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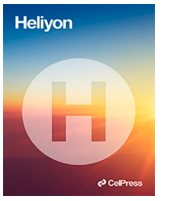

Research article

Multi-probiotic consumption sex-dependently interferes with MSG-induced obesity and concomitant phagocyte pro-inflammatory polarization in rats: Food for thought about personalized nutrition

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ABSTRACT

Epidemic scope which obesity has reached in many countries necessitates shifting the emphasis in medicine from traditional reaction to individualized and personalized prevention. Numerous trials convincingly prove sexual dimorphism of obesity in morbidity, pathophysiology, comorbidity, outcomes and prophylaxis efficacy. Obesity is characterized by chronic systemic low-grade inflammation that creates the preconditions for the emergence of numerous comorbidities. Leading role in the initiation, propagation and resolution of inflammation belongs to tissue resident and circulating phagocytes. The outcome of inflammation largely depends on phagocyte functional polarization, which in turn is governed by environmental stimuli. Gut microbiota (GM), whose disturbances are one of the key pathogenetic features in obesity, substantially affect phagocyte functions and can either aggravate or calm obesity-associated inflammation. Probiotics possess promising physiological functions, including microbiota-restoring and anti-inflammatory traits, that may possibly help prevent obesity. However, sex-specific effects of probiotic supplementation for targeted obesity prevention remain unknown. The aim of the current study was aimed to compare the effect of multi-probiotic preparation used in prophylactic regimen on the adiposity, profile of culturable GM and its short-chain fatty acids as well as on functional profile of phagocytes from different locations in male and female rats with monosodium glutamate (MSG)-induced obesity. Obesity was induced by neonatal MSG injections in male and female rats, who were given the multi-species probiotic during juvenile and adult developmental stages. Culturable fecal and mucosa-associated microbiota of the intestine were examined using selective diagnostic media. Short-chain fatty acid profile in fecal samples was determined by GC-MS. Phagocyte functional profile was evaluated using flow cytometry and colorimetric methods. Probiotic supplementation after the administration of MSG prevented weight gain and fat accumulation, inflammatory phagocyte activation and alterations in GM in female rats. In male MSG-injected rats, probiotic supplementation restricted but did not prevent weight gain and fat deposition, alleviated but did not prevent systemic inflammation, prevented the alterations in GM, but with residual imbalance in the ratio of obligate anaerobic to facultative anaerobic

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bacteria. Our findings emphasize the necessity of sex-centered approaches to the prophylactic use of probiotics in obesity in the context of predictive preventive and personalized medicine.

Nomenclature

AT	Adipose tissue
CFU	Colony-forming unit
GM	Gut microbiota
HBSS	Hank's Balanced Salt Solution
MSG	Monosodium glutamate
NBT	Nitroblue tetrazolium
PhI	Phagocytosis index
PhP	Phagocytosis percentage
PMA	Phorbol 12-myristate 13-acetate
PPs	Peritoneal phagocytes
PPPM	Predictive preventive personalized medicine
ROS	Reactive oxygen species
SCFAs	Short-chain fatty acids
SVF	Stromal vascular fraction
WAT	White adipose tissue

1. Introduction

The development of sex-centered approaches in biological research and medical practice is an important milestone in the progress of predictive preventive and personalized medicine (PPPM), since many diseases exhibit sex differences in prevalence, pathophysiology, clinical manifestation, course, and response to treatment [1–3]. Sexual dimorphism is a distinctive feature of the majority of highly prevalent chronic inflammatory diseases including obesity [4–6]. The World Health Organization (WHO) considers obesity a growing global epidemic and the "Millennium Disease". More than 600 million people suffer from obesity worldwide [7–11]. At the same time, women obesity prevails over men obesity in spite of the variability across countries [12]. Differential metabolism/disease risks in man and woman offer sex dimorphism with obesity incidence, course and outcomes [13]. Overweight and obesity are associated with numerous costly co-morbidities, including dyslipidemia, type 2 diabetes, chronic pain, cardiovascular diseases, hepatobiliary disorders, sleep apnea, osteoarthritis, respiratory problems, and certain cancers [14–16]. Male and female patients with obesity usually present with different comorbidities, what necessitates their stratification in different clusters at least [17]. The growing incidence of obesity in childhood and adolescence is of particular concern: more than 340 million overweight or obese children and adolescents are registered in 2016 [18,19]. Childhood obesity is also accompanied with cardiometabolic, neurological, gastrointestinal, and other comorbidities, and current data concerning sex differences in childhood obesity-associated concomitant pathology are sparse [20]. Middle through late adolescence is the period of the greatest risk for the transition from overweight to obesity, and this risk is higher in female than in male adolescents [21]. There is a significant sex dichotomy in multifactorial pathophysiology of obesity. Total content of adipose tissue (AT) is higher in women. Steroid hormone mediated sex differences in adipokine profile (increased serum level of leptin in a woman during puberty compared with that in a man) predispose a woman to the increased adiposity. Distribution pattern of AT in a woman differs from that in a man (gynoid and android patterns correspondingly) [22]. The autonomic nervous system imbalance, which is inherent in obesity, is manifested in different ways in male and female obese individuals: sympathetic activity is more prominent in women than in men [23]. Poorly explored but commonly admitted component of complex pathophysiology of the obesity is peculiar composition of gut microbiota (GM), the so called 'obese microbiota'. Controversial but numerous literature data concerning this issue altogether indicate species-specific variations in GM in obese individuals, which nevertheless vary significantly depending on age, sex, disease triggers and concomitant comorbidities etc. [24]. Abnormalities in gut microbial dysbiosis can be both the consequence of obesity and its trigger (dysbiosis-induced obesity) [25]. Abnormal GM can also substantially drive the development of comorbidities in obese individuals [26]. Numerous mechanisms are involved in the participation of abnormal GM in the pathophysiology of obesity. Short-chain fatty acid (SCFA) production and pro-inflammatory immunomodulation are the most important among others. GM *per se* is characterized by sex differences [27]. In addition, Min Y. et al. reported about the existence of sex-specific microbiome patterns associated with fat distribution in male and female individuals [28].

Inflammation, including those aggravated by GM disturbances, is one of the key driving forces in the course and progression of obesity. Besides its role as energy reservoir, AT can be considered an immune organ since it contains diversity of resident and recruited immune cells and is the source of metabolically active adipokines [29]. In obesity, tissue-resident macrophages are key players in initiating chronic systemic low-grade or meta-inflammation [30]. Tissue resident macrophages, as well as phagocytes as a whole, are highly plastic cells, responding precisely to slightest alterations in tissue microenvironment by changing their metabolism. According

to the current hypothesis concerning phagocyte metabolic plasticity, these cells can acquire numerous states of polarized activation ranging from proinflammatory or classic (M1) to anti-inflammatory or alternative (M2) [31]. Pro-inflammatory AT resident macrophage polarization caused by still unclear triggers (free-fatty acids, high glucose, Damage-Associated Molecular Patterns (DAMP) from dead adipocytes, GM metabolites etc.) is accompanied by releasing inflammatory mediators, which gradually spread into distant tissues and organs initiating the development of chronic systemic inflammation [32,33]. Pro-inflammatory polarized activation (M1) of these cells causes the recruitment of circulation immune cells into the inflamed tissues and in such a way maintains obesity-associated meta-inflammation [30]. M1-driven inflammation in obesity may be also supported by CD14-dependent activation of resident macrophages (AT macrophages, peritoneal macrophages etc.) and recruited phagocytes from circulation caused by LPS infusion from impaired intestinal microbiota. These M1 cells are characterized by increase in ROS production, iNOS activation and proinflammatory cytokine expression (e.g. IL-6, IL-1β, TNF-α), while M2 polarized cells exhibit enhanced arginase-1 and inhibited iNOS expression [34]. The possibility to manipulate M1/M2 shift of phagocytes and thereby ameliorate obesity was hypothesized by Shapiro H [30]. Sexual dimorphism of immune reactivity, including functional activity of phagocytic cells, is well documented [35,36]. Therefore, meta-inflammation in obesity has obvious sex context, which necessitates sex-centered deep insight for the understanding the immunobiology of the obesity [37,38].

The current situation requires a shift from reactive medicine to predictive and preventive approaches to the obesity problem *per se*, as well as a risk factor for the development of comorbid pathological states [16,39]. GM violations coupled with pro-inflammatory polarized activation of tissue-resident phagocytes can be attractive targets for these preventive approaches. GM modifiers such as probiotics are considered promising tools in these preventive interventions [24,40]. Numerous literature data demonstrate successful use of probiotics to prevent and treat obesity [24,41,42]. However, many issues remain uninvestigated, in particular the ones concerning the mechanisms of their efficacy and possible limitations of their compositions. Beneficial effects of probiotics on body weight and metabolism are considered to be a strain specific, and only several strains of *Lactobacillus* and *Bifidobacterium* genera demonstrate high prophylactic and therapeutic efficacy [40]. In addition, sex-dependence of the capacity of probiotic preparations to prevent and treat obesity still requires deep insight [43]. In our previous study, we demonstrated the immunomodulatory action of multi-probiotic "Symbiter acidophilus", containing 14 strains of probiotic microorganisms including *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Propionibacterium, Acetobacter,* that was expressed in functional activation of MALT-associated phagocytes [44]. Other research groups elicited anti-inflammatory effects of this probiotic supplements in patients and experimental animals with non-alcoholic fatty liver disease [45,46].

