Journal of Vasyl Stefanyk Precarpathian National University. Biology
V.13 (2026) pp. 18-28

Vasyl Stefanyk Carpathian National University

DOI: 10.15330/jpnubio.13.18-28

ISSN(Online): 3083-662X
ISSN(Print): 3083-6611

UDC: 613.25:635.356:678.048

Selective effects of broccoli sprout consumption on the activity of antioxidant enzymes in the cerebral cortex of cafeteria diet-fed mice

Marian Ivanochko[1*], Viktoriia Chyr[1], Iryna Yatskiv[1], Volodymyr Lushchak[1]

[1]*Department of Biochemistry and Biotechnology, Vasyl Stefanyk Carpathian National University, 57 Shevchenko Str., Ivano-Frankivsk, 76018, Ukraine*

*Corresponding author: M. Ivanochko: marian.ivanochko@cnu.edu.ua

Received: 07 January 2026; **Revised:** 15 February 2026; **Accepted:** 25 February 2026; **Published:** 05 March 2026

Abstract: Excess energy from a cafeteria diet causes the development of obesity and increases the risk of other health problems, like oxidative stress in the brain and neurodegenerative diseases. Broccoli sprouts are regarded as an anti-obesity and antioxidant supplement due to the potential of their compounds to stimulate cellular protective and regulatory systems, like Nrf2. This study aimed to investigate the effect of consuming broccoli sprouts on the activity of antioxidant and auxiliary enzymes in the cerebral cortex of cafeteria diet-fed mice. For 20 weeks, eight-month-old C57BL/6J males consumed a basal diet, a cafeteria diet, a basal diet with 5% broccoli sprouts, or a combination of a cafeteria diet with 2.5, 5, and 10% broccoli sprouts. Results demonstrated that only 10% broccoli sprouts with a cafeteria diet had an improving effect on the activity of glutathione reductase in the cerebral cortex of mice, which was ~ 1.8-fold higher compared to the basal diet group. In contrast, consumption of 5% broccoli sprouts with a basal diet caused ~ 2-fold higher values of activities of glutathione S-transferase, glutathione reductase, glucose-6-phosphate dehydrogenase, and NAD(P)H-quinone oxidoreductase 1 compared to the other groups. In conclusion, consumption of broccoli sprouts stimulates antioxidant defense and detoxification processes via Nrf2 target proteins in the cerebral cortex of mice fed a basic diet but not a hypercaloric cafeteria diet.

Keywords: antioxidant enzymes, broccoli sprouts, cafeteria diet, cerebral cortex, mice, Nfr2, obesity.

Abbreviations: ARE, Antioxidant Response Element; BD, basal diet; BS, broccoli sprouts; CD, cafeteria diet; GPx, glutathione peroxidase; GST, glutathione S-transferase; GR, glutathione reductase; G6PDH, glucose-6-phosphate dehydrogenase; HFFD, high fat high fructose diet; LOOH, lipid peroxides; NQO1, NAD(P)H-quinone oxidoreductase 1; Nrf2, Nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species.

1. INTRODUCTION

Obesity is becoming a global health problem in our society. One of the factors that affects this trend is the constant consumption of high-calorie food. The so-called cafeteria diet (CD) is one option for high-calorie nutrition that is rich in carbohydrates, fats, flavor enhancers, and flavorings. Many chronic consequences for the body are already known after long-term consumption of CD, for example, obesity, insulin resistance, and cardiovascular and hepatic diseases; however, new data indicate possible negative consequences for the central nervous system as well (Cavaliere et al. 2022; Johnson et al. 2016; Salas-Venegas et al. 2022). The human brain has a constant need for energy supply and consumes approximately 20% of the body's total energy at rest, which makes it vulnerable to oxidative damage from reactive oxygen species (ROS) (Cavaliere et al. 2022; Garaschuk et al. 2018; Raichle & Gusnard 2002). Consumption of high-calorie food has been found to cause brain inflammation in rats, and expression levels of genes encoding TNF-α and IL-6 were increased in the mediolateral hypothalamus (MBH), which is a key energy balance control center. These changes were also accompanied by the development of inflammation and oxidative stress in the liver and white adipose tissue (De Souza et al. 2005).

Nuclear factor erythroid 2-related factor 2 / Antioxidant Response Element (Nrf2/ARE) pathway typically activates the expression of cytoprotective genes (Bayliak & Abrat 2020; Ivanochko et al. 2024). Long-term consumption of high-calorie foods has been linked to a decrease in Nrf2 activity in the nucleus in the liver, white adipose tissue, and hippocampus, leading to a decrease in the expression level of enzymes such as NAD(P)H-quinone oxidoreductase 1 and heme oxygenase-1 (Bayliak & Abrat 2020; Lushchak 2021; Morrison et al. 2010). The above-mentioned mechanisms have a significant impact on the vital activity of cortex cells and make it possible to investigate whether compounds present in antioxidant and anti-obesity supplements like broccoli sprouts (BS) can increase the resistance of nervous system cells to oxidative stress. Broccoli sprouts showed a potential against oxidative stress and obesity in peripheral organs – liver, muscles, and hypothalamus, increasing the activity of glutathione-dependent antioxidant enzymes in the liver of mice (Ivanochko et al. 2023; Derkachov et al. 2023). Sulforaphane, a component of BS, activates Nrf2/ARE signaling, which upregulates Phase II enzymes of detoxification for antioxidant actions and maintains reduced glutathione levels (Ivanochko et al. 2024; Zhou et al. 2014). In addition, sulforaphane can cross the blood-brain barrier and accumulate in nerve tissue, enabling it to activate defence mechanisms inside the brain (Mao et al. 2019). In the brain, sulforaphane-enriched broccoli sprouts reduced neuroinflammation and improved memory impairment in pharmacological injury models, including scopolamine-induced amnesia and phencyclidine-induced cognitive impairment (Shirai et al. 2015; Subedi et al. 2019).

