

METABOLIC EFFECTS OF BROCCOLI SPROUTS IN MICE WITH CAFETERIA DIET-INDUCED OBESITY

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*Broccoli sprouts (BS) are rich in bioactive compounds with reported antioxidant and anti-inflammatory properties. In this study, a cafeteria diet (CD) was used as a model to study diet-induced obesity in animals. The aim of the study was to evaluate the effects of dietary BS supplementation on metabolic parameters in middle-aged male mice subjected to a cafeteria diet (CD) containing such additional components (w/w) as sweet peanuts (28%), milk chocolate (28%) and chocolate cracker (11%). Mice were fed on CD over 20 weeks, after that, blood was collected, mice were sacrificed, liver and adipose tissue were collected and weighed. The levels of glucose, triacylglycerides (TAG), and cholesterol were determined with a diagnostic kit (Reagent, Dnipro, Ukraine), that of IL-1 β – by ELISA. Paraoxonase (PON) activity in blood was determined by monitoring *p*-nitrophenol formation. Mice fed on the CD alone exhibited higher caloric intake without significant body mass gain, but demonstrated elevated liver mass, hyperglycemia, hypertriglyceridemia, and decreased PON activity relative to those fed on the standard diet. Inclusion of BS (2.5, 5 or 10% w/w) in the CD prevented the rise in TAG level and preserved PON activity. However, BS in higher doses (5 and 10%) increased visceral fat accumulation and further elevated blood glucose levels. In contrast, BS supplementation in a standard diet reduced circulating TAG and inflammatory markers without affecting adipose tissue distribution. These findings indicate a dual role of BS in metabolic regulation: while beneficial in reducing oxidative and inflammatory markers, BS may aggravate visceral adiposity and glycemic imbalance in an obesogenic context.*

Key words: *broccoli sprouts, cafeteria diet, inflammation, metabolic health, paraoxonase, triacylglycerol, visceral fat.*

Obesity is one of the most common lifestyle-related health problems in the world. The World Health Organization (WHO) defined obesity as excessive or abnormal fat accumulation related to an energy imbalance between calories consumed and expended. Thus, excess energy is associated with increased calorie intake, i.e., overeating, which contributes to body mass gain and comorbidities [1-3]. For example, the so-called cafeteria diet (CD) – a diet high in fat, sugar, and salt, characterised by a variety of cafeteria-type foods (such as milk chocolate, chocolate crackers, and peanuts) – promotes obesity and related metabolic disturbances in rodents, mimicking obesity in humans [4]. Such disturbances include increased body mass, fatty liver, excessive body fat accumulation, elevated blood glucose levels, disbalanced blood lipid profile, signs of inflammation and oxidative stress [4-8].

Diets based on functional plant foods, such as broccoli sprouts, are a promising intervention against obesity and related complications [9, 10]. Although broccoli sprouts are not a standardised supplement, they are a very prospective nutritional additive due to their variety of applications in cooking. The high levels of fibres, vitamins, and minerals contribute to the beneficial properties of broccoli [11, 12]. Also, the low-calorie content of broccoli may be a significant advantage for subjects suffering from overweight and obesity. Sulforaphane, a biologically active isothiocyanate abundant in broccoli, is believed to be the primary mediator responsible for its health-promoting properties [13]. A systematic review and meta-analysis by Du and colleagues [14] showed the benefits of sulforaphane (SFN) for weight loss and improvement in lipid profile in ten trials. Sulforaphane is formed by the hydroly-

sis of glucosinolate glucoraphanin, a biologically stable precursor, by an enzyme called myrosinase. Normally, myrosinases are stored separately from glucosinolates and are in contact with them when plant tissues are damaged, which results in SFN production. Therefore, intact plants contain negligible sulforaphane levels [13, 15]. The content of sulforaphane in broccoli plants depends on the stage of plant development and is 10-100 times higher in broccoli sprouts (BS) in comparison to broccoli florets [16]. A diet based on broccoli sprouts and rich in sulforaphane can be a potential intervention for preventing/treating obesity [10, 13]. For example, BS supplementation has been shown to reduce body mass in mice with bisphenol A-induced obesity, as well as fat accumulation and inflammatory markers in overweight adult subjects [17, 18]. Some research suggests that broccoli sprouts, owing to the antioxidant and anti-inflammatory activities of their phytochemicals, can reduce body mass in overweight people and decrease inflammation and oxidative stress in people with type 2 diabetes [17, 19]. However, the anti-obesity potential of broccoli has not been sufficiently studied.

This study aimed to investigate the potential of long-term consumption of three-day-old broccoli sprouts at different doses to improve metabolic health in mice fed a high-calorie cafeteria diet.

Materials and Methods

Cultivation of broccoli sprouts. Green broccoli seeds (*Brassica oleracea* var. *italica*, cultivar *calabrese*) from “SemyaSvet” Company (Odesa, Ukraine) were used for obtaining broccoli sprouts. The seeds were cultivated on a moist cotton substrate in sealed transparent plastic containers at a 12-hour light/dark cycle (6 a.m./6 p.m.) and 25°C. The three-day-old broccoli sprouts (germinated seeds) were frozen in liquid nitrogen and stored at -20°C. The chemical composition of BS is given in Table 1.

Experimental design. Fig. 1 illustrates the design of the experiment. Eight-month-old C57BL/6J male mice were bred in our animal facility and randomly divided into six groups:

1) The control group (denoted as BD group) consumed the standard granular rodent chow (“Vita” company, Obukhiv, Ukraine) containing 20% proteins, 4.8% fats, 70% carbohydrates, and 4.7% fiber per 100 g. The caloric content of the standard food was 310 kcal per 100 g.

Table 1. Composition of three-day-old broccoli sprouts. Data are presented as means $\pm$ SD for six independent replicates

Broccoli parameter	Value
Total polyphenols	4.63 $\pm$ 0.05 mg GAE/gFW
Total flavonoids	1.96 $\pm$ 0.14 mg QE/gFW
Fructose	0.060 $\pm$ 0.002 mg/gFW
Vitamin C	35.2 mg vit C per 100 g
Vitamin P	0.12%
Vitamin B ₁	1.67 $\pm$ 0.01 mg/gFW
Total protein	26.7 $\pm$ 0.2 mg/gFW
Starch	1.80 $\pm$ 0.01 mg/gFW

Note. GAE – gallic acid equivalents; QE – quercetin equivalents; TAC – total antioxidant capacity; gFW – gram of fresh weight. More information in [12]

2) The cafeteria diet (CD) group consumed a hypercaloric, obesogenic food (459 kcal per 100 g) containing 19.3 % proteins, 22.6% fats, and 49.4% carbohydrates per 100 g and consisting of (w/w) sweet peanuts (28%), milk chocolate (28%), chocolate cracker (11%), casein (10%), and powdered rodent chow (23%) [20]. The CD diet was prepared manually by mixing all ingredients and forming pellets using a granulator.