Taking into account sex dichotomy in the pathophysiology of the obesity, as well as sex-centered vector of personalized functional nutrition approaches, the study of the efficacy of probiotics in the prevention of the obesity development in male and female individuals is of particular interest. This study was aimed to compare the effect of multi-probiotic preparation used in prophylactic regimen on the adiposity, profile of culturable GM and its SCFAs as well as on functional profile of phagocytes from different locations in male and female rat with monosodium glutamate (MSG)-induced obesity.

2. Materials and methods

2.1. Experiment design

New-born Wistar female (n = 24) and male (n = 24) rats (bred in the animal facility of the ESC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv, Ukraine) were used in the experiment. The experimental male and female rats (48 in total) were randomly selected and arranged into 6 groups: two control groups of male and female rats (n = 8 in each group), two groups of MSG-administered male and female rats (n = 8 in each group), two groups of MSG-administered male and female rats treated with multi-probiotic (n = 8 in each group). All of the experimental rats, except the control group, were subcutaneously administered MSG at the dose of 4 mg/g of body weight on the second, fourth, sixth, eighth and tenth days of their life. MSG was previously dissolved in saline; the volume of injected MSG solution was 8 μL/g [47]. The same volume of saline was subcutaneously injected in control new-born rats at the same time points. The multi-probiotic "Symbiter acidophilus" were being administered *per os* to the rats of corresponding groups from the first month of their life to the end of the fourth month: 2-week-long intervals were between 2-week-long introduction of the multi-probiotic at the dose of 14 mg/g of body weight with a volume of 200 μL, which was previously dissolved in saline solution [44,46,48].

The multi-probiotic "Symbiter acidophilus" was provided by the Scientific and Production Company „O.D. Prolisok" (Kyiv, Ukraine). It contained 14 strains of probiotic microorganisms: *Lactobacillus* and *Lactococcus*–1.0×10^9 CFU/mL, *Bifidobacterium*–1.0×10^8 CFU/mL, *Propionibacterium*–3.0×10^7 CFU/mL, *Acetobacter*–1.0×10^5 CFU/mL.

The rats were held in standard conditions of the vivarium, and were allowed *ad libitum* access to food and water. Body weight and body length (nose-to-anus distance, cm) were assessed for 4 months after birth. Lee index (weight $(g)^{0.33}$/nose-to-anus length (cm)) was used to confirm obesity development. Four-month-old rats were euthanized by decapitation. White adipose tissue (WAT): visceral (retroperitoneal, epididymal (perigonadal) and mesenteric fat pads), as well as subcutaneous (inguinal) fat pads, were excised and weighed. Body adiposity index was characterized by the weight values (absolute and relative, % body weight) of the total adipose tissue and the fat depots of various localization [47,50]. Heparinized blood was used for examining functional activity of circulating phagocytes (monocytes and granulocytes) by flow cytometry. Rat peritoneal phagocytes (PPs) were isolated by standard method [49] to determine the activity of resident phagocyte population in the peritoneal cavity. Isolated cells were centrifuged at 300 g for 5 min at 4 °C, washed twice with Hanks balanced salt solution (HBSS). Cell viability determined by trypan blue exclusion was ≥95%.

Ethical approval of this study was obtained from the Ethics committee of ESC "Institute of Biology and Medicine", Taras

Shevchenko National University of Kyiv (protocol N 4 issued October 10, 2018), according to the Animal Welfare Act guidelines. The study was conducted in accordance with the standards of the "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes" (1997), the ethical principles of animal experiments, approved by the First National Congress on Bioethics Ukraine (September 2001), and national law (LAW OF UKRAINE # 3447-IV) issued by the Cabinet of Ministers of Ukraine (2006). The animal research facility was accredited in accordance with the "Standard rules on arrangement, equipment and maintenance of experimental biological clinics (vivarium)". Instruments used for the experiments were metrologically controlled.

2.2. Study of fecal and mucosa-associated microbiota of the colon intestine of rats

The colon samples of 1 cm^2 lengths (2 cm from the anus) was collected during autopsy for the bacteriological analysis of the mucosa-associated microbiota [51]. Selective diagnostic media (all produced by HiMedia Laboratories Pvt. Ltd., India): Bifidobacterium Agar, MRS agar, Endo agar, Mannitol Salt Agar, Iron Sulphite Agar, Simmons Citrate Agar, and Blood Agar Base (with aseptically added 5% sterile sheep's blood) were used for quantitative and qualitative analysis of microbiota composition. After inoculation, media were incubated in a thermostat at 37 °C for 24–48 h. Bacteria identification was performed using Bergey's Manual of determinative Bacteriology. The morphological and tinctorial characteristics of bacteria were assessed using reaction to plasma coagulation, DNA activity test, lysozyme and phosphatase production tests, oxidase test, carbohydrate fermentation tests, Voges-Proskauer's reaction, mobility test. Sensitivity to novobiocin was determined to differ *S. aureus, S. epidermidis* from *S. saprophyticus*. Lactose-negative *E. coli* were differentiated from opportunistic enterobacteria by detecting hydrogen sulphide formation. The results are presented as M ± m lg CFU/g and M ± m lg CFU/cm^2.

2.3. Analysis of short-chain fatty acids (SCFAs)

Samples of feces weighing 1 g were blended in 2 mL of 0.02 N HCl and then were held at room temperature for 30 min extraction in air-tight containers for preventing the loss of volatile SCFAs [52]. After this, samples were centrifuged for 10 min at 11 000 g, and 300 μL of supernatants were carried to an autosampler vial for GC-MS analysis and mixed with 100 μL of 0.05% internal standard (4-methyl-valeric acid, Sigma-Aldrich, Germany). The gas chromatographic (GC) analysis was performed at the Center for collective usage, D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine using an Agilent 6890 N GC system (Agilent Technologies, USA) equipped with an automatic liquid sampler Agilent 7683B (Agilent Technologies, USA). A fused-silica capillary column with a free fatty acid phase DB_FFAP, 0.25 μm × 0.25 mm × 30 m (Agilent Technologies Inc., USA) was used to perform separation. Helium was applied as the carrier gas at a flow rate of 1 mL/min. The initial oven temperature was 100 °C, maintained for 5 min, raised to 190 °C at 10 °C/min. The injection port was maintained at 250 °C. The samples were injected in a volume of 1 μL with 1:20 split ratio. The GC run time for each sample was 16 min. Detection of SCFAs was performed using a single quadrupole mass spectrometer Agilent, 5973 inert MSD (Agilent Technologies Inc., USA). Chem Station Data Analysis D.01.02.16 software was employed for data analysis. The SCFAs were identified on chromatograms by their specific retention times of standard SCFA mixture of acetic, propionic, i-butyric, n-butyric, valeric, i-valeric, n-caproic acids (Sigma-Aldrich, Germany) under the mentioned above GC conditions. Quantitative evaluation of the spectrum of short-chain fatty acids was performed by the method of normalization of the area of their peaks relative to the peak area of the internal standard and was expressed in mg/g of feces. Profiles (Cn/Σ (C2 + C3 + C4)) of acetic (C2), propionic (C3), butyric (C4) acid and values of anaerobic indexes ($AI = \sum \frac{C3+C4}{C2}$) were also calculated.

2.4. Stromal vascular fraction (SVF) isolation from adipose tissue

SVF cells were isolated from subcutaneous or visceral white adipose tissue by the Weisberg S.P. et al. with minor modifications [53, 54]. In brief, adipose tissues were weighed, rinsed with PBS, and gently minced under sterile conditions. Then the tissue samples were digested by adding 0.1% collagenase solution (Sigma-Aldrich) in HBSS (Sigma-Aldrich) for 1 h at 37 °C. Cell suspensions were then filtered and precipitated by centrifuging at 400 g. Cell viability determined using Trypan blue exclusion test was over 93%. Isolated cells were analysed for endocytic (phagocytic) activity, reactive oxygen species (ROS) generation (refer to 2.7 and 2.8) as well as for CD14 expression (positive cell proportion and the level of surface expression) by flow cytometry. Phycoerythrin (PE)-labelled anti-CD14 antibodies (Becton Dickinson, Farmingen, USA) were used. The samples were analysed by the FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). The data were analysed using CELLQuest software (BD; Franklin Lakes, NJ, USA).

2.5. ROS release detection

The nitroblue tetrazolium (NBT) test was used to evaluate production of ROS by the SVF cells and by peritoneal macrophages [55]. SVF cells were isolated as described above in 2.4. SVF cells or PPs (2×10^5/well) were incubated in HBSS containing 1 mg/mL of NBT (Sigma-Aldrich) for 1 h at 37 °C in a 5% CO_2 atmosphere. The 2 M KOH solution was added at the volume of 100 μL per well to stop the reaction and the formed formazan was dissolved in 50% dimethyl sulfoxide (100 μL per well). A plate reader was used to determine the optical density in each well at 630 nm. Samples were analysed in triplicate. The results are presented as mean ± SD.

