gyrus; HFHSD = high-fat high-sugar diet; HFHSD-L = high-fat high-sugar diet, treated with liraglutide; HFHSD-M = high-fat high-sugar diet, treated with metformin; IDV = integrated density value; StD = standard diet
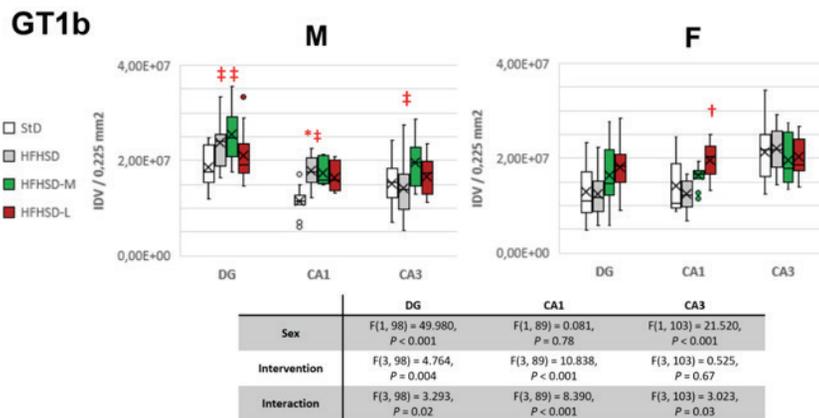
Fig. 5. Immunohistochemical analysis of GT1b expression.

*Significant change compared with the StD group; †significant change compared with the HFHSD group; ‡significant change between the same groups of different sexes; comparisons between the HFHSD-M and HFHSD-L groups of the same sex were excluded. The table within the image shows results of the two-way ANOVA test; CA1 = cornu ammonis 1; CA3 = cornu ammonis 3; DG = dentate gyrus; HFHSD = high-fat high-sugar diet; HFHSD-L = high-fat high-sugar diet, treated with liraglutide; HFHSD-M = high-fat high-sugar diet, treated with metformin; IDV = integrated density value; StD = standard diet
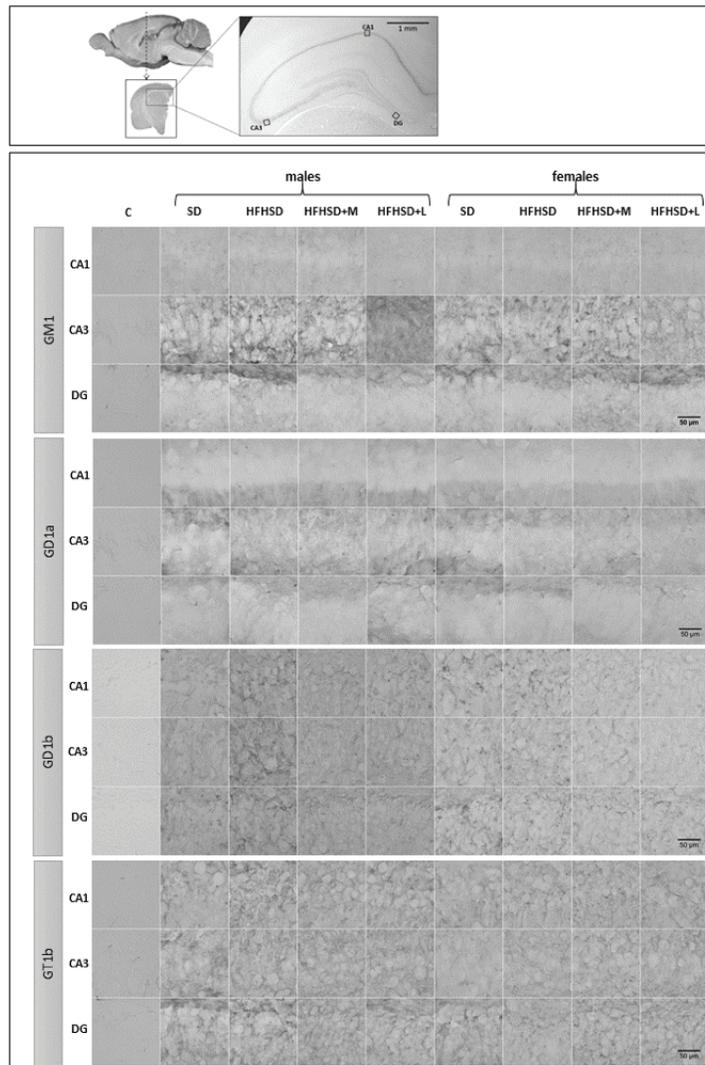
Fig. 6. Immunohistochemical stains for every analyzed ganglioside, separate by sex, group and region of interest.