Despite the above-mentioned facts, the effectiveness of BS against CD-driven oxidative stress in the cortex is not currently clearly confirmed and well-studied. Moreover, most studies focus on the neuroprotective properties of sulforaphane and the use of high-fat diets rather than supplementation of broccoli sprouts and exposure of cafeteria diet. High-fat diet relies on purified fats (35-60%), which alter lipid profile, while cafeteria diet includes highly palatable, varied human junk foods rich in saturated fats, sugars, and additives to induce rapid obesity (Higa et al. 2014; Sampey et al. 2011a). Also, studies that show positive effects usually use direct neurotoxic effects (ischemia, scopolamine, phencyclidine) rather than dietary metabolic stress (Mao et al. 2019; Shirai et al. 2015; Subedi et al. 2019). The appearance of saturated fats, fast carbohydrates, and various production supplements present in a cafeteria diet can create a more stable pathological environment similar to human.

The aim of this work was to study the effect of broccoli sprouts consumed with a cafeteria diet on antioxidant parameters of the murine cerebral cortex.

2. Materials and Methods

Broccoli seeds (*Brassica oleracea var. italica*, cultivar Calabrese) from "SemyaSvet" company (Odesa, Ukraine) were used for obtaining broccoli sprouts. The seeds were cultivated as described before (Ivanochko et al. 2025a,b). Seeds were grown in sealed, transparent plastic containers with a moist cotton substrate, under a 12 h light/dark photoperiod at +25°C and 50-60% humidity. Three-day-old sprouts were quickly frozen in liquid nitrogen and stored at -20°C for biochemical analysis and murine chow production. Biochemical analysis of broccoli sprouts was described in our previous work (Ivanochko et al. 2025a). The stability of bioactive compounds in broccoli sprouts after freezing was proved by several repeated determinations.

Eight-month-old C57BL/6J males were bred in our animal facility with a 12 h light/dark cycle (6 a.m./6 p.m.) at +22 ± 2°C temperature, and 50-60% humidity (Bayliak et al. 2022a,b; Ivanochko et al. 2025b). Access to food and water was unlimited. Mice were randomly assigned to six groups, each consisting of 5-6 mice (2-3 mice per cage). Figure 1 describes the experimental design. The control group (denoted as the BD group) consumed the standard granular rodent chow ("Vita" company, Obukhiv, Ukraine), which contained 20% protein, 4.8% fat, 70% carbohydrates, 4.7% fiber, and a total 310 kcal per 100 g. The cafeteria diet (CD) group consumed a hypercaloric, obesogenic food (459 kcal per 100 g) containing 19.3 % protein, 22.6% fat, and 49.4% carbohydrates per 100 g. This chow consists of (w/w) sweet peanuts (28%), milk chocolate (28%), chocolate cracker (11%), casein (10%), and powdered rodent chow (23%) based on the study of other scientists (Sampey et al., 2011b). The CD diet, as well as the next four chow types, was prepared manually by mixing all ingredients with powdered rodent chow ("Vita" company) and forming pellets using a granulator (TMS company, Cherkasy, Ukraine). The next three groups consumed a CD diet supplemented with 2.5%, 5%, and 10% (w/w) crude shredded broccoli sprouts (BS), denoted as CD2.5BS, CD5BS, and CD10BS, respectively. The content of powdered rodent feed was decreased with the addition of BS, but the content of other CD components remained unchanged. The broccoli sprouts group (BD5BS) received the standard chow supplemented with 5% (w/w) broccoli sprouts. The content of broccoli sprouts was chosen based on previous studies (Alhajri & H. Saad 2023; Zhang et al. 2023). All experimental chow was produced once for the whole duration of feeding, stored in the fridge (at -20°C), and warmed to room temperature before serving to mice.

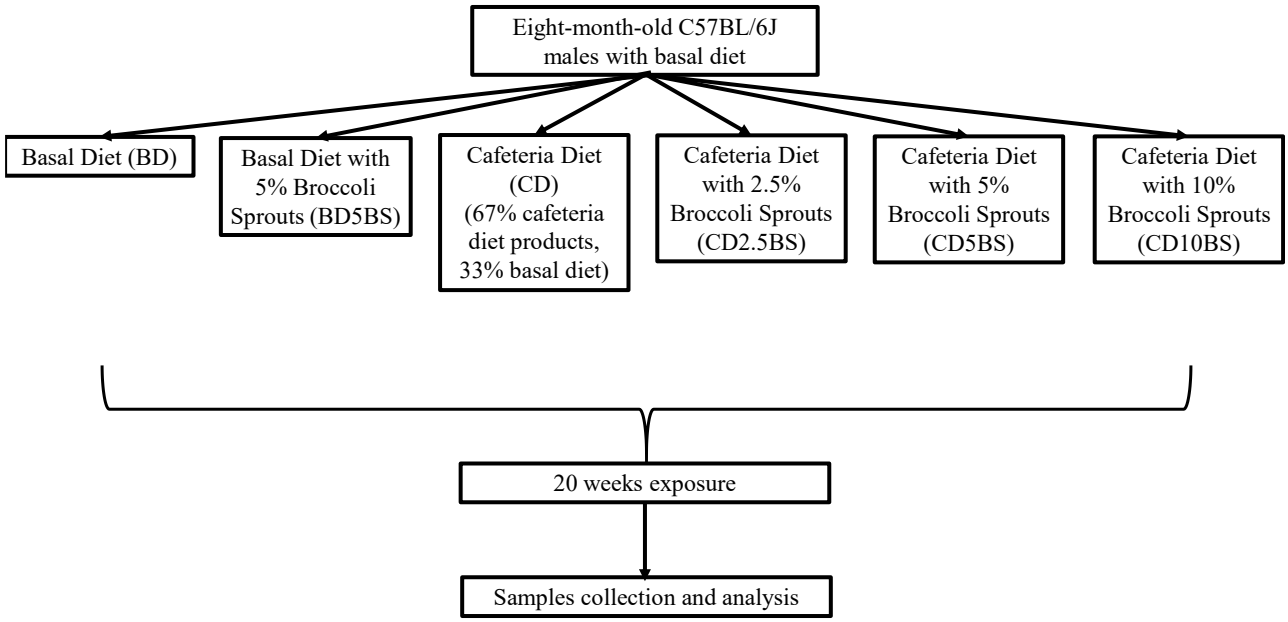

Figure 1. Experimental design.