3-5) The next three groups consumed a CD diet supplemented with broccoli sprouts (BS) at different doses, namely 2.5, 5, and 10% (w/w), and were denoted as CD2.5BS, CD5BS, and CD10BS, respectively. When broccoli sprouts were added to the feed, the content of powdered rodent feed was decreased, but the content of other CD components remained unchanged.

6) The broccoli sprouts group (BD5BS) received the standard chow supplemented with 5% (w/w) shredded broccoli sprouts [21, 22]. This diet was produced manually by mixing shredded broccoli sprouts with powdered rodent chow (“Vita” company, Ukraine) and forming pellets using a granulator (TMS company, Cherkasy, Ukraine).

Animals were kept on the corresponding diets for the next 20 weeks with a 12-hour light/dark cycle (6 a.m./6 p.m.) at 22 $\pm$ 2°C, and 50-60% humidity [23, 24]. Access to food and water was unlimited. Each group consisted of 5-6 mice (2-3 mice per cage). The amounts of food and water consumed were registered every second day. The mouse body

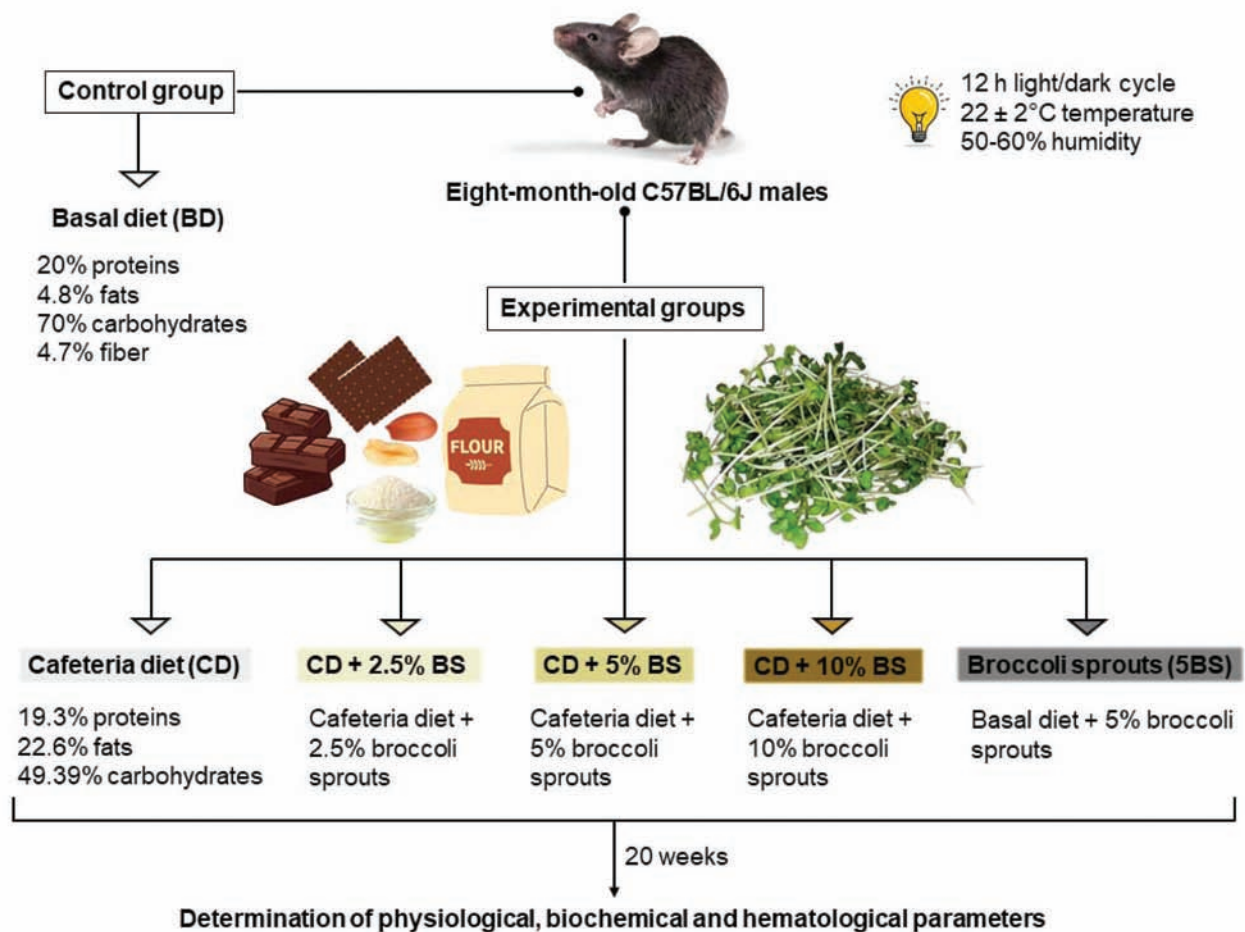


Fig. 1. Experimental design

mass was recorded weekly in the morning, at 9.00-10.00 a.m.

The experiments were approved by the ethics committee of Vasyl Stefanyk Carpathian National University and were conducted in accordance with 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.

Glucose tolerance test and blood glucose assay. A week before the euthanasia procedure, a glucose tolerance test was performed according to a modified protocol [25]. Mice were fasted for 6 h from 7:00 a.m. to 1:00 p.m. with access to water. During the first hour of fasting, the mice were weighed. Fasted mice were administered through a gavage tube with a 40% glucose solution in an amount corresponding to 2 g of glucose per 1 kg of body mass (glucose load). Blood was drawn from the tail vein with local anaesthetic cream before the start of glucose administration (0 hour, fasting glu-

cose) and within 2 h at 15, 30, 60, and 120 min after administration.

The concentration of glucose was determined by using a diagnostic kit (Reagent, Dnipro, Ukraine) according to the instructions.