C = control; CA1 = cornu ammonis 1 region; CA3 = cornu ammonis 3 region; DG = dentate gyrus; HFHSD = high-fat high-sugar diet; HFHSD-L = high-fat high-sugar diet, treated with liraglutide; HFHSD-M = high-fat high-sugar diet, treated with metformin; StD = standard diet

There was a sex difference in GD1b levels in the StD groups. HFHSD groups showed opposite trajectories, i.e., immunoreactivity was increased in male rats and decreased in female rats. These changes were most prominent in the DG. HFHSD-M and HFHSD-L groups of both sexes showed significant changes in immunoreactivity compared to their respective HF-HSD groups, with a decrease in immunoreactivity that reached statistical significance in several regions of interest. Immunoreactivity following either metformin or liraglutide treatment was at similar levels between sexes, which was not observable in the control StD groups. Changes in GD1b immunoreactivity are shown in Figure 4.

Significant changes in GT1b immunoreactivity were observable mostly between sexes and most prominent in the DG. HFHSD-M and HFHSD-L groups of both sexes showed only a couple of region-specific significant changes compared to their respective HF-HSD group. Changes in GD1b immunoreactivity are shown in Figure 5. Stains for every analyzed ganglioside are shown in Figure 6.

Discussion

In this study, we investigated the effects of HF-HSD, as well as treatment with two common pharmacotherapeutics (metformin and liraglutide) on the expression of the most common gangliosides found in the hippocampus to identify the potential links between insulin resistance and neurodegeneration, as well as to observe changes induced by metformin and liraglutide.

Statistical testing and significance of changes in body weight and plasma glucose levels following OGTT were not shown because there were small, but still observable differences in these values at the start of the experiment. These differences could be explained by simply acknowledging the fact that the rats were already 45 weeks of age at the start of the experiment and their biological aging could not be identical despite having the same living conditions. However, our monitoring tests throughout the experiment did show that both male and female HFHSD groups progressively gained weight and showed glucose tolerance impairment, which could be interpreted as developing an insulin resistant state. The HFHSD-M and HF-HSD-L groups, on the other hand, showed positive effects of the pharmacotherapeutics by the end of the experiment, which was reflected in both body weight and OGTT. The female HFHSD-L group, however, was the weakest of the eight groups, with five rats succumbing to unknown causes, even though we speculate that this might be related to their age and burden of such an extensive change in diet[15,16].

Gangliosides are very abundant in the central nervous system and they are often seen in lipid rafts, which have a specific lipid composition and include many important molecules that contribute to the integrity and homeostasis of neurons and myelin sheaths, such as glycosylphosphatidylinositol anchors[11]. Lipid raft functions extend from synaptic integrity and synaptic transmission to signal transduction[18,19]. A key component of lipid rafts is the amyloid precursor protein, which as its name states, gives rise to the pathological Aβ amyloid seen in Alzheimer's disease. Still, it is important to note that amyloid precursor protein, despite its ominous name, has several important physiological functions in the central nervous system that overlap with those of lipid rafts[20]. Still, lipid rafts have been identified as the origin site to pathological amyloidogenesis (carried out by amyloidogenic cleavage of the protein by a group of secretases)[13].

In our analysis of ganglioside composition changes following a HFHSD, as well as metformin and liraglutide treatment, there were two striking findings. The first one were changes in GM1 levels in male rats treated with liraglutide, while the other one were all the extensive changes seen in GD1b levels between both groups and sexes. GM1 levels were higher even in regions of the hippocampus where it is not physiologically found, as described in a 2013 paper by Vajn *et al.* on differential distribution of gangliosides in mice hippocampi[21]. GM1 and GD1a were two gangliosides that showed significantly higher levels in male rats following liraglutide treatment. Female groups that received pharmacotherapy, on the other hand, did not show many significant alterations in ganglioside composition. We cannot claim with full certainty that this lack of changes is not a reflection of the poor physical status of surviving HFHSD-L female rats, but no significant changes were observable in HFHSD-M rats either.

The observed changes for GM1 and GD1b are suggestive of ganglioside restructuring within the neuronal membrane and/or myelin sheath, and these changes could reflect changes related to amyloidogenesis, as both metformin and liraglutide have been shown to alter the expression of both amyloid precursor protein and pTau, another key biomarker of Alzheimer's disease, leading to changes in amyloidogenesis and synthesis of pathological neurofibrillary tangles. While the effects of liraglutide seem to point in the direction of neuroprotection[22], research into the effects of metformin on the central nervous system and the hippocampus, specifically, are opposing, with certain investigations suggesting that metformin promotes amyloidogenesis[23], while others are indicative of its neuroprotective properties[24].