2.6. NO release detection

Nitric oxide (NO) release from peritoneal phagocytes was indirectly measured in cell culture media after 24 h of cultivation through nitrite quantification by Griess reaction assay [49,56]. In brief, Griess reagent (100 μL) containing 2% sulphanilamide in 10% phosphoric acid and 0.2% naphthylethylene diamine dihydrochloride at equal volumes was added to equal volumes of cell culture media and then incubated in a 96-well microplate for 30 min at room temperature in the dark. The absorbance of azo compound was measured at 550 nm using a microplate reader. Sodium nitrite solution was used as a standard to calculate the nitrite concentration. Samples were analysed in triplicate. The absorbance value in each well was divided by the number of viable cells, and results were put as nitrite level per 10^6 cells. The results are presented as mean ± SD calculated with normalized values.

2.7. Intracellular ROS assay

Intracellular ROS production was measured using 2'7'-dichlorodihydro-fluorescein diacetate (H_2DCFDA, Invitrogen) staining by flow cytometry, as described earlier [49,54]. In brief, the isolated SVF cells, PPs or heparinized whole blood samples were incubated with 10 μM carboxy–H_2DCFDA solution in PBS for 30 min at 37 °C. Lysis buffer was used to lyse RBCs in blood samples. The samples were assessed with flow cytometer (excitation: 488 nm, emission: 525 nm). Granulocytes and monocytes were gated based on forward scatter (FSC) and side scatter (SSC) properties of the cells. Results were presented as mean fluorescence per cell. To assess phagocyte non-specific reserve, 10^{-9} M phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich) was added [56]. The functional reserve was established as the modulation coefficient (MC) and determined by the following equation:

$$MC = 100 - \frac{S \times 100}{B}$$

where S is a value of ROS production in sample treated with PMA *in vitro*, B is a value of ROS production in untreated sample (basal value).

2.8. Phagocytosis assay

Phagocytic activity was assessed by FACS analysis as previously described [49,54]. Briefly, 90 ml of heparinized whole blood samples, SVF cells or PPs (2×10^6 cells/ml) were incubated with 5 μL of bacterial suspension (1×10^7 cells/mL) of *Staphylococcus aureus* Cowan I (obtained from the collection of the Department of Microbiology and Immunology ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv), which was previously heat-inactivated and stained with FITC, at 37°C for

Table 1
Morphometric parameters and white adipose tissue (WAT) deposition in rats with MSG-induced obesity treated with multi-probiotic preparation.

Groups of experimental animals Parameter		Female rats			Male rats		
		Control (n = 8)	MSG (n = 8)	MSG + Symbiter (n = 8)	Control (n = 8)	MSG (n = 8)	MSG + Symbiter (n = 8)
Weight, g		212.40 ± 4.99	271.67 ± 3.36*	217.60 ± 5.48$^{\&\&}$	380.50 ± 0.95 ##	353.33 ± 5.6 ## **	333.75 ± 4.75 ## ** $^{\&}$
Body length, cm		18.71 ± 0.53	18.78 ± 0.51	17.50 ± 0.69	25.25 ± 1.24 #	20.67 ± 0.29 #*	23.13 ± 1.39 #
Lee index		0.317 ± 0.01	0.335 ± 0.01	0.346 ± 0.01	0.290 ± 0.01	0.341 ± 0.01*	0.30 ± 0.02 $^{\&}$
Total WAT	Absolute weight, g	7.80 ± 1.13	25.10 ± 6.70*	11.50 ± 2.25	9.55 ± 0.95	45,63 ± 8.78 # **	25.78 ± 1.23 # ** $^{\&}$
	Relative weight, % body weight (adiposity index)	3.1 ± 0.46	10 ± 2.30*	5.72 ± 1.05*	2.51 ± 0.30	12,56 ± 1.62**	7.74 ± 0.46 # ** $^{\&}$
Subcutaneous WAT	Absolute weight, g	Not detected	4.75 ± 1.35 *	1.46 ± 0.42*$^{\&}$	1.90 ± 0.14#	19.63 ± 6.34 #*	9.15 ± 1.97# **
	Relative weight, % body weight	Not detected	1.90 ± 0.50*	0.70 ± 0.19*$^{\&}$	0.50 ± 0.05 #	5.29 ± 1.45 #**	2.76 ± 0.69 #**
Visceral WAT (including perigonadal adipose tissue)	Absolute weight, g	7.80 ± 1.13	20.35 ± 5.35*	10.04 ± 1.96	7.65 ± 1.15	26 ± 4.04**	16.63 ± 1.21 #** $^{\&}$
	Relative weight, % body weight	3.10 ± 0.46	8.10 ± 1.90*	5.01 ± 0.94	2.01 ± 0.31	7.28 ± 0.42**	4.98 ± 0.41** $^{\&\&}$
Perigonadal adipose tissue	Absolute weight, g	5.35 ± 1.85	5.15 ± 0.05	3.90 ± 1.14	4.30 ± 0.30	9.80 ± 0.63 # **	5.45 ± 0.91 $^{\&\&}$
	Relative weight, % body weight	2.13 ± 0.77	2.07 ± 0.06	1.97 ± 0.55	1.13 ± 0.08 #	2.79 ± 0.09 #**	1.62 ± 0.26 $^{\&\&}$

Notes Values are presented as mean ± SD. *, $P < 0.05$ and **, $P < 0.01$ as compared to the control; $^{\&}$, $P < 0.05$ and $^{\&\&}$, $P < 0.01$ as compared to MSG-induced obesity group. Qualitative data was compared using χ^2 test. Statistically significant differences between sexes in a two-way ANOVA are shown as follows: #, $P < 0.05$, ##, $P < 0.01$.

30 min. Chilled stop solution (0.02% EDTA and 0.04% paraformaldehyde in PBS) was added to the samples in volume of 2 mL per well for 10 min to stop the reaction. Then cells were washed twice with PBS. The samples were acquired with FACSCalibur flow cytometer and results were analysed using CellQuest software (Becton Dickinson, USA). Granulocytes and monocytes were gated based on forward scatter (FSC) and side scatter (SSC) properties of the cells. Phagocytosis percentage (PhP) and phagocytosis index (PhI) were calculated by counting the percentage of fluorescence positive cells and the mean fluorescence estimated per one cell with ingested bacteria respectively.

2.9. Statistical analysis

The results are shown as mean ± SD. The *t*-test and non-parametric Mann-Whitney *U* test were used to determine the statistical significance between the animal groups using Microsoft Excel software (Microsoft Excel Corp., USA). χ^2 test was used for qualitative data. Comparative analysis between groups, with influence of sex and obesity factors, was performed using two-way ANOVA test by Statistica 10.0 software (Stasoft Inc., USA). Differences between values were considered significant at $p \leq 0.05$.

3. Results

3.1. Morphometric parameters and white adipose tissue deposition

The primary outcome of this study was evaluated by morphometric parameters and WAT deposition changes in female and male animals with MSG-induced obesity received probiotics prophylactically. After 4 months, MSG-injected female animals showed body weight values larger than those of their control counterparts (Table 1). Female rats receiving MSG injections along with the probiotic presented no weight gain. MSG administration in male rats was associated with weight loss. The weight of those MSG-injected male rats, who received probiotic, was lower than in both control and MSG-injected animals without probiotic. Lee index values were slightly increased in both female and male MSG-administered rats, with prophylactic probiotic supplementation having positive effects on this parameter only in male animals. MSG administration induced significant total WAT deposition gain: more than three times in female animals, and more than four times in males. Total WAT deposition in female rats administered with MSG along with prophylactic probiotic supplementation did not differ from that in control animals. In male rats from probiotic group, total WAT deposition was nearly twice lower than that in their MSG-obese counterparts without probiotic supplementation but did not reach control values. The emergence of subcutaneous fat pads was significantly more pronounced in male than in female MSG-obese rats. Prophylactic use of probiotic substantially prevented subcutaneous WAT deposition in female animals and at lesser extent in male rats. More than twice gain of visceral WAT deposition was observed in both female and male MSG-obese animals. Total visceral WAT deposition in female rats from probiotic group did not differ from that in control rats, whereas in male rats was lower than in MSG-obese rats without probiotic supplementation, but twice higher than in control male rats. Perigonadal WAT depot weight gain was registered only in male MSG-obese rats. Prophylactic probiotic supplementation along with MSG administration moderately prevented perigonadal WAT deposition in male animals.

Table 2
Culturable gut microbiota isolated from faces of rats with MSG-induced obesity treated with multi-probiotic preparation.