Tissue collection. Mice were fasted from 9:00 p.m. to 9:00 a.m. They were then anaesthetised using light CO₂ anaesthesia. After that, blood was collected from the retroorbital sinus using a glass capillary. A part of blood was collected into microcentrifuge tubes (with heparin anticoagulant) and centrifuged at 4300 g for 10 min at 4°C to obtain plasma. The plasma samples were then frozen and stored at -80°C. The length of the mouse from nose to anus was measured in anesthetized mice. After that, mice were sacrificed by cervical dislocation. Liver and adipose tissue (epididymal, mesenteric, and perirenal) were collected, weighed, frozen in liquid nitrogen, and stored at -80°C for further biochemical analyses [23, 24].

Determination of hematological parameters.

The concentration of total hemoglobin was determined in whole blood with Drabkin's reagent using a commercial kit (Reagent, Dnipro, Ukraine). The hematocrit, which is the ratio of the volume of blood cells to the total volume of blood, was determined in sterile capillaries, which were filled with freshly collected blood. The ends of the capillaries were sealed and the capillaries were centrifuged at 2000 g for 15 min at room temperature. The ratio of the height of the red blood cell column (mm) to the height of the blood column (mm) was calculated and expressed as a percentage [23]. Fresh blood samples were diluted 200-fold in 3% NaCl to count red blood cells and 40-fold diluted in 5% acetic acid with methylene blue staining to count white blood cells. The numbers of red and white blood cells were then counted using a Goryaev chamber under a light microscope [23].

Determination of plasma biochemical parameters. The concentrations of triacylglycerols (TAG) and total cholesterol were measured using the respective diagnostic kits from the "Reagent" company (Dnipro, Ukraine) according to the manufacturer's instructions. Protein concentration was determined using Bradford assay at 595 nm with Coomassie brilliant blue reagent [26]. Paraoxonase activity was determined by monitoring p-nitrophenol formation at 405 nm in the reaction mixture containing 50 mM Tris-HCl (pH 7.0), 1 mM CaCl_2 , 3 mM 4-nitrophenyl acetate, and a plasma aliquot [27]. The reaction was started by the addition of 4-nitrophenyl acetate. The activity of paraoxonase was expressed in U/l. The activity of myeloperoxidase (MPO) was determined by the colourimetric method based on MPO-catalysed oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide to brown-colour oxybenzidine with maximum absorption at 450 nm [28]. The MPO activity was expressed in U/l.

Determination of IL-1 β . Blood plasma was diluted 5-fold in phosphate-buffered saline (PBS) (pH 7.4). 100 μl of diluted plasma was added to the 96-well microplate and incubated for 2 h for protein immobilization, followed by three washes with PBS. Then, 200 μl of 4% (bovine serum albumin (BSA) was added to each well for blocking, following incubation for 1 h at room temperature. After washing with PBS, primary antibodies anti-interleukin-1 β (Abcam, #ab9722, diluted with distilled water to a concentration of 100 $\mu\text{g}/\text{ml}$) were diluted in 4% BSA (1:100) and added to the samples with incubation for 2 h at room temperature. Probes were then

washed three times with PBS. Secondary anti-rabbit antibodies (CellSignaling, #7074S) were diluted in 4% BSA (1:2000) and added to each well and incubated for 2 h at room temperature. After washing with PBS, 80 μl of 3,3', 5,5'-tetramethylbenzidine dihydrochloride (substrate for horseradish peroxidase linked with secondary antibodies) was added to the samples. The samples were incubated at 37°C for 30 min until a blue color developed. After incubation, the reaction was stopped by the addition of 100 μl of 2 M H_2SO_4 . Absorption was determined at 450 nm using Biosan HiPo Microplate Photometer MPP-96. The results were expressed as the relative level of IL-1 β versus the mean level of the control group.

Statistical analysis. GraphPad Prism version 8.3.1 and MS Excel were used to perform statistical analysis and visualization. Data were subjected to one-way ANOVA followed by Tukey's tests for multiple comparisons. Results were presented as means $\pm$ SEM.

Results and Discussion

Food and water consumption. Mice on the cafeteria diet (CD group) consumed $\sim 14\%$ less food compared to the control mice fed the basic diet (BD group) (Fig. 2, A). Adding broccoli sprouts to the CD decreased food consumption. In particular, CD2.5BS, CD5BS, and CD10BS mice consumed ~ 22 , 25, and 31% less food, compared to the BD group. In the BD5BS group, food consumption was slightly increased, by 6%, compared to the BD group. In addition, the BD5BS group consumed more food compared to the CD5BS group. Thus, the mice on the cafeteria diet consumed less food than those on the basal diet. Lower food intake on high-calorie diets was previously observed in young mice [8, 29]. Here we found the same for middle-aged male mice. However, when we calculated the total energy received from food, the opposite situation to food consumption was observed. The BD group received 18.3 ± 0.3 kcal/mouse/day, and CD mice received 23.3 ± 0.4 kcal/mouse/day, which was $\sim 27\%$ higher than in the BD group, and it was the highest calorie intake among all experimental groups (Fig. 2, B).

Earlier studies with 10% (w/w) broccoli florets or stalks reported that broccoli did not change food intake [30]. In addition, administration of broccoli microgreens' juice (20 g/kg bw) decreased food consumption in mice [29]. In our experiment, a com-