Animals were kept on the corresponding diets for the next 20 weeks. At the end of the experiment, they were anaesthetised using light CO_2 anaesthesia followed by cervical dislocation. The whole murine cerebral cortex was divided from the rest of the brain, collected and frozen in liquid nitrogen, and stored at -80°C for further biochemical analyses (Bayliak et al. 2022a; Demianchuk et al. 2024, 2025). Nutrition, body mass, and blood parameters of mice were analysed in our previous paper (Ivanochko et al. 2025b). The experiments were approved by the ethics committee of Vasyl Stefanyk Carpathian National University and assigned it the number PNU-2023-M-010. Procedures were conducted following Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

The lipid peroxide content (LOOH) and activities of enzymes in murine cerebral cortex were determined spectrophotometrically as described previously (Demianchuk et al. 2024, 2025). For the LOOH assay, frozen samples were homogenised on ice with 96% ethanol (1:10, w:v) and centrifuged (10,000×g, 10 min, +4°C). LOOH content was measured in supernatants based on the formation of Fe(III)-xylenol orange complex (Hermes-Lima et al. 1995; O. V. Lushchak et al. 2008). Absorbance was determined at 580 nm using a Ulab 102U spectrophotometer (Ulab Scientific Instruments, China). Cumene hydroperoxide was used as a standard to build a calibration curve. Content of LOOH was determined as nmol equivalents of cumene hydroperoxide per gram of wet mass (nmol CHP eqv/gwm).

Cerebral cortex samples were homogenised 1:10 (w:v) in a medium containing (final concentrations): 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM phenylmethylsulfonyl fluoride (PMSF). Homogenates were centrifuged (16,100×g, 15 min, +4°C). In the received supernatants, activities of glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), and NAD(P)H-quinone oxidoreductase 1 (NQO1) were determined by methods described in our previous works (Bayliak et al. 2022a; Demianchuk et al. 2024, 2025). The activity was normalised on the content of total protein, determined in supernatants using the Bradford assay (Bradford 1976).

GraphPad Prism version 8.3.1 and MS Excel were used for statistical analysis and visualisation. Data were subjected to one-way ANOVA followed by Tukey's tests for multiple comparisons. Results were presented as means ± standard errors of the mean (SEM).

3. Results and Discussion

Consumption of chow was lower in all CD-fed groups compared to the BD group, while the amount of total energy from food was higher in CD, CD2.5BS, and CD5BS groups compared to the BD group (Ivanochko et al. 2025b). In the BD, CD10BS, and BD5BS groups, the amount of received calories was similar. These results indicated that consumption of CD did not cause overeating behaviour in mice and animals received enough calories (Ivanochko et al. 2025b). Consumption of CD did not cause critical body mass gain, and the addition of broccoli sprouts to the CD did not demonstrate significant changes either (Ivanochko et al. 2025b). However, the mass of visceral adipose tissue was significantly higher in the CD5BS group compared to BD, BD5BS, and even the CD group. This parameter was also higher in the CD10BS group compared to the BD group, suggesting that BS did not counteract the accumulation of visceral fat (Ivanochko et al. 2025b).

Previously, consumption of a high-fat, high-fructose diet (HFFD) was found to be one of the main stimuli for lipid peroxidation and higher LOOH content in the murine cerebral cortex (Demianchuk et al. 2024). In the current study, lipid peroxide levels did not differ between groups, suggesting that the CD diet did not induce lipid peroxidation in the cerebral cortex (Fig. 2).

Excess energy molecules from the catabolism of carbohydrates and lipids (NADH, $FADH_2$) can overload the electron transport chain of mitochondria, resulting in excessive production of ROS. In mitochondria, ROS are harmful not only to membrane lipids but also to fatty acids that undergo

β-oxidation, increasing the risks of oxidative damage. In our study, the content of LOOH was similar between groups, suggesting that the murine cerebral cortex is constitutively well-protected or non-affected during the described scenario of oxidative stress development. In a similar study with nine-month-old C57BL/6J females or eight-month-old C57BL/6J males, consumption of CD for 12 weeks and 16 weeks, respectively, did not cause high LOOH levels in the murine cerebral cortex (Demianchuk et al. 2025; Derkachov et al. 2023). On the contrary, eight-week consumption of the HFFD caused lipid peroxidation in the liver and cortex of mice (Bayliak et al. 2022a; Demianchuk et al. 2024). As a suggestion, short-term consumption of HFFD causes oxidative stress, while protective systems of the murine cortex respond and adapt to prolonged hypercaloric conditions to keep the brain healthy. In addition, the HFFD and CD contain different types of lipids, as well as carbohydrates and harmful compounds like advanced glycation end products (AGEs), flavour enhancers, etc. These compounds can reach the brain, and antioxidant plus detoxification proteins can respond in different ways to their presence, chemical type, and amount.