This study showcased sex-specific differences in the hippocampal ganglioside composition in a high-fat high-sugar diet model, with metformin treatment showing little effect and liraglutide treatment mainly affecting GM1 and GD1b. Further investigation that would correlate these findings with changes in the expression of Alzheimer's disease biomarkers could prove useful in detecting specific structural changes in the hippocampal neurons that would reflect an increase or decrease in amyloidogenesis. This also highlights an important translational potential of this investigation.

References

1. Hojo Y, Higo S, Ishii H, Ooishi Y, Mukai H, Murakami G, *et al*. Comparison between hippocampus-synthesized and circulation-derived sex steroids in the hippocampus. Endocrinology. 2009;150(11):5106-12. DOI: 10.1210/en.2009-0305

2. Kwon HS, Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. Transl Neurodegener. 2020;9(1):42. DOI: 10.1186/s40035-020-00221-2

3. Taoufik E, Kouroupi G, Zygogianni O, Matsas R. Synaptic dysfunction in neurodegenerative and neurodevelopmental diseases: an overview of induced pluripotent stem-cell-based disease models. Open Biol. 2018;8(9):180138. DOI: 10.1098/rsob.180138

4. Winner B, Winkler J. Adult neurogenesis in neurodegenerative diseases. Cold Spring Harb Perspect Biol. 2015;7(4):a021287. DOI: 10.1101/cshperspect.a021287

5. Mango D, Saidi A, Cisale GY, Feligioni M, Corbo M, Nistico R. Targeting synaptic plasticity in experimental models of Alzheimer's disease. Front Pharmacol. 2019;10:778. DOI: 10.3389/fphar.2019.00778

6. Chen XQ, Mobley WC. Alzheimer disease pathogenesis: insights from molecular and cellular biology studies of oligomeric A and tau species. Front Neurosci. 2019 Jun 21;13:659. DOI: 10.3389/fnins.2019.00659

7. Sedzikowska A, Szablewski L. Insulin and insulin resistance in Alzheimer's disease. Int J Mol Sci. 2021;22(18):9987. DOI: 10.3390/ijms22189987

8. Kang S, Lee YH, Lee JE. Metabolism-centric overview of the pathogenesis of Alzheimer's disease. Yonsei Med J. 2017;58(3):479-88. DOI: 10.3349/ymj.2017.58.3.479

9. Ardura-Fabregat A, Boddeke E, Boza-Serrano A, Brioschi S, Castro-Gomez S, Ceyzeriat K, *et al*. Targeting neuroinflammation to treat Alzheimer's disease. CNS Drugs. 2017;31(12):1057-82. DOI: 10.1007/s40263-017-0483-3

10. Sipione S, Monyor J, Galleguillos D, Steinberg N, Kadam V. Gangliosides in the brain: physiology, pathophysiology and therapeutic applications. Front Neurosci. 2020;14:572965. DOI: 10.3389/fnins.2020.572965

11. Tsui-Pierchala BA, Encinas M, Milbrandt J, Johnson Jr EM. Lipid rafts in neuronal signaling and function. Trends Neurosci. 2002 Aug;25(8):412-7. DOI: 10.1016/s0166-2236(02)02215-4

12. Grassi S, Giussani P, Mauri L, Prioni S, Sonnino S, Prinetti A. Lipid rafts and neurodegeneration: structural and functional roles in physiologic aging and neurodegenerative diseases. J Lipid Res. 2020 May;61(5):636-54. DOI: 10.1194/jlr.TR119000427

13. Rushworth JV, Hooper NM. Lipid Rafts: Linking Alzheimer's amyloid-β production, aggregation, and toxicity at neuronal membranes. Int J Alzheimers Dis. 2010 Dec 27;2011:603052. DOI: 10.4061/2011/603052

14. Marques C, Meireles M, Norberto S, Leite J, Freitas J, Pestana D, *et al*. High-fat diet-induced obesity rat model: a comparison between Wistar and Sprague-Dawley rat. Adipocyte. 2016;5(1):11-21. DOI: 10.1080/21623945.2015.1061723

15. Altun M, Bergman E, Edstrom E, Johnson H, Ulfhake B. Behavioral impairments of the aging rat. Physiol Behav. 2007;92(5):911-23. DOI: 10.1016/j.physbeh.2007.06.017

16. Duffy PH, Seng JE, Lewis SM, Mayhugh MA, Aidoo A, Hattan DG, *et al*. The effects of different levels of dietary restriction on aging and survival in the Sprague-Dawley rat: implications for chronic studies. Aging (Milano). 2001;13(4):263-72. DOI: 10.1007/BF03353422

17. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, *et al*. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012;9(7):676-82. DOI: 10.1038/nmeth.2019

18. Brusés, JL, Chauvet N, Rutishauser U. Membrane lipid rafts are necessary for the maintenance of the (α)7 nicotinic acetylcholine receptor in somatic spines of ciliary neurons. J Neurosci. 2001 Jan 15;21(2):504-12. DOI: 10.1523/JNEUROSCI.21-02-00504.2001