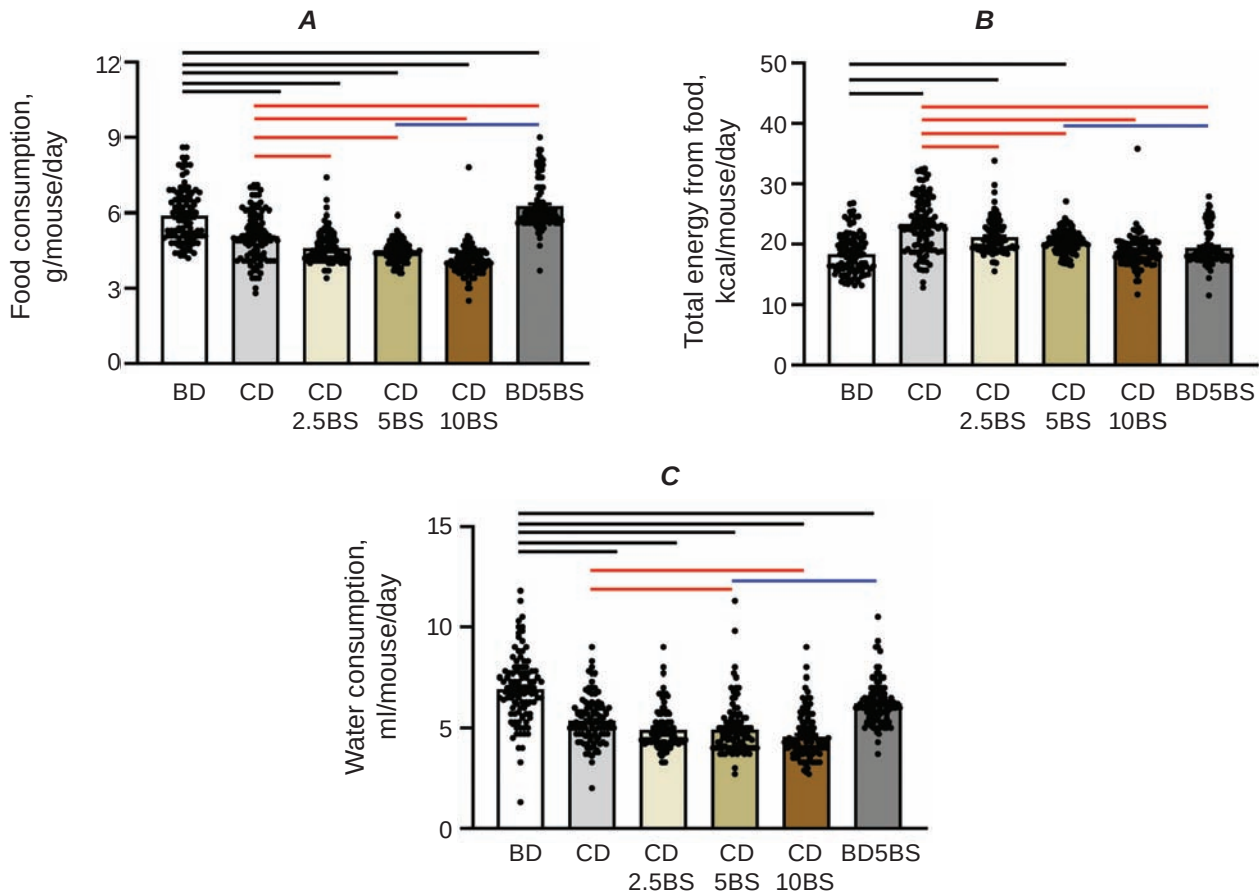


Fig. 2. Food consumption (A), calorie intake (B) and water consumption (C) by mice fed a basic diet alone (BD group) and in combination with 5% broccoli sprouts (BD5BS group), a cafeteria diet alone (CD group) and with addition of 2.5, 5 and 10% of broccoli sprouts (CD2.5BS, CD5BS and CD10BS groups) for 20 weeks. Food intake and water intake were measured every second day. Data are presented as means $\pm$ SEM from the number of measurements in two cages during the entire experiment (104 measurements are shown as black points within each group). Two cages were in each group, with 5-6 mice in each group. Lines above two bars denote a significant difference ($P < 0.05$) between groups using one-way ANOVA followed by Tukey's test for multiple comparisons

bination of broccoli sprouts with the cafeteria diet reduced food intake in a dose-dependent manner. Mice in the CD group with 2.5 and 5% of broccoli sprouts received 15 and 11% more calories, respectively, than the BD group. At the same time, with 10% broccoli sprouts in the CD, caloric intake was similar to that of the BD group. Combining basal food with broccoli sprouts (BD5BS group) did not affect calorie intake compared to the BD group, and CD5BS mice consumed more calories than BD5BS mice. Thus, broccoli sprouts have different effects on food consumption when added to the basic diet or to the cafeteria diet.

Lower water intake was detected in all experimental groups compared to the control BD group

(Fig. 2, C). As food consumption, water intake decreased with increasing broccoli sprouts' content in the CD (Fig. 2, C). Mice on the CD alone consumed 23% less water, and CD10BS mice consumed 31% less water than the BD group. Similar results in decreased water intake were observed when mice were fed a high-fat diet and broccoli microgreens juice [29].

Body mass and visceral fat. The cafeteria food caused no dramatic increase in body mass, only $\sim 5\%$, during 20 weeks of feeding (Fig. 3, A) despite the increased calorie intake (Fig. 2, B). Adding broccoli sprouts to CD did not prevent a minimal increase in body mass in mice at all concentrations of broccoli used. It should be noted that previous studies