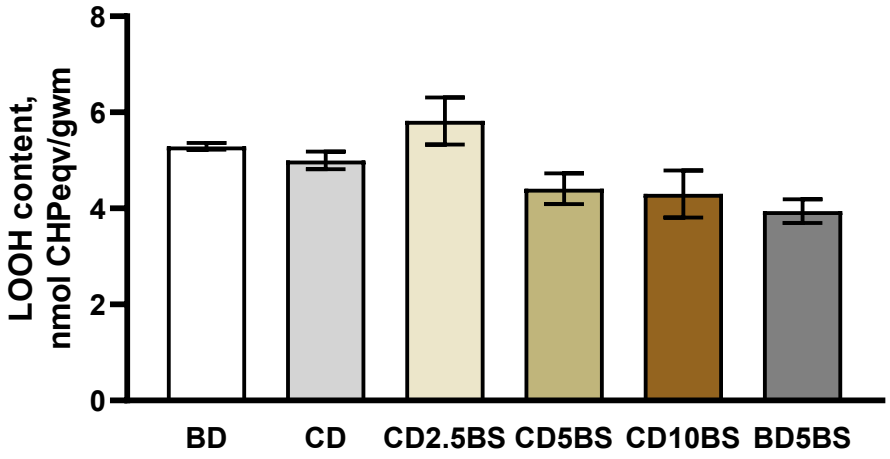

Figure 2. Lipid peroxide content in the cerebral cortex of mice fed a basal diet (BD), cafeteria diet (CD), cafeteria diet with 2.5%, 5% or 10% broccoli sprouts (CD2.5BS, CD5BS, CD10BS, respectively), and a basal diet with 5% broccoli sprouts (BD5BS) for 20 weeks. Data are presented as means ± SEM, n = 4-6.

Clear evidence of the antioxidant action of BS on the termination of lipid peroxidation and coping with its consequences was not determined in the present study, as LOOH values were similar between groups (Fig. 2). Broccoli sprouts have several compounds with antioxidant properties like polyphenols, vitamins, pigments, and others that have the potential to terminate lipid peroxidation (Ivanochko et al. 2025a). In our study, the antioxidant potential of BS compounds might be poorly realized in the murine brain. Alternatively, this potential could be overshadowed by the action of antioxidant enzymes that utilize ROS as a part of adaptation to long-term hypercaloric conditions and oxidative stress. Therefore, we measured the activity of some antioxidant enzymes in the murine cortex.

The activity of GPx in the CD5BS group had a trend to be lower compared to the other groups (Fig. 3A). The rest of the groups did not differ in this parameter. Glutathione peroxidase is an enzyme that reacts to mild oxidative stress and can reduce lipid peroxides, while catalase is the main scavenger of H_2O_2 and has no potential to terminate lipid peroxidation. Activation of Nrf2 upregulates GPx as a part of the antioxidant response (Cheng et al. 2015). Broccoli sprouts have many useful compounds that have the potential to activate Nrf2 for improved antioxidant and detoxification actions, as was determined in the liver of mice from a similar investigation (Ivanochko et al. 2023). In the present study, the activity of GPx in the murine cortex remained mainly unchanged with CD or BS consumption (Fig. 3A). The decreased GPx activity in the cortex was

previously described in mice on HFFD or CD (Demianchuk et al. 2024, 2025). Authors of the mentioned study linked the lower GSSG levels with the lower activities of GST and GPx in the cortices of HFFD mice (Demianchuk et al. 2024). In our study, we showed both trends towards lower GPx activity and lower LOOH levels on CD with the addition of BS. This can suggest that GPx did not play a key role in reducing LOOH, or LOOH can be produced at lower rates or more quickly metabolized by other pathways.

The activity of glutathione S-transferase (GST) was significantly ~ 2-fold higher in the BD5BS compared to the CD group (Fig. 3B). Between other groups, differences in GST activity were not found. This result suggests that the consumption of BS with the basic food had the potential to intensify Nrf2-related mechanisms of xenobiotic detoxification in the murine brain, since GST is a target of the Nrf2 transcriptional factor (Cheng et al. 2015). This feature was not realized in CD with BS groups. It was reported that the activity of GST and GSSG levels was lower in mice under consumption of HFFD (Demianchuk et al. 2024). In addition, CD with similar nutritional composition and 12-week exposure in females demonstrated lower activity of GST (Demianchuk et al. 2025). In the present study, GST activity was higher in BD5BS male mice and similar between the rest of the groups, indicating a potential of BS supplementation to stimulate detoxification processes that was not realized under hypercaloric conditions.

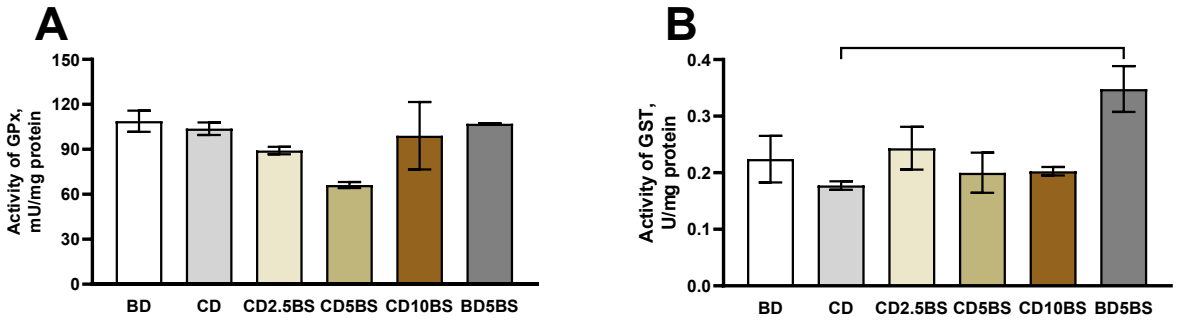

Figure 3. Activity of glutathione peroxidase (A) and glutathione S-transferase (B) in the cerebral cortex of mice fed a basal diet (BD), cafeteria diet (CD), cafeteria diet with 2.5%, 5% or 10% broccoli sprouts (CD2.5BS, CD5BS, CD10BS, respectively), and a basal diet with 5% broccoli sprouts (BD5BS) for 20 weeks. Data are presented as means ± SEM, n = 4-6. Bracket indicates a significant difference ($P < 0.05$) between groups using one-way ANOVA followed by Tukey's test for multiple comparisons.

Glutathione reductase reduces the GSSG to GSH for the protective action of GPx and GST. The activity of GR was approximately twice higher in CD10BS and BD5BS groups compared to the rest of the groups (Fig. 4A). Values of CD10BS and BD5BS were not significantly different.