19. Tansey MG, Baloh RH, Milbrandt J, Johnson Jr EM. GFRα-mediated localization of RET to lipid rafts is required for effective downstream signaling, differentiation, and neuronal survival. Neuron. 2000 Mar;25(3):611-23. DOI: 10.1016/s0896-6273(00)81064-8

20. Priller C, Bauer T, Mitteregger G, Krebs B, Kretzschmar HA, Herms J. Synapse formation and function is modulated by the amyloid precursor protein. J Neurosci. 2006;26(27):7212-21. DOI: 10.1523/JNEUROSCI.1450-06.2006

21. Vajn K, Viljetić B, Večeslav Degmečić I, Schnaar RL, Heffer M. Differential distribution of major brain gangliosides in the adult mouse central nervous system. PLoS One. 2013 Sep 30;8(9):e75720. DOI: 10.1371/journal.pone.0075720

22. Jantrapirom S, Nimlamool W, Chattipakorn N, Chattipakorn S, Temviriyanukul P, Inthachat W, *et al*. Liraglutide suppresses tau hyperphosphorylation, amyloid beta accumulation through regulating neuronal insulin signaling and BACE-1 activity. Int J Mol Sci. 2020 Mar 3;21(5):1725. DOI: 10.3390/ijms21051725

23. Picone P, Nuzzo D, Caruana L, Messina E, Barera A, Vasto S, Di Carlo M. Metformin increases APP expression and processing *via* oxidative stress, mitochondrial dysfunction and NF-κB activation: use of insulin to attenuate metformin's effect. Biochim Biophys Acta. 2015 May;1853(5):1046-59. DOI: 10.1016/j.bbamcr.2015.01.017

24. Gorgich EAC, Parsaie H, Yarmand S, Baharvand F, Sarbishegi M. Long-term administration of metformin ameliorates age-dependent oxidative stress and cognitive function in rats. Behav Brain Res. 2021 Jul 23;410:113343. DOI: 10.1016/j.bbr.2021.113343

Sažetak

PRIMJENA METFORMINA I LIRAGLUTIDA UTJEČE NA STRUKTURU HIPOKAMPUSNIH GANGLIOZIDA U MODELU ŠTAKORA NA DIJETI OBOGAĆENOJ MASTIMA I UGLJIKOHIDRATIMA

M. Kuharić, V. Ivić, M. Zjalić, A. Matić, I. Drenjančević, S. G. Vari, A. Včev i M. Heffer

Inzulinska rezistencija ima negativan učinak na središnji živčani sustav, uključujući razvoj i potencijaciju neurodegenerativnih promjena. Iako postoje brojna saznanja o patogenezi Alzheimerove bolesti u kontekstu amiloidnih depozita i neurofibrilarnih vlakana, veza između inzulinske rezistencije i Alzheimerove bolesti još uvijek nije u potpunosti razjašnjena. Gangliozidi su prisutni u membranama neurona i mijelinskim ovojnicama, osobito u sklopu lipidnih splavi, koje su identificirane kao mjesto patološke amiloidogeneze. Ukupno je 64 Sprague Dawley štakora jednake spolne distribucije podijeljeno u četiri spolno specifične skupine: kontrolnu skupinu na standardnoj dijeti, skupinu na dijeti obogaćenoj mastima i ugljikohidratima (HFHSD), skupinu na HFHSD uz liječenje metforminom te skupinu na HFHSD uz liječenje liraglutidom. Provedena je *free-floating* imunohistokemijska analiza promjena četiri najzastupljenija gangliozida u neuronskim membranama i mijelinskim ovojnicama: GM1, GD1a, GD1b i GT1b. Skupine na HFHSD pokazale su poremećenu toleranciju glukoze te povećanje tjelesne mase na kraju eksperimenta, dok su skupine koje su primale farmakoterapeutike pokazale poboljšanje tolerancije glukoze te smanjenje tjelesne mase na kraju eksperimenta. Najviše promjena zabilježeno je kod gangliozida GM1 i GD1b. Pozitivna imunoreaktivnost za GM1 zabilježena je kod mužjaka koji su liječeni liraglutidom i u regijama hipokampusa gdje u fiziološkim uvjetima nisu prisutni. Promjene nakon HFHSD i liječenja liraglutidom upućuju na restrukturiranje hipokampusnih gangliozida, što može imati implikacije za patološku amiloidogenezu. Liječenje metforminom nije značajno utjecalo na hipokampusnu strukturu gangliozida u oba spola.

Ključne riječi: *Gangliozid; Hipokampus; Inzulinska rezistencija; Liraglutid; Metformin; Neurodegeneracija*