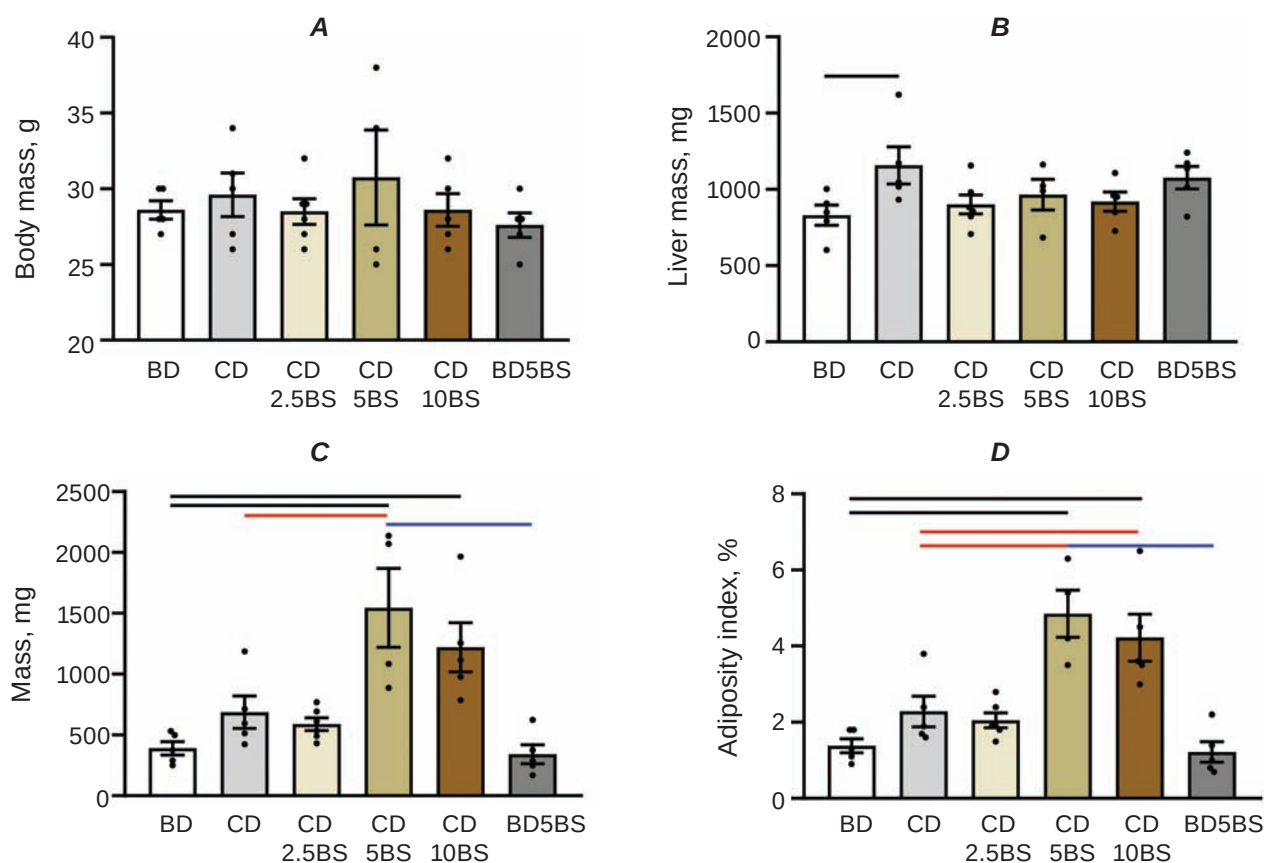


Fig. 3. Final body mass of mice (A), liver mass (B), mass of total visceral adipose tissue (C), and adiposity index (D). Data are presented as means $\pm$ SEM, $n = 4-6$ (shown as black points within each group). Other information is as in Fig. 2

reported on body mass loss in mice by feeding with broccoli microgreens [29] or sulforaphane isolated from broccoli sprouts [31, 32].

It is known that the development of metabolic disorders induced by high-calorie diets is not always accompanied by body mass gain, but, in rodents, the mass of visceral fat or such organs as the liver or spleen may increase [8, 24]. Fig. 3, B shows that CD mice had the heaviest liver (1158 ± 122 mg) among the groups. This value was 40% higher compared to the BD group. At the same time, groups fed a CD with broccoli sprouts did not differ in the liver mass from the control BD mice (Fig. 3, B). The total mass of visceral adipose tissue tended only to be higher in CD mice, compared with BD mice. Unexpectedly, mice in groups CD5BS and CD10BS had 3.9- and 2.9-fold more visceral fat, respectively, than the BD group (Fig. 3, C). More specifically, CD5BS and CD10BS mice had higher content of all three types of visceral fat, namely epididymal, perirenal, and mesenteric, than the BD and BD5BS groups (Table 2).

Accordingly, adiposity indexes were statistically higher in CD5BS and CD10BS mice and tended to be higher in the CD group and CD2.5BS group compared with the BD group (Fig. 3, D). Other indices of obesity, namely the Lee obesity index and body mass index, did not differ significantly between experimental groups due to high intragroup variability (Table 2).

Thus, although hypercaloric cafeteria food caused a decrease in food consumption, the total number of calories received was higher. At the same time, the body mass of the mice remained almost unchanged, with a slight increase in visceral adipose tissue. The broccoli sprout-induced increase in visceral fat in mice fed a CD may indicate an enhancement of triacylglycerol synthesis or the inflammation development. Increased TAG synthesis in visceral tissue is often accompanied by leukocyte infiltration, followed by inflammation and oxidative stress development [1]. Therefore, next, we measured certain hematological parameters and parameters of oxidative stress and immune status in experimental mice.

Table 2. Adiposity-related and several blood biochemical parameters in mice

	BD	CD	CD2.5BS	CD5BS	CD10	BD5BS
Epididymal adipose tissue, mg	217±32	495±82	431±25	1046±237	802±144	217±54
Perirenal adipose tissue, mg	114±25	122±25	113±11	230±39	207±32	104±21
Mesenteric adipose tissue, mg	73±17	117±29	151±38	268±66	211±35	34±3
Lee obesity index	0.328±0.007	0.320±0.005	0.329±0.007	0.320±0.007	0.322±0.005	0.334±0.009
Body mass index	0.34±0.01	0.32±0.02	0.34±0.02	0.33±0.03	0.32±0.01	0.34±0.02
Blood cholesterol, mmol/l	2.53±0.56	2.30±0.49	2.73±0.51	3.67±0.94	3.03±0.39	2.05±0.31
Blood protein, mg/ml	78.49±10.46	65.41±5.28	89.25±3.17	95.10±1.89	94.44±2.47	96.08±2.07

Note. Data are presented as means ± SEM, n = 4-6

Hematological parameters. The hemoglobin level did not differ between the groups (Fig. 4, A) and was within the range of values reported in the literature [23, 33]. No significant differences or significant deviations were observed between groups in hematocrit levels, except that CD2.5BS mice showed ~ 45% lower hematocrit levels compared to BD mice (Fig. 4, B). In addition, erythrocyte counts and total leukocyte counts were similar between groups (Fig. 4, C, D). Thus, CD diet alone and in combination with broccoli sprouts did not affect hematological and immune system parameters in middle-aged male mice.

Levels of glucose, triacylglycerides, cholesterol, and PON activity in the blood. Cafeteria diet alone and in combination with broccoli sprouts did not affect total cholesterol levels in the murine blood plasma (Table 2), which was reported previously in a similar study of type 2 diabetes and sulforaphane [37]. The level of total protein in plasma was lower in the CD mice, and, accordingly, in all groups that received broccoli sprouts, the blood protein level was higher compared to the CD mice, but did not differ from that of the BD mice (Table 2).

In the CD group, blood glucose levels were 25% higher than in the BD group (Fig. 5, A), a typical effect associated with hypercaloric or obesogenic diets [34]. Mice from the CD2.5BS and CD5BS groups had 1.5-fold higher blood glucose levels compared to BD mice. The CD10BS group showed 1.3-fold higher glucose levels compared to the BD group. Thus, mice fed CD with 2.5 and 5% broccoli sprouts had higher glucose levels than mice fed CD alone. In BD5BS mice, the blood glucose level was similar to the value in the BD group. Previously, it was reported that glucose levels were increased by broccoli in mice fed high-calorie food [30, 35]. But in our experiment, the presence of broccoli sprouts in the cafeteria diet promoted even higher blood glucose levels than the cafeteria diet alone did. When broccoli sprouts were added to the basic diet (BD5BS group), we did not observe this effect, and the blood glucose level was within the values of the control BD group.