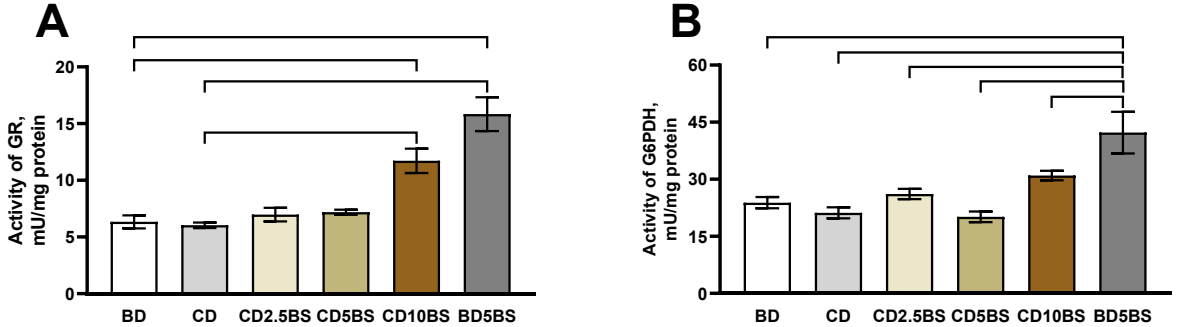

Figure 4. Activity of glutathione reductase (A) and glucose-6-phosphate dehydrogenase (B) in the cerebral cortex of mice fed a basal diet (BD), cafeteria diet (CD), cafeteria diet with 2.5%, 5% or

10% broccoli sprouts (CD2.5BS, CD5BS, CD10BS, respectively), and a basal diet with 5% broccoli sprouts (BD5BS) for 20 weeks. Data are presented as means ± SEM, n = 4-6. Brackets indicate a significant difference ($P < 0.05$) between groups using one-way ANOVA followed by Tukey's test for multiple comparisons.

Hypothetically, increased GR activity indicates cellular GSH necessity that could be depleted during obesity and lead to comorbidities in the liver (Vairetti et al. 2021). In the present study, consumption of an obesogenic CD alone did not affect the activity of GR in the cerebral cortex of mice. A high content of broccoli sprouts in the CD10BS group stimulated the activity of GR. It seems that 10% of broccoli sprouts stimulated GSSG reduction regardless of the oxidative-reductive state of murine brain cells, while in the BD5BS group, activity of GR was higher, as well as GST activity. Higher GR activity can provide a higher pool of reduced glutathione, which itself has antioxidant properties; in particular, GSH can react with LOOH, reducing the latter (Kennedy et al. 2020; V. I. Lushchak 2012). Similar to GST and GR, the activity of G6PDH was higher in the BD5BS group compared to the other groups (Fig. 4B).

The activity of NAD(P)H-quinone oxidoreductase 1 (NQO1) in the BD5BS group was significantly higher compared to BD, CD, CD2.5BS, and CD5BS groups (Fig. 5). The CD5BS value of NQO1 activity was also lower compared to CD10BS.

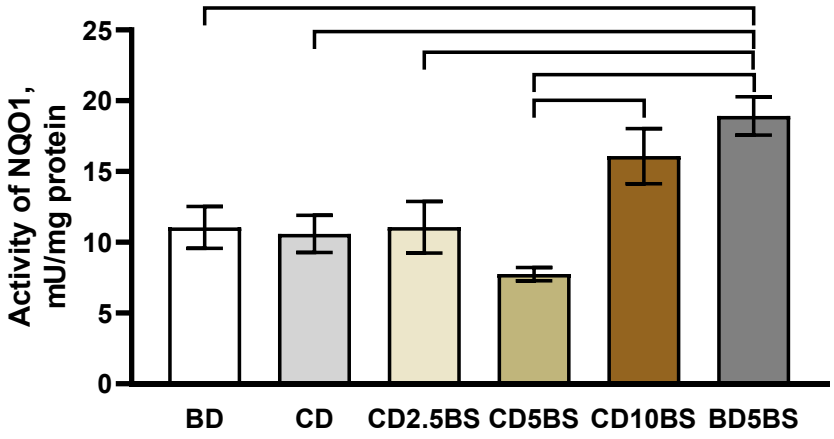

Figure 5. Activity of NAD(P)H-quinone oxidoreductase 1 in the cerebral cortex of mice fed a basal diet (BD), cafeteria diet (CD), cafeteria diet with 2.5%, 5% or 10% broccoli sprouts (CD2.5BS, CD5BS, CD10BS, respectively), and a basal diet with 5% broccoli sprouts (BD5BS) for 20 weeks. Data are presented as means ± SEM, n = 4-6. Brackets indicate a significant difference ($P < 0.05$) between groups using one-way ANOVA followed by Tukey's test for multiple comparisons.

The cerebral cortex was reported to be impenetrable to the influence of obesogenic diets, while beneficial compounds of broccoli sprouts can reach brain structures. It was described that the activity of GPx and GST was lower in the HFFD murine cortex, and the activity of GPx, GST, G6PDH, and NQO1 was lower in CD-fed females (Demianchuk et al. 2024, 2025). In our study, such an effect was present for NQO1 only in the CD5BS group. CD10BS and BD5BS groups demonstrated several similarities, suggesting that the positive effects of BS in the CD were realized at 10% dose and at 5% in the basal diet. Hypothetically, these doses are critical for the intervention of sulforaphane or other broccoli sprouts compounds for acting on the Nrf2/ARE system and protecting against ROS and xenobiotics. Regarding the activation of Nrf2, we tried to determine the level of Nrf2 by Western blot, but unfortunately, we were unable to detect an appropriate signal (apparently due to the instability of the antibodies). This limitation is common for several studies of Nrf2. Based on the determination of enzyme activity, we can only assume the involvement of Nrf2. Future research

should study each stage in the chain of the Nrf2 response, including relative expression of key target genes and Western blot of proteins, as well as changes in bioenergetic parameters of the murine brain. Such comprehensive analyses would allow a more definitive confirmation of Nrf2 pathway activation and help clarify the molecular mechanisms underlying the neuroprotective effects of broccoli sprouts consumption under an obesogenic diet.