Group of microorganisms	Number (lg CFU/g feces)					
	Female rats			Male rats		
	Control (n = 8)	MSG (n = 8)	MSG + Symbiter (n = 8)	Control (n = 8)	MSG (n = 8)	MSG + Symbiter (n = 8)
Bifidobacterium	8.7 ± 0.3	7.8 ± 0.7	8.0 ± 0.4	9.0 ± 0.2	8.1 ± 0.5	8.3 ± 0.2
Lactobacillus	9.2 ± 0.6	7.4 ± 0.3 *	8.8 ± 0.3 &&	8.0 ± 0.5	6.0 ± 0.1 ## **	8.0 ± 0.5 &&
E. coli lactose-fermenting	3.8 ± 0.5	4.9 ± 0.5	4.2 ± 0.1	4.2 ± 1.2	5.9 ± 0.5	4.0 ± 0.2 &&
E. coli lactose non-fermenting	0.8 ± 0.4	0.9 ± 0.1	0.6 ± 0.4	1.2 ± 0.9	0.9 ± 0.3	3.1 ± 0.4 ## * &&
Opportunistic enterobacteria	0	0	0	0	0	0
Staphylococcus aureus	5.4 ± 0.3	8.1 ± 0.9 *	6.0 ± 0.3 &	4.2 ± 0.2 ##	6.1 ± 1.2	4.6 ± 0.2 ##
Staphylococcus sp. (mannitol negative)	3.9 ± 0.6	4.4 ± 1.2	2.1 ± 0.2 * &	3.0 ± 0.9	3.4 ± 1.0	1.8 ± 0.1
Clostridium sp.	3.4 ± 0.2	3.5 ± 0.3	3.4 ± 0.2	2.9 ± 0.1 #	2.9 ± 0.2	2.9 ± 0.2 #
Candida sp.	2.4 ± 0.1	3.3 ± 0.1 **	2.9 ± 0.6	3.4 ± 0.5 #	4.2 ± 0.5 #	3.0 ± 0.9
Hemolytic bacteria	1.2 ± 0.2	3.6 ± 0.2 **	1.7 ± 0.5 &&	3.0 ± 0.5 ##	4.5 ± 0.1 ## *	2.8 ± 0.9 &

Notes: Values are presented as mean ± SD. *, $P < 0.05$ and **, $P < 0.01$ as compared to the control; &, $P < 0.05$ and &&, $P < 0.01$ as compared to MSG-induced obesity group. Qualitative data was compared using χ^2 test. Statistically significant differences between sexes in a two-way ANOVA are shown as follows: #, $P < 0.05$, ##, $P < 0.01$.

3.2. Culturable gut microbiota and SCFA profiles

Compositional changes in culturable GM were registered in both female and male MSG-obese rats. A significant quantitative decrease in lactobacilli along with an increase in staphylococci and microbiota with hemolytic activity were most characteristic among others of both male and female rats (Table 2). Prophylactic administration of the probiotic prevented these changes in both male and female rats. It is worthy to note that quantitative indices of lactobacilli and clostridia in male rats of both control and probiotic groups were slightly lower than those in female animals.

The same compositional changes as in the fecal microbiota were observed in the parietal microbiota of the intestine: quantitative decrease in lactobacilli along with the appearance of staphylococci and slight increase in microbiota with hemolytic activity (Table 3). Prophylactic administration of the probiotic prevented these alterations. Like the fecal microbiota, parietal microbiota of the intestine in male rats from probiotic group was characterized by slightly lower number of lactobacilli than that in female rats.

The concentration of three major SCFAs (acetic, propionic, and butyric acids) excreted in the feces was lower in both female and male MSG-obese rats in comparison with their control counterparts (Table 4, Fig. 1), and this reduction was more pronounced in male animals. It is necessary to note, that total amount of these SCFAs in fecal samples of control female rats was lower than that in male ones. Prophylactic use of the probiotic prevented this phenomenon completely in female MSG-injected rats, and partially - in male rats. The concentration of acetate in fecal samples of MGS-injected male rats receiving probiotic did not reach control values, and acetate: propionate ratio in these animals was 2 times lower than that in MGS-administered female rats receiving probiotic. The anaerobic index ($AI = \sum \frac{C3+C4}{C2}$) was also increased in male rats from probiotic group compared to control one, which indicates slight residual imbalance in the ratio of obligate anaerobes to facultative anaerobic microorganisms in their GM (Table 3).

3.3. Polarized activation of adipose tissue phagocytes

The stromal vascular fraction (SVF) of adipose tissue contains highly heterogenous call populations. Tissue-resident phagocytes comprise ~20% of rodent adipose SVF, and ~60% of adipose tissue–resident hematopoietic cells [57,58]. Among these adipose tissue-resident phagocytes, tissue-resident macrophages comprise the largest proportion (~80%), myeloid (monocytic) angiogenic cells (MACs) constitute small fraction, and tissue-resident granulocytes compose tiny fraction [59]. In obesity, SVF innate immunity cell fraction is replenished significantly with recruited circulating phagocytes: first with neutrophils, and then with monocytes [60]. In our experiments, quantitative values of SVF fraction in visceral adipose tissue (including the perigonadal one) of control female rats (Fig. 2a) were 2.8 times higher than that in male rats, which coincides with the literature data concerning sex dimorphism in adipose tissue composition [61]. In MSG-obese female rats, total SVF cell number tended to grow, whereas in male rats, it tended to reduce. At the same time, SVF fraction in male but not in female obese rats was highly enriched with $CD14^+$ granulocytes, key effector cells of the inflammation (Fig. 2b, c). Surface expression of CD14 was substantially higher in male SVF phagocytes than in female ones (Fig. 2d, e), which indicates the presence of more mature cells slightly shifted to the M1 activation profile [62,63]. Functional profile of WAT phagocytes in female rats was notable for moderate decrease of phagocytic activity (Fig. 3a–c) along with increased ROS generation (Fig. 4a–c). It indicates pro-inflammatory polarized activation of AT phagocytes in female obese rats. Parameters of the phagocytosis (Fig. 3c-d) and oxidative metabolism (Fig. 4a–c). in obese male AT phagocytes did not change significantly as compared to those in control male animals.

Prophylactic use of the probiotic along with the administration of MSG prevented SVF enlargement in female but not in male rats and facilitated the enrichment of the adipose tissue SVF with CD14 + phagocytes in both female and male animals. Nevertheless, in

Table 3
Parietal microbiota isolated from the intestine of rats with MSG-induced obesity treated with multi-probiotic preparation.

Group of microorganisms	Number (lg CFU/cm^2)					
	Female rats			Male rats		
	Control (n = 8)	MSG (n = 8)	MSG + Symbiter (n = 8)	Control (n = 8)	MSG (n = 8)	MSG + Symbiter (n = 8)
Bifidobacterium	3.4 ± 0.2	3.2 ± 0.2	3.8 ± 0.2 $^{\&}$	4.0 ± 0.6	4.2 ± 0.2 ##	4.5 ± 0.2 #
Lactobacillus	4.8 ± 0.1	3.9 ± 0.5 *	5.6 ± 0.5 $^{\&}$	3.9 ± 0.5 #	3.0 ± 0.2	5.0 ± 0.2 * $^{\&\&}$
E. coli lactose-fermenting	0	0	0.3 ± 0.1 * $^{\&}$	0	0	0.6 ± 0.2 * $^{\&}$
E. coli lactose non-fermenting	0	0	0.1 ± 0.2	0	0	0.3 ± 0.2
Opportunistic enterobacteria	0	0	0	0	0	0
Staphylococcus aureus	0	1.9 ± 0.2 **	0 $^{\&\&}$	0	1.0 ± 0.2 ## **	0 $^{\&\&}$
Staphylococcus sp. (mannitol negative)	0.6 ± 0.7	0	0	0.8 ± 0.9	0	0
Clostridium sp.	0	0	0	0	0	0
Candida sp.	0	0	0	0	0	0
Hemolytic bacteria	1.0 ± 0.4	1.2 ± 0.2	0.8 ± 0.2	1.5 ± 0.9	1.9 ± 0.7	1.2 ± 0.2

Notes: Values are presented as mean ± SD. *, $P < 0.05$ and **, $P < 0.01$ as compared to the control; $^{\&}$, $P < 0.05$ and $^{\&\&}$, $P < 0.01$ as compared to MSG-induced obesity group. Qualitative data was compared using χ^2 test. Statistically significant differences between sexes in a two-way ANOVA are shown as follows: #, $P < 0.05$, ##, $P < 0.01$.

Table 4
SCFA profiles and the anaerobic indexes in feces of rats with MSG-induced obesity treated with multi-probiotic preparation.

Groups SCFA indexes	Female rats			Male rats		
	Control (n = 8)	MSG (n = 8)	MSG + Symbiter (n = 8)	Control (n = 8)	MSG (n = 8)	MSG + Symbiter (n = 8)
Acetic acid (C2), mg/g of feces	0.494 ± 0.221	0.346 ± 0.220	0.634 ± 0.223	0.604 ± 0.198	0.393 ± 0.240	0.407 ± 0.220
Propionic acid (C3), mg/g of feces	0.286 ± 0.186	0.184 ± 0.165	0.296 ± 0.140	0.367 ± 0.099	0.223 ± 0.212	0.338 ± 0.117
Butyric acid (C4), mg/g of feces	0.222 ± 0.206	0.172 ± 0.199	0.272 ± 0.189	0.230 ± 0.53	0.184 ± 0.120	0.345 ± 0.187
The anaerobic index	+1.5789473	+1.5317919	+1.5668769	+1.5213068	+1.56743	+1.8304668

Notes: The anaerobic index was calculated as the proportion of the sum of total propionate and butyrate concentration to acetate concentration (AI = (C3 + C4)/C2). Values are presented as mean ± SD.