The results of the glucose tolerance test are shown in Fig. 5, B. Curves demonstrate the typical bell-shaped dynamics of glucose level in the blood of mice after glucose loading, although a statistically significant increase in glucose levels after loading

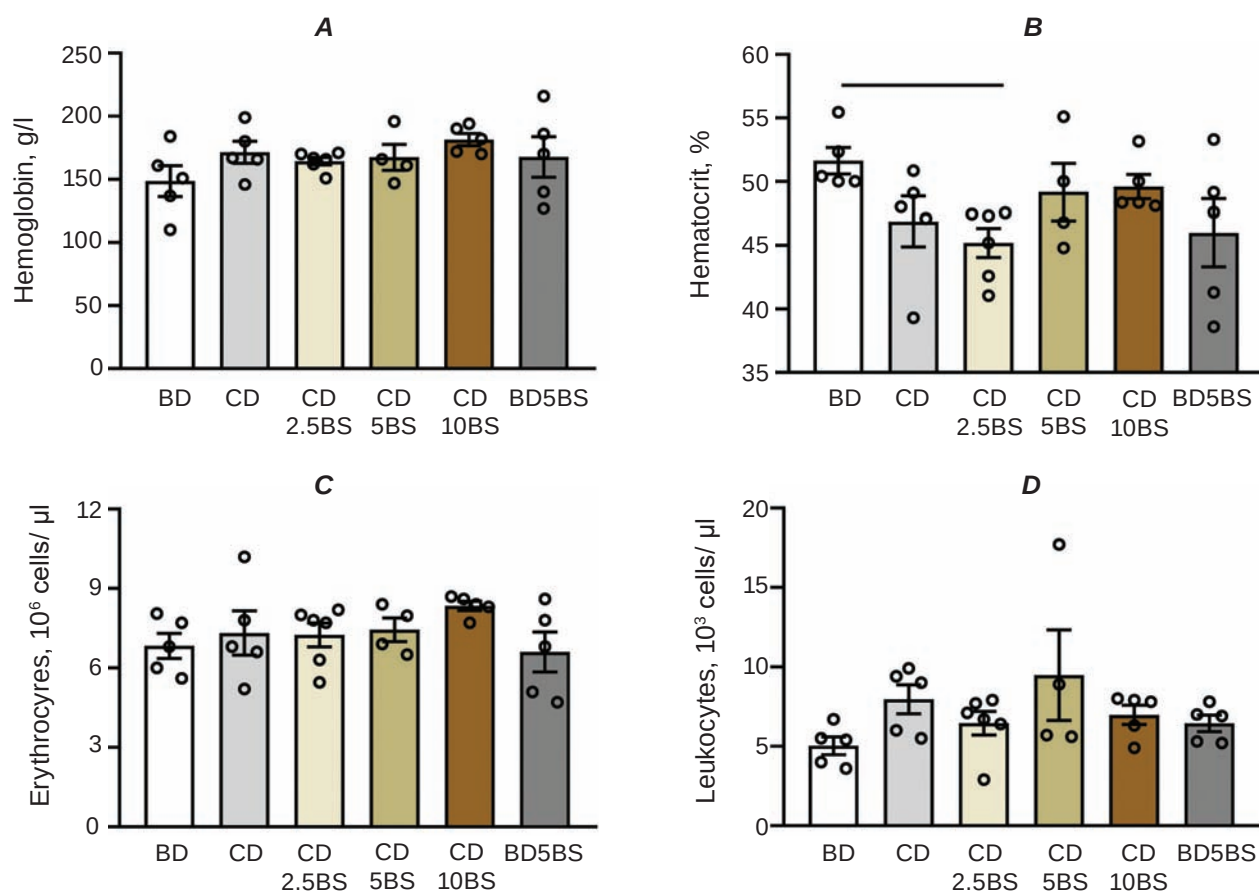


Fig. 4. Hemoglobin levels (A), hematocrit (B), erythrocyte (C), and total leukocyte (D) counts in the mouse blood. Data are presented as means $\pm$ SEM, $n=4-6$ (shown as black points within each group). Other information is as in Fig. 2

was not observed. Earlier, it was also found that there was a smaller increase in blood glucose concentration at oral glucose administration compared to one at an intraperitoneal injection [36]. Our results showed the lowest values of blood glucose level curves in the BD and BD5BS groups, the highest in the CD2.5BS and CD5BS groups, and intermediate values in the CD and CD10BS groups. Fasting glucose levels and glucose tolerance test values were consistent.

Plasma triacylglycerol (TAG) level was ~ 2.2 -fold higher in CD mice compared to the BD group (Fig. 6, A). At the same time, plasma TAG levels decreased with increasing dose of broccoli sprouts in the CD and reached control levels in the CD5BS and CD10BS groups. In the BD5BS mice, TAG levels were $\sim 90\%$ lower than in the control BD group.

These results suggest TAG-lowering effects of broccoli sprouts. Similar results were obtained in several previous experiments with mice fed a high-fat diet, with sulforaphane from broccoli [37, 38].

Our investigation demonstrated that the addition of broccoli sprouts effectively prevented an increase in TAG levels in murine plasma.

Mice fed CD alone had 56% lower activity of plasma paraoxonase (PON) compared with the BD group (Fig. 6, B). Addition of 2.5, 5 and 10% broccoli sprouts to CD had an upward trend in PON activity compared to BD (Fig. 6, B). In contrast, the BD5BS group had $\sim 47\%$ lower PON activity than the BD group (Fig. 6, B). Thus, the addition of 5% broccoli to the cafeteria diet (CD5BS) and to the basic food (BD5BS) had opposite effects on the PON activity.