4. CONCLUSIONS

Broccoli sprouts did not have any gradual effects when consumed with a long-term cafeteria diet. Consumption of 10% broccoli sprouts with a cafeteria diet did not support antioxidant defense directly, but by enhancing glutathione reductase activity. The presence of 5% broccoli in basal feed activated the antioxidant defense involved in xenobiotic detoxification and maintenance of reduced glutathione. As an interpretation, broccoli sprouts activate Nrf2 to affect targets that support antioxidant protection rather than the main enzymes that directly neutralize xenobiotics and ROS.

Acknowledgments. The authors are grateful to Professor, D.Sc., Head of Department of Biochemistry and Biotechnology Maria Bayliak for supervision of this work. The authors are grateful to two anonymous reviewers for their critical analysis of our work, which has significantly improved the manuscript. The authors are grateful to the defenders of Ukraine, whose heroic deeds made this research possible.

Author contributions. M. Ivanochko – performance of experiments (biochemical assays), data analysis, visualisation and writing of original graft (Materials and Methods, Results and Discussion, Conclusion); V. Chyr — writing of original graft (Introduction); I. Yatskiv — performance of experiments (maintenance and feeding of mice, biochemical assays); V. Lushchak — conceptualisation, writing — review and editing, funding acquisition.

Funding. This research was supported by a grant from the Ministry of Education and Science of Ukraine (#0122U000894) to Volodymyr Lushchak.

Data availability. All data are available on request.

Declarations

Conflict of interest. The authors have no competing interests to declare relevant to this article's content.

Research involving human participants and/or animals. The experiments were approved by the ethics committee of Vasyl Stefanyk Carpathian National University and assigned it the number PNU-2023-M-010. Procedures were conducted following Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

REFERENCES

Alhajri, A. S., & H. Saad, H. (2023). The Biological, Biochemical, and Immunological Impact of Broccoli and Green Pea Sprouts on Acrylamide Intoxicated Rats. *Current Research in Nutrition and Food Science Journal*, *11*(3), 1243–1262. https://doi.org/10.12944/CRNFSJ.11.3.28

Bayliak, M. M., & Abrat, O. B. (2020). Role of Nrf2 in Oxidative and Inflammatory Processes in Obesity and Metabolic Diseases. In H. Deng (Ed.), *Nrf2 and its Modulation in Inflammation* (Vol. 85, pp. 153–187). Springer International Publishing. https://doi.org/10.1007/978-3-030-44599-7_7

Bayliak, M. M., Sorochynska, O. M., Kuzniak, O. V., Drohomyretska, I. Z., Klonovskyi, A. Ya., Hrushchenko, A. O., Vatashchuk, M. V., Mosiichuk, N. M., Storey, K. B., Garaschuk, O., & Lushchak, V. I. (2022b). High stability of blood parameters during mouse lifespan: Sex-specific effects of every-other-day fasting. *Biogerontology*, *23*(5), 559–570. https://doi.org/10.1007/s10522-022-09982-x

Bayliak, M. M., Vatashchuk, M. V., Gospodaryov, D. V., Hurza, V. V., Demianchuk, O. I., Ivanochko, M. V., Burdyliuk, N. I., Storey, K. B., Lushchak, O., & Lushchak, V. I. (2022a). High fat high fructose diet induces mild oxidative stress

and reorganizes intermediary metabolism in male mouse liver: Alpha-ketoglutarate effects. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1866*(12), 130226. https://doi.org/10.1016/j.bbagen.2022.130226

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cavaliere, G., Catapano, A., Trinchese, G., Cimmino, F., Penna, E., Pizzella, A., Cristiano, C., Lama, A., Crispino, M., & Mollica, M. P. (2022). Butyrate Improves Neuroinflammation and Mitochondrial Impairment in Cerebral Cortex and Synaptic Fraction in an Animal Model of Diet-Induced Obesity. *Antioxidants*, *12*(1), 4. https://doi.org/10.3390/antiox12010004

Cheng, M.-L., Lu, Y.-F., Chen, H., Shen, Z.-Y., & Liu, J. (2015). Liver expression of Nrf2-related genes in different liver diseases. *Hepatobiliary & Pancreatic Diseases International*, *14*(5), 485–491. https://doi.org/10.1016/S1499-3872(15)60425-8

De Souza, C. T., Araujo, E. P., Bordin, S., Ashimine, R., Zollner, R. L., Boschero, A. C., Saad, M. J. A., & Velloso, L. A. (2005). Consumption of a Fat-Rich Diet Activates a Proinflammatory Response and Induces Insulin Resistance in the Hypothalamus. *Endocrinology*, *146*(10), 4192–4199. https://doi.org/10.1210/en.2004-1520

Demianchuk, O., Bayliak, M., Vatashchuk, M., Gospodaryov, D., Hurza, V., Derkachov, V., Berezovskyi, V., & Lushchak, V. I. (2025). Alpha-ketoglutarate promotes anxiety, activates autophagy, and suppresses antioxidant enzymes in the cerebral cortex of female mice on cafeteria diet. *Brain Research Bulletin*, *222*, 111255. https://doi.org/10.1016/j.brainresbull.2025.111255

Demianchuk, O., Vatashchuk, M., Gospodaryov, D., Hurza, V., Ivanochko, M., Derkachov, V., Berezovskyi, V., Lushchak, O., Storey, K. B., Bayliak, M., & Lushchak, V. I. (2024). High-fat high-fructose diet and alpha-ketoglutarate affect mouse behavior that is accompanied by changes in oxidative stress response and energy metabolism in the cerebral cortex. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1868*(1), 130521. https://doi.org/10.1016/j.bbagen.2023.130521

Derkachov V. P., Ivanochko M.V., Bayliak M.M. (2023). The effect of broccoli sprouts on oxidative stress markers in mice fed with cafeteria diet. *Biotechnologia Acta*, 16(2), 18–20. https://doi.org/10.15407/biotech16.02.018