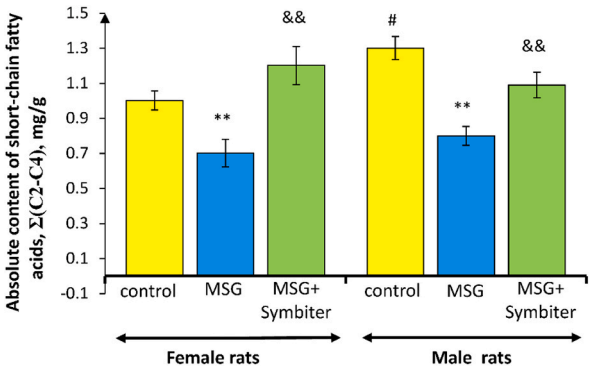

Fig. 1. Absolute content of short-chain fatty acids in feces of female and male rats with MSG-induced obesity treated with multi-probiotic preparation (n = 8). Values are presented as mean ± SD. **, $P < 0.01$ as compared to the control animal group; &&, $P < 0.01$ as compared to the group of rats with MSG-induced obesity. Statistically significant differences between sexes in a two-way ANOVA are shown as follows: #, $P < 0.05$.

female rats from probiotic group these phagocytes were characterized by increased phagocytic function (Fig. 3a-b), typically involved in the resolution of inflammation [64]. By contrast, SVF phagocytes in male rats from probiotic group were notable for the decrease of phagocytosis (Fig. 3a-b). Altogether, these findings demonstrate that prophylactic administration of the probiotic prevented pro-inflammatory polarized activation of adipose tissue phagocytes in female rats, but not in male animals.

3.4. Functional features of peritoneal phagocytes

MSG-induced obesity was associated with substantial enlargement of the pool of phagocytizing cells in the peritoneal cavity of female animals, and with the reduction of this pool in male rats along with moderate decrease in phagocytic activity of these cells (Fig. 5a–d). ROS generation of peritoneal granulocytes was increased, as compared to control one, in both MSG-obese male and female rats (Fig. 5e–g). Whereas oxidative metabolism of peritoneal mononuclear phagocytes was augmented only in male MSG-administered animals. Peritoneal phagocytes from MSG-obese male animals were also characterized by the increase in NO production (Fig. 5h), while those from female rats by the decrease in this function. Taken together, these results indicate pro-inflammatory polarized activation of peritoneal phagocytes in male but not in female MSG-obese rats.

Prophylactic use of probiotic along with MSG administration was accompanied by further enlargement of phagocytizing fraction of mononuclear cells in the peritoneal cavity of female animals, and by sharp rise of the fraction of both phagocytizing mono- and polymorphonuclear phagocytes in the peritoneal fluid of male rats (Fig. 5a–d). Both peritoneal phagocyte populations from female animals in probiotic group were characterized by the normal values of their oxidative metabolism and NO-generation (Fig. 5e–h). By contrast, peritoneal phagocytes from male animals in probiotic group were notable for increased ROS- and NO-generation. These results indicate that prophylactic consumption of probiotic along with MSG administration prevented pro-inflammatory shift of peritoneal phagocyte activation in female rats, but nor in male ones.

3.5. Functional profile of circulating phagocytes

MSG-induced obesity was associated with increased fraction of phagocytizing granulocytes and monocytes in peripheral blood of both female and male animals (Fig. 6a, b). Functional profiles of circulating monocytes and granulocytes from female MSG-obese rats were characterized by the normal values of their phagocytic activity and slightly decreased oxidative metabolism (Fig. 6c–f).

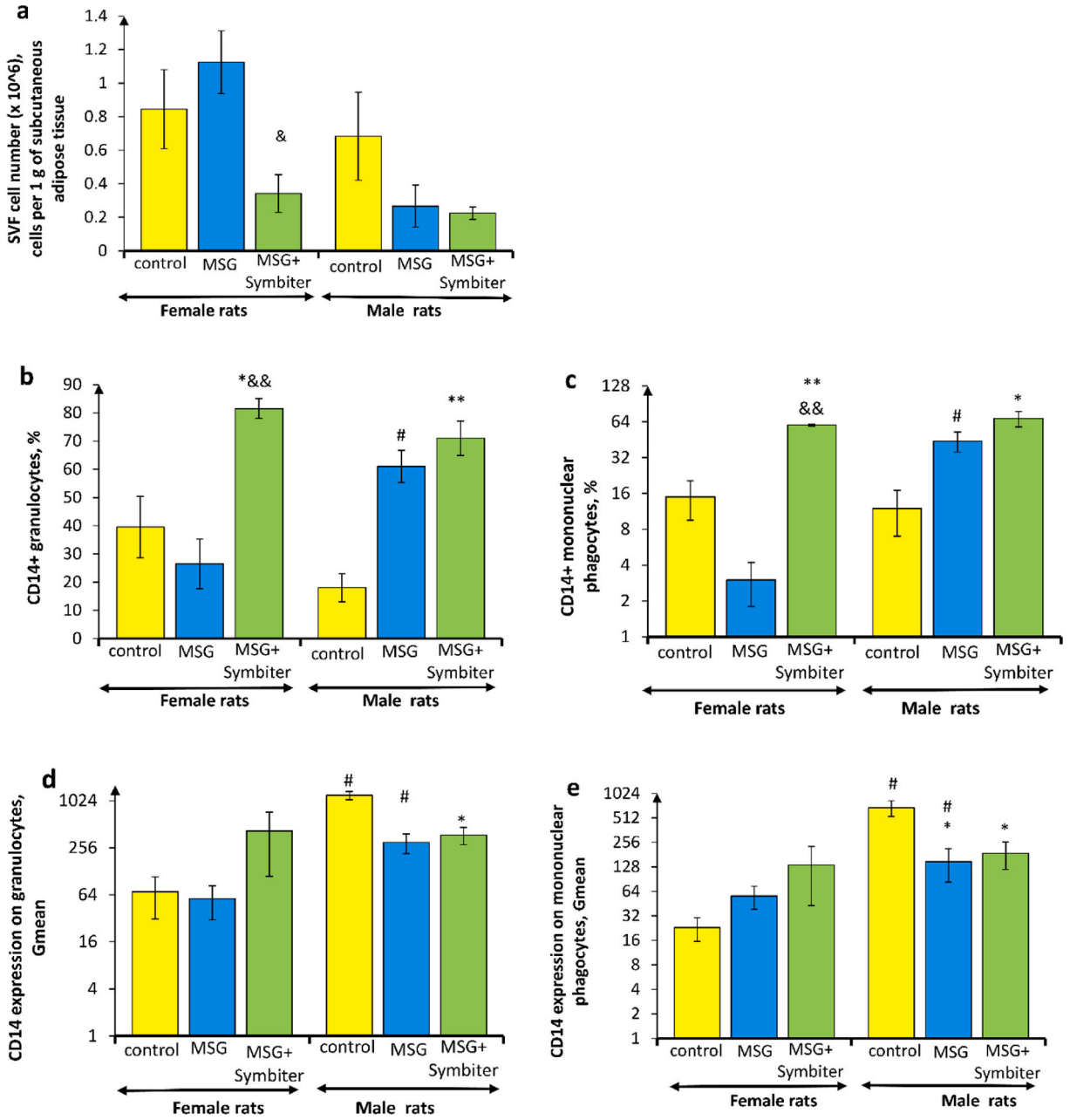

Fig. 2. The amount of $CD14^+$ phagocytes in adipose tissue in female and male rats with MSG-induced obesity treated with multi-probiotic preparation (n = 8). (a) number of SVF cells; (b, c) number of $CD14^+$ granulocytes and mononuclear phagocytes, respectively; (d, e) CD14 expression levels. Values are presented as mean ± SD. *, $P < 0.05$ and **, $P < 0.01$ as compared to the corresponding values of the control animal group; $^{\&}$, $P < 0.05$ and $^{\&\&}$, $P < 0.01$ as compared to the values in group of rats with MSG-induced obesity. Statistically significant differences between sexes in a two-way ANOVA are shown as follows: #, $P < 0.05$. SVF, stromal vascular fraction.

Circulating granulocytes from MSG-obese male animals demonstrated increased phagocytic activity and oxidative metabolism (Fig. 6a–f). Moreover, metabolic reserve of ROS generation in cells from male rats, after *in vitro* stimulation by PMA, was absent (Fig. 6e, f). It signifies that oxidative metabolism of these cells was activated to the utmost. Taken together, these data indicate pro-inflammatory polarization shift of circulating phagocytes in male but not in female rats with obesity induced by MSG administration.

Prophylactic use of probiotic along with MSG administration prevented pro-inflammatory skew of circulating phagocyte activity in both female and male animals (Fig. 6a–f).