Paraoxonase is an enzyme that prevents the oxidation of low-density lipoproteins, a process involved in the development of atherosclerosis as a comorbidity of obesity. It had been reported that consumption of a high-fat diet caused a decrease in paraoxonase activity in the blood of mice [39]. In addition, an investigation with male rats reported that paraoxonase activity and PON1 protein levels were decreased in the blood serum. Authors linked their

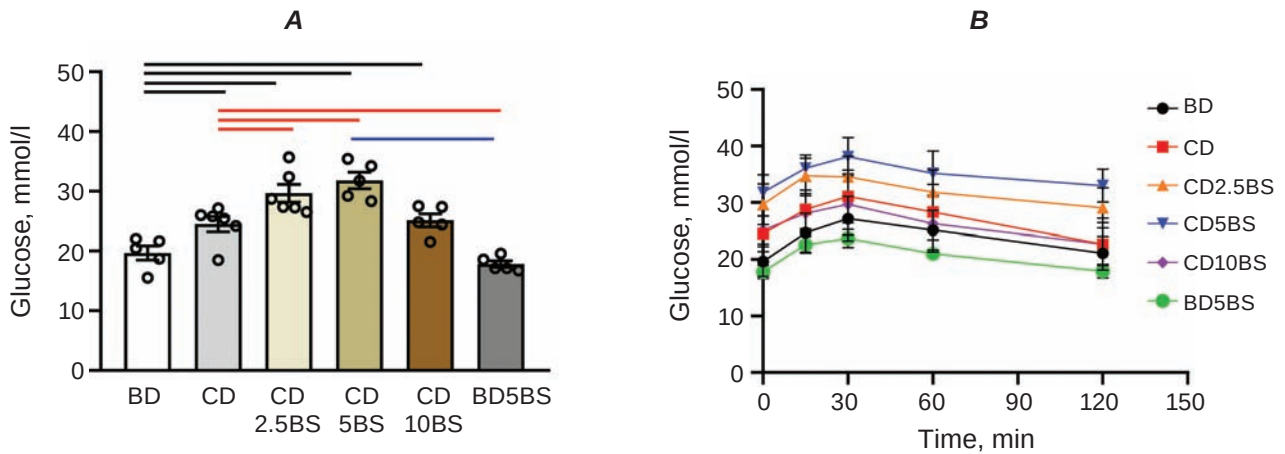


Fig. 5. Fasting blood glucose levels (A) and glucose tolerance test (B). Data are presented as means $\pm$ SEM, $n = 4-6$ (shown as black points within each group in panel A). Other information is as in Fig. 2

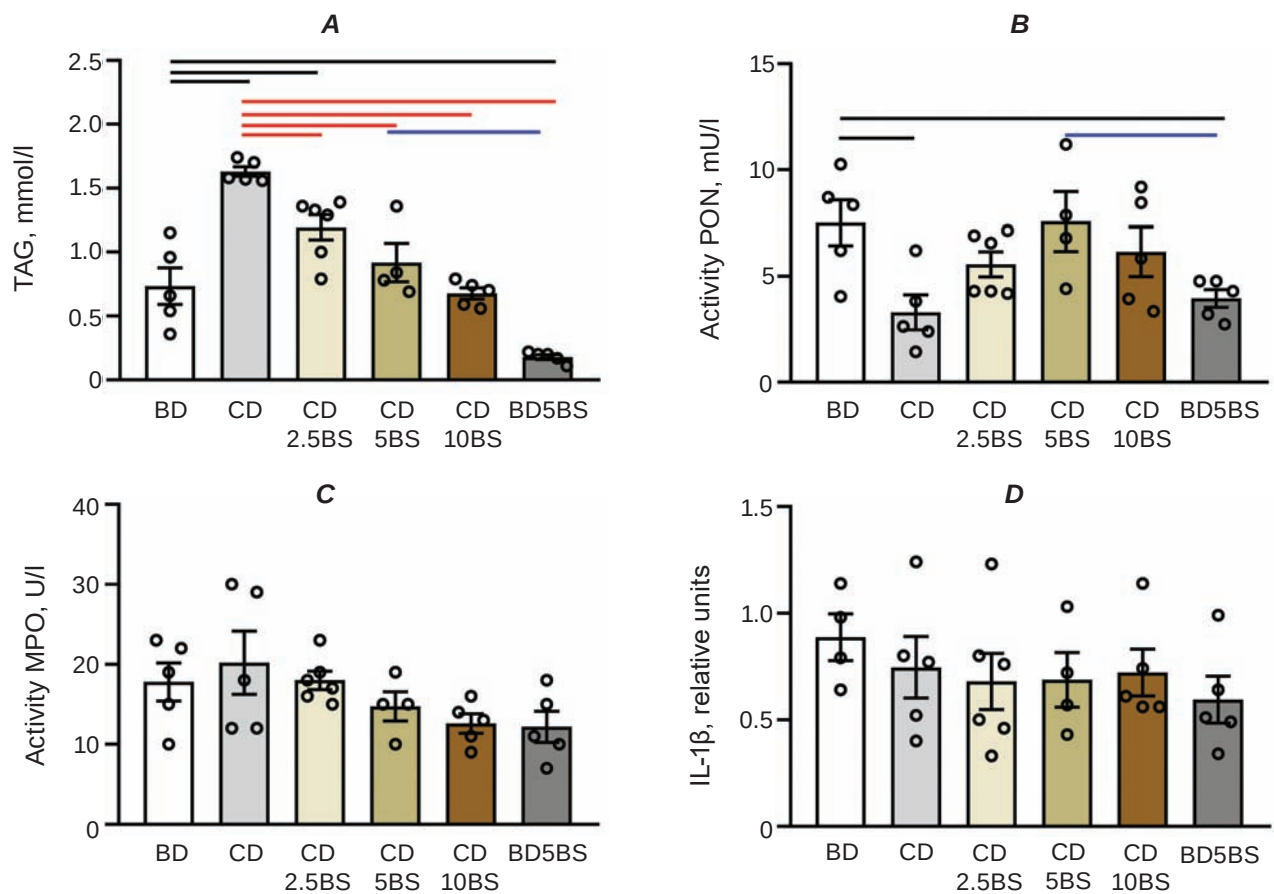


Fig. 6. Triacylglycerol levels (A), activities of paraoxonase (B) and myeloperoxidase (C), as well as relative level of IL-1 β (D) in blood plasma. Data are presented as means $\pm$ SEM, $n = 4-6$ (shown as black points within each group). Other information is as in Fig. 2

findings to decreased PON1 mRNA levels in males and proved a positive correlation between paraoxonase activity and PON1 mRNA levels [40].

In our study, we determined lower activity of paraoxonase in the CD group and BD5BS group compared to the BD group. At the same time, PON

activity was higher in groups fed CD with broccoli sprouts. It can be suggested that BS supplementation improved PON activity and lipid profile, in particular lowering TAG levels, which were impaired by eating of cafeteria diet.

The activity of myeloperoxidase, a central defence enzyme of immune cells - neutrophils, and levels of proinflammatory cytokine IL-1 β were not affected by any type of diet, but with the increase in BS content in the diet tended to be lower (Fig. 6, C, D). Myeloperoxidase level was increased during obesity-driven oxidative stress, inflammation, lipoprotein oxidation, and atherosclerosis [41]. Also, it was reported that plasma IL-1 β levels increased in obese humans [42]. In our study with mice, a cafeteria diet did not affect these immune markers, but BS supplementation showed a trend to lower these indices compared with both the BD group and the CD group. This may suggest the potential anti-inflammatory properties of broccoli sprouts.