Garaschuk, O., Semchyshyn, H. M., & Lushchak, V. I. (2018). Healthy brain aging: Interplay between reactive species, inflammation and energy supply. *Ageing Research Reviews*, *43*, 26–45. https://doi.org/10.1016/j.arr.2018.02.003

Hermes-Lima, M., Willmore, W. G., & Storey, K. B. (1995). Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. *Free Radical Biology and Medicine*, *19*(3), 271–280. https://doi.org/10.1016/0891-5849(95)00020-X

Higa, T. S., Spinola, A. V., Fonseca-Alaniz, M. H., & Evangelista, F. S. (2014). Comparison between cafeteria and high-fat diets in the induction of metabolic dysfunction in mice. *Int J Physiol Pathophysiol Pharmacol*, 13; 6(1):47-54

Ivanochko M.V., Demianchuk O.I., Bayliak M.M., Lushchak V. I. (2023). Consumption of broccoli sprouts increased the activity of glutathione-dependent antioxidant enzymes in murine liver. *Biotechnologia Acta*, 16(2), 26–29. https://doi.org/10.15407/biotech16.02.026

Ivanochko, M. V., Dmytriv, T. R., Yatskiv, I. M., Bayliak, M. M., Lushchak, V. I. (2025b). Metabolic effects of broccoli sprouts in mice with cafeteria diet-induced obesity. *The Ukrainian Biochemical Journal*, 97(6), 65–78. https://doi.org/10.15407/ubj97.06.065

Ivanochko, M. V., Fediv, K. V., Shvadchak, V. V., Bayliak, M. M., & Lushchak, V. I. (2025a). Nutritional analysis of aqueous and ethanol broccoli sprout extracts. *Journal of Plant Biochemistry and Biotechnology*. https://doi.org/10.1007/s13562-025-00974-9

Ivanochko, M. V., M. M. BaylIak, & Lushchak, V. I. (2024). Potential of isothiocyanate sulforaphane from broccoli to combat obesity and type 2 diabetes: Involvement of NRF2 regulatory pathway. *The Ukrainian Biochemical Journal*, *96*(6), 17–28. https://doi.org/10.15407/ubj96.06.017

Johnson, A. R., Wilkerson, M. D., Sampey, B. P., Troester, M. A., Hayes, D. N., & Makowski, L. (2016). Cafeteria diet-induced obesity causes oxidative damage in white adipose. *Biochemical and Biophysical Research Communications*, *473*(2), 545–550. https://doi.org/10.1016/j.bbrc.2016.03.113

Kennedy, L., Sandhu, J. K., Harper, M.-E., & Cuperlovic-Culf, M. (2020). Role of Glutathione in Cancer: From Mechanisms to Therapies. *Biomolecules*, *10*(10), 1429. https://doi.org/10.3390/biom10101429

Lushchak, O. V., Kubrak, O. I., Nykorak, M. Z., Storey, K. B., & Lushchak, V. I. (2008). The effect of potassium dichromate on free radical processes in goldfish: Possible protective role of glutathione. *Aquatic Toxicology*, *87*(2), 108–114. https://doi.org/10.1016/j.aquatox.2008.01.007

Lushchak, V. I. (2012). Glutathione Homeostasis and Functions: Potential Targets for Medical Interventions. *Journal of Amino Acids*, *2012*, 1–26. https://doi.org/10.1155/2012/736837

Lushchak, V. I. (2021). Interplay between bioenergetics and oxidative stress at normal brain aging. Aging as a result of increasing disbalance in the system oxidative stress–energy provision. *Pflügers Archiv - European Journal of Physiology*, *473*(5), 713–722. https://doi.org/10.1007/s00424-021-02531-4

Mao, L., Yang, T., Li, X., Lei, X., Sun, Y., Zhao, Y., Zhang, W., Gao, Y., Sun, B., & Zhang, F. (2019). Protective effects of sulforaphane in experimental vascular cognitive impairment: Contribution of the Nrf2 pathway. *Journal of Cerebral Blood Flow & Metabolism*, *39*(2), 352–366. https://doi.org/10.1177/0271678X18764083

Morrison, C. D., Pistell, P. J., Ingram, D. K., Johnson, W. D., Liu, Y., Fernandez-Kim, S. O., White, C. L., Purpera, M. N., Uranga, R. M., Bruce-Keller, A. J., & Keller, J. N. (2010). High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: Implications for decreased Nrf2 signaling. *Journal of Neurochemistry*, *114*(6), 1581–1589. https://doi.org/10.1111/j.1471-4159.2010.06865.x

Raichle, M. E., & Gusnard, D. A. (2002). Appraising the brain's energy budget. *Proceedings of the National Academy of Sciences*, *99*(16), 10237–10239. https://doi.org/10.1073/pnas.172399499

Salas-Venegas, V., Flores-Torres, R. P., Rodríguez-Cortés, Y. M., Rodríguez-Retana, D., Ramírez-Carreto, R. J., Concepción-Carrillo, L. E., Pérez-Flores, L. J., Alarcón-Aguilar, A., López-Díazguerrero, N. E., Gómez-González, B., Chavarría, A., & Konigsberg, M. (2022). The Obese Brain: Mechanisms of Systemic and Local Inflammation, and Interventions to Reverse the Cognitive Deficit. *Frontiers in Integrative Neuroscience*, *16*, 798995. https://doi.org/10.3389/fnint.2022.798995

Sampey, B. P., Vanhoose, A. M., Winfield, H. M., Freemerman, A. J., Muehlbauer, M. J., Fueger, P. T., Newgard, C. B., & Makowski, L. (2011a). Cafeteria Diet Is a Robust Model of Human Metabolic Syndrome With Liver and Adipose Inflammation: Comparison to High-Fat Diet. *Obesity*, *19*(6), 1109–1117. https://doi.org/10.1038/oby.2011.18