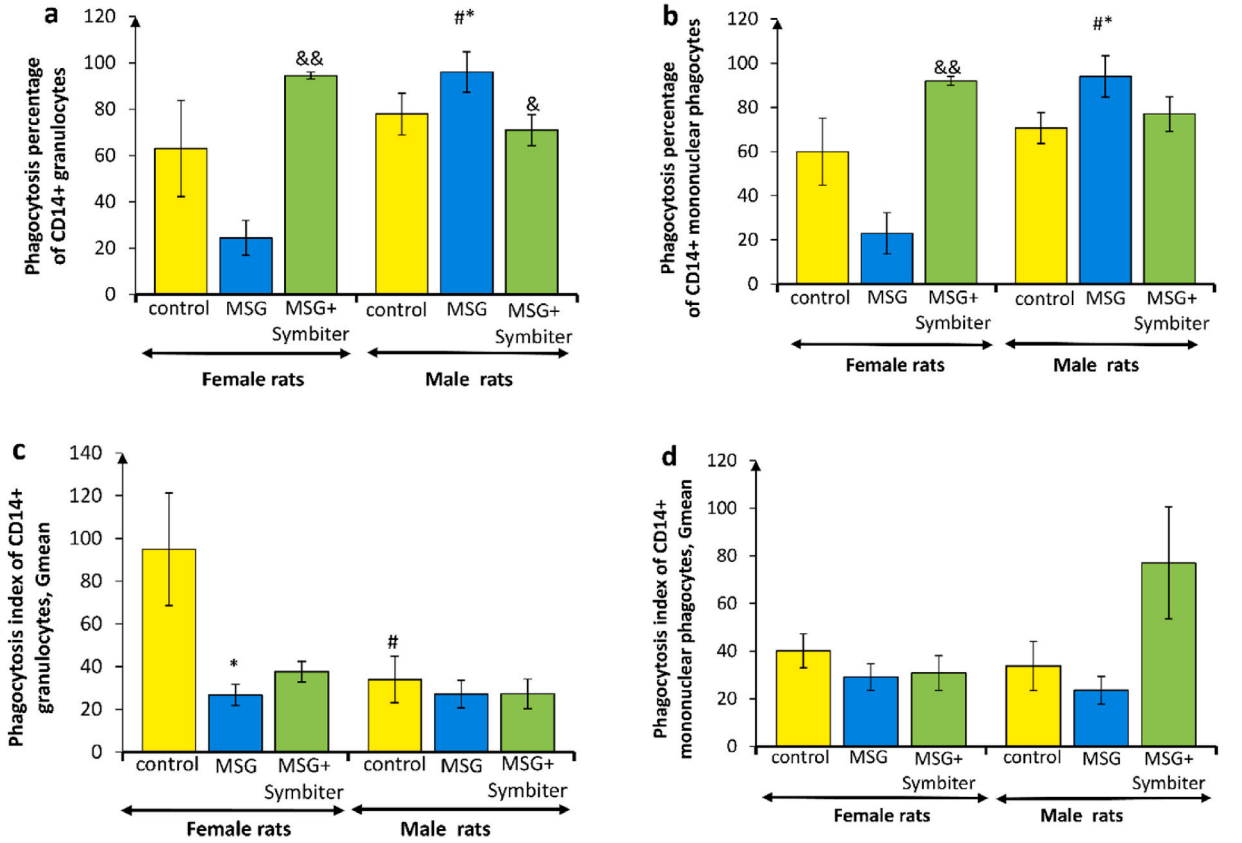

Fig. 3. Phagocytosis activity of $CD14^+$ phagocytes of adipose tissue in female and male rats with MSG-induced obesity treated with multi-probiotic preparation (n = 8). (a, b) the relative amount of $CD14^+$ granulocytes and mononuclear phagocytes, respectively; (c, d) phagocytosis index of $CD14^+$ granulocytes and mononuclear phagocytes, respectively. Values are presented as mean ± SD. *, $P < 0.05$ as compared to the corresponding values of the control group; $^{\&}$, $P < 0.05$ and $^{\&\&}$, $P < 0.01$ as compared to the values in group of rats with MSG-induced obesity. Statistically significant differences between sexes in a two-way ANOVA are shown as follows: #, $P < 0.05$.

4. Discussion

Epidemic scope, which obesity has reached in many countries, necessitates shifting the emphasis in medicine from traditional reaction to personalized prevention [16,65]. Constantly growing number of literature data convincingly proves the effect of sex at cellular and molecular levels on obesity in morbidity, pathophysiology, comorbidity, outcomes and treatment efficacy [4,5] Understanding biomedical scientific findings concerning obesity in the context of sex differences can facilitate applying research-derived knowledge in personalized approaches to the management and prophylaxis of this pathologic state. Sex hormones control and regulate many aspects of immune response and in general they are considered key mediators of sex dimorphism of immune reactivity including tissue resident and circulating phagocyte responses in obesity-associated local and systemic inflammation [38]. Hormones could also drive sex differences in the microbiota that has been termed the 'microgenderome' [66]. On the other hand, microbiome can influence sex hormone levels in different ways accordingly modulating their effect on immune cells, whose expression of receptors specific to these hormones has been proved by many works [67,68]. Moreover, another mechanism that connects obesity, immune system and GM is that adipose tissue can be a source of sex hormones that mediates sex differences of GM [69].

Promising approaches to manipulating obesity thereby are those targeted GM. Experts have significantly contributed to the extension of the current knowledge concerning microbiota alterations associated with metabolic disorders including obesity. The results of these efforts have pointed the necessity of integrative and personalized approaches to the preventive and therapeutic intervention affecting GM [70,71]. In microbiology terms, obesity represents sophisticated imbalance in GM [25–28], whose patterns and consequences are rather unexplored. Nevertheless, considering the proven involvement of GM in the obesity pathophysiology, this newly recognized "indispensable metabolic organ" has been regarded as attractive target with great therapeutic and prophylactic potential [40]. Taking into account the sex-specific immunomodulating ability of GM and its metabolites that may include not only the role in shaping resident macrophage metabolism in the intestine, but also in regulation of distant phagocytes as microglia [72], possibly through the signaling mediated by microbial short-chain fatty acids, probiotic therapy of GM disturbances should be reconsidered in relation to these effects.

The objectives of the current study were to examine the influences of multi-probiotic preparation on the animal adiposity, profile of

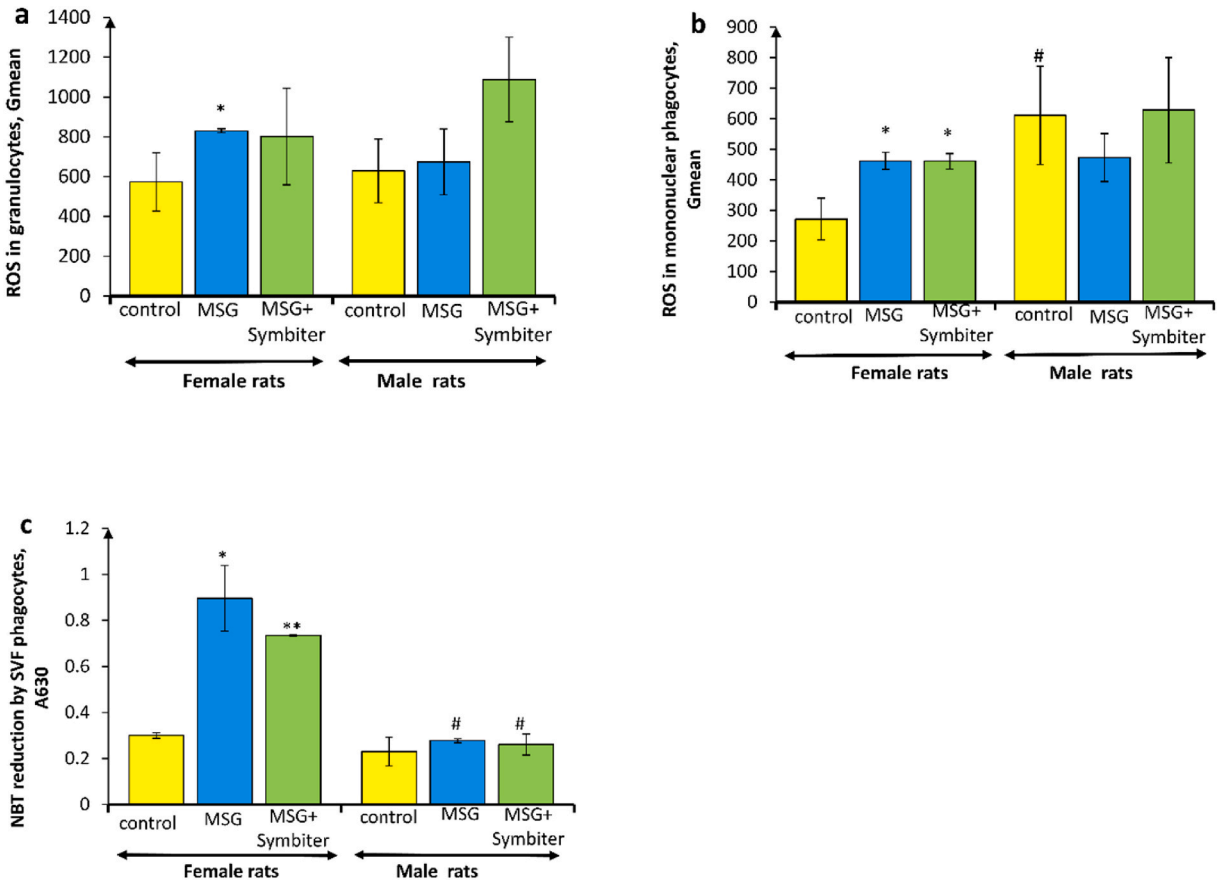

Fig. 4. Oxidative metabolism of adipose tissue phagocytes in female and male rats with MSG-induced obesity treated with multi-probiotic preparation (n = 8). (a, b) intracellular production of ROS by granulocytes and mononuclear phagocytes, respectively; (c) ROS production by stromal vascular fraction cells measured in NBT-test. Values are presented as mean ± SD. *, $P < 0.05$ and **, $P < 0.01$ as compared to the corresponding values of the control group. Statistically significant differences between sexes in a two-way ANOVA are shown as follows: #, $P < 0.05$. SVF, stromal vascular fraction.