Broccoli sprouts have different effects with a basic vs. a cafeteria diet. In our experiment, a cafeteria diet for 20 weeks did not cause visible signs of obesity in mice. It can be connected with the age of mice. We used middle-aged mice, which are more resistant to obesity development than young ones.

Despite the absence of obesity, CD led to some metabolic disturbances in mice, including higher liver mass, higher blood TAG, fasting glucose, and lower PON activity, as well as a tendency to higher visceral fat content. The liver is enlarged during pathological conditions like hepatic steatosis (fatty liver), which leads to cirrhosis. It contains fatty deposits and has abnormal metabolic functions due to obesity or type 2 diabetes mellitus [43]. An enlarged liver was determined only in the CD group, while the rest of the cafeteria-fed mice's liver mass was within the control range. High triacylglycerol levels may result in problems with vessels, arteriosclerosis, and cardiovascular diseases [44]. Paraonase hydrolyses the oxidized phospholipids present in low-density lipoproteins (LDL). As a result, the lack of paraonase response to a high-fat diet is considered a risk factor that leads to obesity-driven atherosclerosis and inflammation. This enzyme attenuates the proinflammatory effect of high-calorie diets and the development of atherosclerosis [39, 45].

Thus, long-term CD leads to metabolic restructuring with unfavourable long-term consequences. Unexpectedly, supplementation of the cafeteria diet with broccoli sprouts aggravated some CD-induced

metabolic disturbances, in particular, increased blood glucose levels, and caused an increase in visceral adipose tissue content. Our study also noted that the mice accumulated the most epididymal adipose tissue among the three types of adipose tissue measured, with the highest accumulation in the groups receiving cafeteria food with 5 or 10% mass fraction of broccoli supplementation. At the same time, broccoli sprouts prevented liver mass increase, a decrease in PON activity, and an increase in TAG levels in the blood of mice fed CD. Also, BS showed a tendency to lower MPO activity, level of IL-1 β , and to increase total cholesterol and protein levels. These results suggest the complicated effects of broccoli sprouts. Broccoli sprouts contain a lot of biologically active compounds, including phenolic compounds and glucosinolates, which are powerful activators of the Nrf2 signaling pathway [13, 18]. Activation of Nrf2 may play a dual role in obesity, having the ability to both prevent and promote obesity progression due to the impact on adipocyte proliferation and differentiation [5, 46].

When mice consumed broccoli sprouts with the basic food, they did not differ from mice that consumed the basic food alone in body mass, visceral fat content, hematological parameters, blood glucose and total cholesterol levels, but had lower blood TAG levels and lower activity of blood PON and tended to have lower MPO activity and IL-1 β . Generally, this may indicate altered lipid metabolism and antioxidant response and a potential, albeit mild, anti-inflammatory effect. Decreased levels of TAG and anti-inflammatory effects of broccoli sprouts may be associated with Nrf2 activation by broccoli sulforaphane and polyphenols [47-52].

Conclusions. This study revealed that a cafeteria diet (CD) in middle-aged mice caused metabolic disturbances, including elevated blood glucose, TAG, and liver mass, alongside lower PON activity, without significant body mass gain. Broccoli sprouts supplementation prevented TAG increase and PON decline but unexpectedly increased visceral fat accumulation and hyperglycemia in CD-fed mice, particularly at higher doses (5–10%). In contrast, BS added to a standard diet lowered TAG levels and inflammation without affecting fat distribution. These findings suggest BS exerts dual effects: protective ones against lipid oxidation but potentially harmful in obesogenic contexts by promoting adiposity and glucose dysregulation. Future research should investigate dose-dependent mechanisms, such as Nrf2

signaling and adipocyte metabolism, to optimize BS dosing for metabolic health. These results caution against broad recommendations for BS in high-calorie diets and underscore the need for personalised nutrition strategies in obesity management.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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МЕТАБОЛІЧНІ ЕФЕКТИ ПРОРОСТКІВ БРОКОЛІ У МИШЕЙ З ОЖИРІННЯМ, СПРИЧИНЕНИМ КАФЕТЕРІЙНОЮ ЇЖЕЮ

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Проростки броколі (ПБ) є багатими на біоактивні сполуки з встановленими антиоксидантними та протизапальними властивостями. У цьому дослідженні кафетерійна їжа (КІ) використовувалася як модель для вивчення спричиненого харчування ожиріння у тварин. Метою дослідження було оцінити вплив харчових добавок із ПБ на метаболічні параметри у мишей середнього віку чоловічої статі, яких годували кафетерійною їжею (КІ), що містила (за масою) солодкий арахіс (28%), молочний шоколад (28%) і шоколадні крекери (11%). Мишей годували КІ протягом 20 тижнів, після чого брали кров, евтаназували мишей, збирали та зважували печінку і жирову тканину. Рівень глюкози, триацилгліцеридів (ТАГ) та холестерину

визначали за допомогою діагностичного набору (Reagent, Дніпро, Україна), а рівень ІЛ-1β – методом ELISA. Активність параоксонази (PON) в крові визначали шляхом моніторингу утворення р-нітрофенолу. Миші, яких годували виключно КІ, отримували більше калорій з їжею без значного збільшення маси тіла, але мали більшу масу печінки, гіперглікемію, гіпертригліцеридемію та нижчу активність PON порівняно з мишами, яких годували стандартним раціоном. Споживання ПБ (2,5, 5 або 10% маси) з КІ запобігало підвищенню рівня ТАГ і зберігало активність PON. Однак споживання ПБ у вищих дозах (5 і 10%) збільшувало накопичення вісцерального жиру і ще більше підвищувало рівень глюкози в крові. Проте споживання ПБ із стандартним раціоном знижувало рівень ТАГ та маркерів запалення в крові, не впливаючи на розподіл жирової тканини. Ці результати вказують на подвійну роль ПБ у регуляції метаболізму: хоча проростки броколі є корисними для зниження маркерів окислення та запалення, ПБ можуть сприяти вісцеральному ожирінню та глікемічному дисбалансу.

Ключові слова: проростки броколі, кафетерійна їжа, запалення, метаболічне здоров'я, параоксоназа, триацилгліцероли, вісцеральний жир.

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