Sampey, B. P., Vanhoose, A. M., Winfield, H. M., Freemerman, A. J., Muehlbauer, M. J., Fueger, P. T., Newgard, C. B., & Makowski, L. (2011b). Cafeteria Diet Is a Robust Model of Human Metabolic Syndrome With Liver and Adipose Inflammation: Comparison to High-Fat Diet. *Obesity*, *19*(6), 1109–1117. https://doi.org/10.1038/oby.2011.18

Shirai, Y., Fujita, Y., Hashimoto, R., Ohi, K., Yamamori, H., Yasuda, Y., Ishima, T., Suganuma, H., Ushida, Y., Takeda, M., & Hashimoto, K. (2015). Dietary Intake of Sulforaphane-Rich Broccoli Sprout Extracts during Juvenile and Adolescence Can Prevent Phencyclidine-Induced Cognitive Deficits at Adulthood. *PLOS ONE*, *10*(6), e0127244. https://doi.org/10.1371/journal.pone.0127244

Subedi, L., Cho, K., Park, Y. U., Choi, H. J., & Kim, S. Y. (2019). Sulforaphane-Enriched Broccoli Sprouts Pretreated by Pulsed Electric Fields Reduces Neuroinflammation and Ameliorates Scopolamine-Induced Amnesia in Mouse Brain through Its Antioxidant Ability via Nrf2-HO-1 Activation. *Oxidative Medicine and Cellular Longevity*, *2019*, 1–19. https://doi.org/10.1155/2019/3549274

Vairetti, M., Di Pasqua, L. G., Cagna, M., Richelmi, P., Ferrigno, A., & Berardo, C. (2021). Changes in Glutathione Content in Liver Diseases: An Update. *Antioxidants*, *10*(3), 364. https://doi.org/10.3390/antiox10030364

Zhang, T., Holman, J., McKinstry, D., Trindade, B. C., Eaton, K. A., Mendoza-Castrejon, J., Ho, S., Wells, E., Yuan, H., Wen, B., Sun, D., Chen, G. Y., & Li, Y. (2023). A steamed broccoli sprout diet preparation that reduces colitis via the gut microbiota. *The Journal of Nutritional Biochemistry*, *112*, 109215. https://doi.org/10.1016/j.jnutbio.2022.109215

Zhou, R., Lin, J., & Wu, D. (2014). Sulforaphane induces Nrf2 and protects against CYP2E1-dependent binge alcohol-induced liver steatosis. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1840*(1), 209–218. https://doi.org/10.1016/j.bbagen.2013.09.018

Marian Ivanochko, PhD student, Department of Biochemistry and Biotechnology Vasyl Stefanyk Carpathian National University, Ivano-Frankivsk, Ukraine;

ORCID ID: https://orcid.org/0000-0002-1947-2564

Victoriia Chyr, bachelor's degree student, Department of Biochemistry and Biotechnology Vasyl Stefanyk Carpathian National University, Ivano-Frankivsk, Ukraine;

ORCID ID: https://orcid.org/0009-0007-9572-0412

Iryna Yatskiv, bachelor, Department of Biochemistry and Biotechnology Vasyl Stefanyk Carpathian National University, Ivano-Frankivsk, Ukraine;

ORCID ID: https://orcid.org/0009-0006-7615-4200

Volodymyr Lushchak, Professor, Doctor of Science, Department of Biochemistry and Biotechnology Vasyl Stefanyk Carpathian National University, Ivano-Frankivsk, Ukraine;

ORCID ID: https://orcid.org/0000-0001-5602-3330

Address: M. Ivanochko, V. Chyr, I. Yatskiv, V. Lushchak, Vasyl Stefanyk Carpathian National University, 57 Shevchenko Str., Ivano-Frankivsk, 76018 Ukraine.
E-mail: marian.ivanochko@cnu.edu.ua, viktoriia.chyr.25@cnu.edu.ua, iryna.yatskiv.21@pnu.edu.ua, volodymyr.lushchak@cnu.edu.ua.

Іваночко М., Чир В., Яцків І., Лущак В. (2026) Вибірковий вплив споживання проростків броколі на активність антиоксидантних ферментів у корі головного мозку мишей з кафетерійним харчуванням. *Журнал Прикарпатського національного університету імені Василя Стефаника. Біологія* 13: 18-28.

Надлишок енергії від кафетерійної їжі призводить до розвитку ожиріння та збільшує ризик інших проблем зі здоров'ям, таких як оксидативний стрес у мозку та нейродегенеративні захворювання. Проростки броколі вважаються харчовим засобом проти ожиріння та оксидативного стресу завдяки потенціалу їх сполук стимулювати клітинні захисні та регуляторні системи, такі як Nrf2. Метою цього дослідження було вивчення впливу споживання проростків броколі на активність антиоксидантних та допоміжних до них ферментів у корі головного мозку мишей, яких годували кафетерійною їжею. Протягом 20 тижнів восьмимісячні самці C57BL/6J споживали базову їжу, кафетерійну їжу, базову їжу з 5% проростками броколі або комбінацію кафетерійної їжі з 2,5, 5 та 10% проростками броколі. Результати показали, що лише 10% проростків броколі з кафетерійною їжею мали сприятливий вплив на активність глютатіонредуктази в корі головного мозку мишей, яка була приблизно в 1,8 разів більша порівняно зі значенням групи базової їжі. Натомість споживання 5% проростків броколі з базовою їжею спричинило приблизно вдвічі вищі показники активності глютатіон-S-трансферази, глютатіонредуктази, глюкозо-6-фосфатдегідрогенази та НАД(Ф)Н-хінон оксидоредуктази 1 порівняно з іншими групами. Як висновок, споживання проростків броколі стимулює антиоксидантний захист і процеси детоксикації через білки-мішені Nrf2 в корі головного мозку мишей, яких годували базовою їжею, але не гіперкалорійною кафетерійною їжею.

Ключові слова: антиоксидантні ферменти, кафетерійна їжа, кора головного мозку, миші, проростки броколі, Nrf2.