culturable GM and its SCFAs as well as on functional profile of phagocytic cells from different locations in rats with MSG-induced obesity in the context of sex differences. MSG-induced obesity model was chosen for several reasons. MGS-induced obesity in rodents is a model characterized by central adiposity, with glucose intolerance and metabolic syndrome [73]. This model also reproduces such important components of the obesity clinical manifestation as systemic inflammation and general oxidative stress [74,75]. Neurointoxication with MSG in neonate rats reconciles aforementioned features with increased parasympathetic activity, which is an important component of obesity pathophysiology [76]. In addition, MSG-induced obesity allows to shed the light on unexplored aspect of the alterations of GM in obesity. GM alterations in obesity can be considered either one of the triggers or one of the consequences of adiposity [77]. In high-fat diet-induced obesity models, alterations of GM are caused actually by dietary intervention, fluctuations in bile acid metabolism etc., and contribute significantly to the adiposity development. Therefore, microbiota alteration in high-fat diet-induced obesity models can be considered one of the obesity triggers [78]. MSG *per se* does not affect significantly GM composition and metabolism [79,80]. Therefore, GM alterations in MSG-induced obesity, if any, can be considered a consequence of metabolic abnormalities such as imbalance of autonomic nervous system, inflammation and oxidative stress.

Current study confirmed our previous observations concerning significant sexual differences in adipose tissue deposition and inflammation in MSG-induced obesity [54]. Adiposity was more severe in MSG-obese male than in female rats. Key mechanism of MSG-induced obesity is neurointoxication followed by the imbalance of vegetative nervous system [73]. The anti-apoptopic effects of estrogen can be one of the reasons for less severe MSG-induced obesity in female rats [81]. Large amounts of phagocytes were accumulated in adipose tissue in both female and male MSG-obese animals. There are two main mechanisms of this phenomenon. Pro-inflammatory molecules produced by adipose tissue, as well as increased local extracellular lipid concentrations, altogether promote adipose tissue phagocyte accumulation [82]. Nevertheless, cellular composition of SVF phagocytes in male and female obese rats differed and was characterized by the increased number of neutrophils in male animals. It indicates more pronounced inflammatory milieu in AT of obese male rats. Sex differences in adipokine profile conditioned, inter alia, by the adipokine-sex hormone cross-talk can be one of the reasons for this phenomenon [83]. Excess accumulation of fat facilitates the release of free fatty acids by

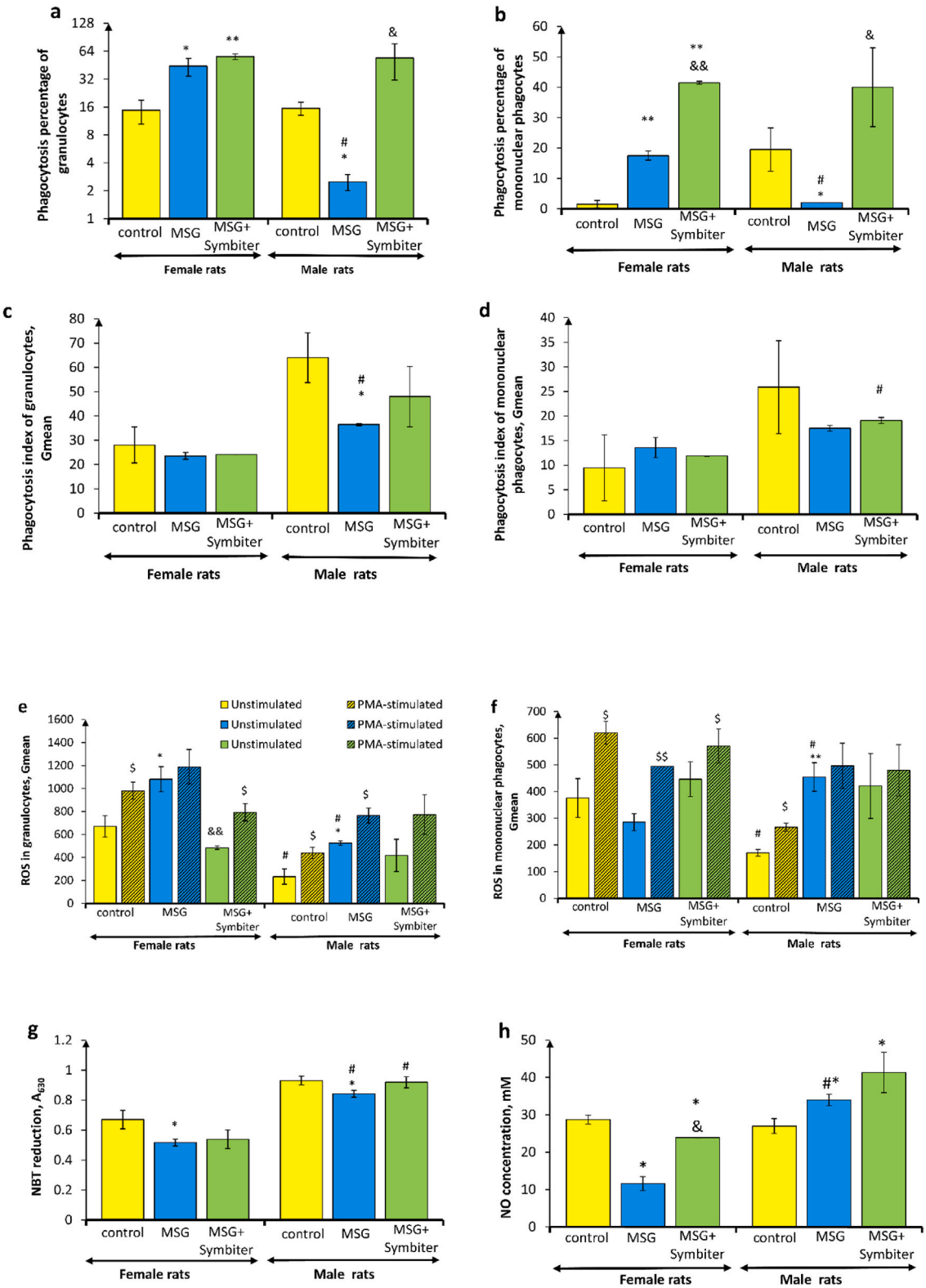

Fig. 5. Functional activity of peritoneal phagocytes in female and male rats with MSG-induced obesity treated with multi-probiotic preparation (n = 8). (a, b) Percentage of phagocytizing granulocytes and mononuclear phagocytes, respectively; (c, d) phagocytosis index of granulocytes and mononuclear phagocytes, respectively; (e, f) intracellular production of ROS by granulocytes and mononuclear phagocytes, respectively; (g) ROS production measured in NBT-test; (h) NO production (quantified as nitrite level). Values are presented as mean ± SD. *, $P < 0.05$ and **$P < 0.01$ as

compared to the corresponding values of the control group. [&], $P < 0.05$ and [&&], $P < 0.01$ as compared to the values in group of rats with MSG-induced obesity. [$], $P < 0.05$ and [$$], $P < 0.01$ as compared to the values of the corresponding unstimulated cells. Statistically significant differences between sexes in a two-way ANOVA are shown as follows: #, $P < 0.05$.

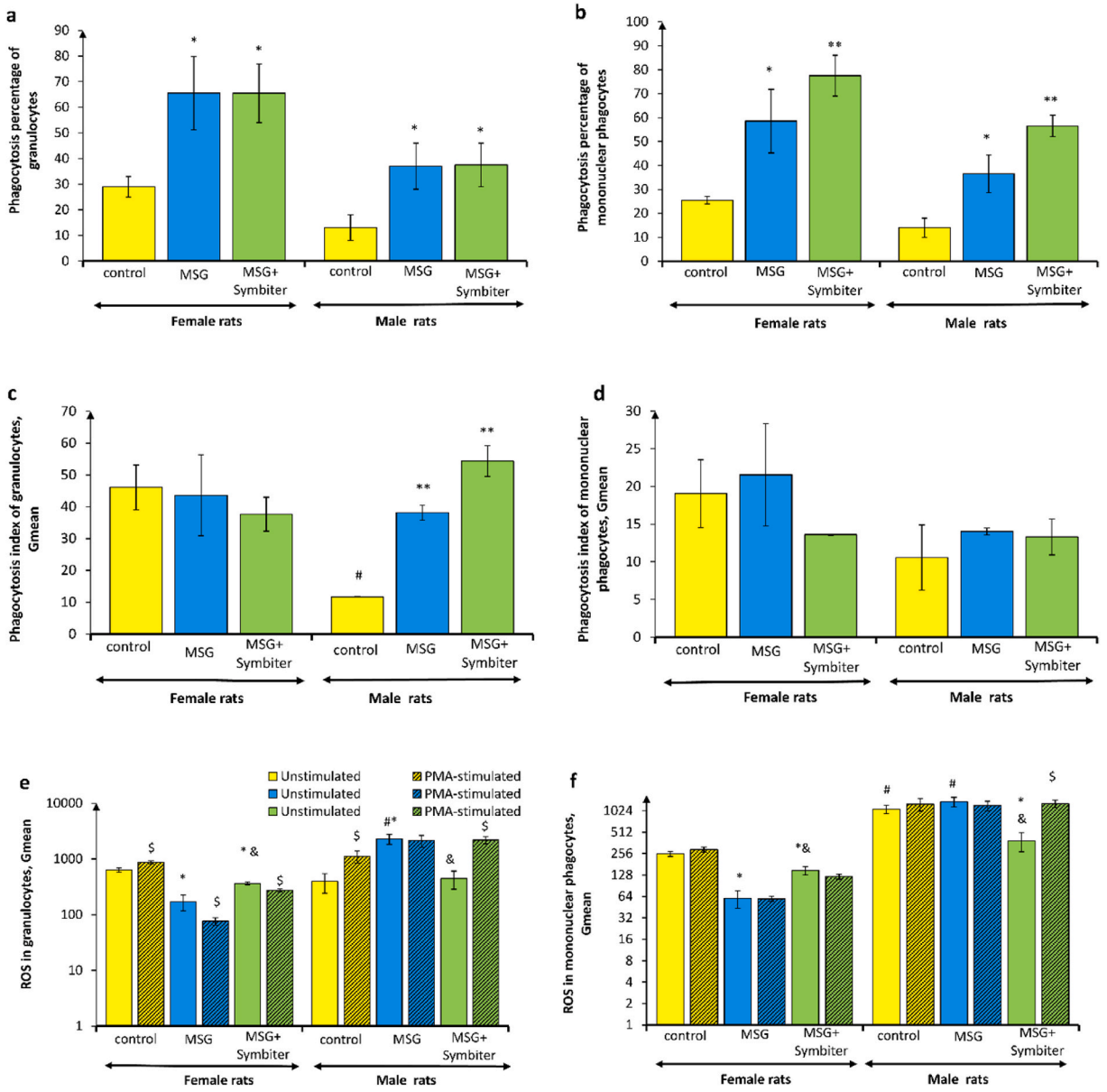

Fig. 6. Functional activity of peripheral blood phagocytes in female and male rats with MSG-induced obesity treated with multi-probiotic preparation (n = 8). (a, b) Percentage of phagocytizing granulocytes and mononuclear phagocytes, respectively; (c, d) phagocytosis index of granulocytes and mononuclear phagocytes, respectively; (e, f) intracellular production of ROS by granulocytes and mononuclear phagocytes, respectively. Values are presented as mean ± SD. *, $P < 0.05$ and **$P < 0.01$ as compared to the corresponding values of the control group. [&], $P < 0.05$ as compared to the values in group of rats with MSG-induced obesity. [$], $P < 0.05$ as compared to the values of the corresponding unstimulated cells. Statistically significant differences between sexes in a two-way ANOVA are shown as follows: #, $P < 0.05$.

adipocytes into the circulation, which may be a crucial factor in pro-inflammatory activating of distant phagocytes including peritoneal macrophages and circulating cells [84]. In our experiment, only male obese rats demonstrated signs of pro-inflammatory shift of distant (peritoneal and circulating) phagocytes. It indicates more pronounced systemic inflammation in MSG-obese male animals. Systemic inflammatory phagocyte shift in male rats can be partly explained by a higher adiposity and more prominent AT inflammation.

There is a lot of literature data concerning glutamatergic signaling along the microbiota-gat-brain axis. All these data regard bidirectional communication system between the gastrointestinal tract and the brain with the emphasis on the ability of glutamate produced by GM to affect glutamatergic pathways along the gut-brain axis [85,86]. Nevertheless, there is a lack of information concerning the consequences of the disturbances in glutamatergic signaling for GM. To our knowledge, it is the first report about the alterations in GM in rodent model of MSG-induced obesity (with subcutaneous (parenteral) MSG introduction). In our experiment, slight but numerous sex differences were revealed in the alterations of composition and metabolism of culturable GM in MSG-obese animals. Quantitative decrease in lactobacilli was registered in all animals, but to a greater extent in male rats. Amount of GM with hemolytic activity was increased in all obese rats, but to a greater extent in male animals. Decrease in SCFAs in feces was characteristic of all obese animals, but the acetate decline in male rats was more dramatic than in female animals etc. More prominent alterations in GM composition and metabolism in male obese rats can be partly explained by more pronounced inflammation. Pro-inflammatory phagocyte activation is accompanied by the ROS generation contributing to the systemic oxidative stress, which is characteristic of obesity [87]. ROS are supposed to constrain microbial growth in important habitats including gut and indeed of shaping bacterial communities [88]. Our findings regarding greater expression of CD14 by adipose tissue phagocytes, which are in agreement with the results of Marriott et al. [89] that proved the constitutively greater levels of CD14 and TLR-4 surface expression by macrophages in male mice, can explain some contribution of scavenger receptor expression difference in sexual dimorphism of GM-mediated inflammation associated with obesity. Nevertheless, deep insight in the reasons for the GM alterations in MSG-induced obesity necessitates the understanding of the effects of the disturbances in glutamatergic pathways on GM quorum sensing, metabolism etc. It is worthy to note, that strongly decreased acetate amount can be the reason for the aggravation of the inflammation in male obese rats, thereby creating the vicious circle between the inflammation and GM alteration in the pathophysiology of obesity [90].

In our prophylactic interventions, we have used multi-probiotic “Symbiter acidophilus”, containing 14 different live probiotic strains (*Lactobacillus, Bifidobacterium, Propionibacterium, Acetobacter* genera). In previous experimental studies and randomized clinical trials, the beneficial effect of probiotic preparations of Symbiter group was proven in different inflammatory disorders including obesity [44–48,91,92]. According to the results of current study, probiotic supplementation of MSG-injected animals prevented weight gain and fat accumulation, inflammatory phagocyte activation and alterations in GM in female rats. In male MSG-injected rats, probiotic supplementation restricted but did not prevent weight gain and AT deposition, alleviated but did not prevent systemic inflammation, prevented the alterations in GM, but with residual imbalance in the ratio of obligate anaerobic to facultative anaerobic bacteria.

Taken together, our results indicate that prophylactic supplementation with multi-probiotic “Symbiter acidophilus” of MSG-administered rats in their early life prevents obesity development in female animals and strongly restrains but does not prevent in male animals. The main reason for this phenomenon is significant sex differences in the MSG-induced obesity course, which in turn are stipulated by sex-determined distinctive features of AT deposition, innate immunity, glutamatergic signaling and GM composition and metabolism.

However, some limitations of this study should be addressed. We did not have the opportunity to evaluate the levels of cytokine expression in our experiment. But we expect that significant functional changes in phagocyte populations should be supported by the release of inflammatory related molecules during MSG-induced obesity, and probiotic treatment could prevent it in female rats. This suggestion is based on the results obtained by Shastri et al. [93], where anti-inflammatory IL-10 levels were higher in female rats treated with prebiotic supplements, while increased pro-inflammatory IL-6 levels were detected in males. Sex hormones, though we did not assess their levels, are the major contributors in sex diversification of immune cell response in immunopathology, and their influence on MSG-induced obesity still needs to be revealed. The interactions between hormones, immune cells, and GM in obesity appear to be very complex, it is mediated by the various specific receptors for hormones, DAMPs and PAMPs (pathogen-associated molecular patterns), and each of them has its own patterns of expression in different sexes. Probiotic treatment of immunopathological disorders offer natural balance solution by contrast to artificial strictly specific synthetics, but in the current state of the study it is obvious that sex-centered approach is needed to analyze and reassess gained to date knowledge to predict probiotic therapeutic efficacy in obesity.

5. Conclusions

Probiotic supplementation can be considered pathogenetic preventive strategy in obesity. Multifactorial pathophysiology of the obesity includes several key components which can be beneficially affected by probiotic microorganisms considering their ability to exert effects in tissues and organs distant from their habitat. All variables in the pathophysiology of the obesity are characterized by sexual dimorphism. Thus, our findings emphasize the necessity of sex-centered approaches to the prophylactic use of probiotic preparation in obesity in the context of PPPM, considering profound sexual dimorphism in multifactorial pathophysiology of the disease.

Author contribution statement

Mariia Rudyk: Performed the experiments; Analysed and interpreted the data; Wrote the paper.

Yevheniia Hurmach: Performed the experiments.

Tetiana Serhiichuk: Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Iryna Akulenko: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Larysa Skivka: Conceived and designed the experiments; Analysed and interpreted the data; Wrote the paper.

Tetiana Berehova: Conceived and designed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Liudmyla Ostapchenko: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of interest's statement

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