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Probiotics and nutraceuticals as a new frontier in obesity prevention and management

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ABSTRACT

Introduction: The beneficial interaction between the microbiota and humans is how bacteria contained within the gut 'talk' to the immune system and in this landscape, probiotics and nutraceuticals play a major role. The study aims to determine whether probiotics plus nutraceuticals such as smectite or omega-3 are superior to probiotic alone on the monosodium glutamate (MSG) induced obesity model in rats.

Methods: Totally, 75 rats divided into five groups were included (n = 15, in each). Rats in group I were intact. Newborn rats in groups II–V were injected with MSG. Group III (Symbiter) received 2.5 ml/kg of multiprobiotic "Symbiter" containing concentrated biomass of 14 probiotic bacteria genera. Groups IV (Symbiter-Omega) and V (Symbiter-Smectite) received a combination of probiotic biomass supplemented with flax and wheat germ oil (250 mg of each, concentration of omega-3 fatty acids 1–5%) or smectite gel (250 mg), respectively.

Results: In all interventional groups, significant reductions of total body and visceral adipose tissue weight as compared to MSG-obesity were observed. However, the lowest prevalence of obesity was noted for Symbiter-Omega (20% vs 33.3% as compared to other interventional groups). Moreover, supplementation of probiotics with omega-3 lead to a more pronounced decrease in HOMA-IR (2.31 ± 0.13 vs 4.02 ± 0.33, $p < 0.001$) and elevation of adiponectin levels (5.67 ± 0.39 vs 2.61 ± 0.27, $P < 0.001$), compared to the obesity group.

Conclusion: Probiotics and nutraceuticals led to a significantly lower prevalence of obesity, reduction of insulin resistance, total and VAT weight. Our study demonstrated that supplementation of probiotics with omega-3 may have the most beneficial antiobesity properties.

Abbreviations: ACAT, acyl-coenzyme A:cholesterol acyltransferases; BSH, bile salt hydrolase; CFU, colony-forming unit; FAS, fatty acid synthase; FXR, farnesoid X receptor; GA, gum Arabic; GLP, glucagon-like peptides; HDL-C, high density lipoproteins cholesterol; HFD, high-fat diet; IL, interleukins; INF-γ, interferon γ; IR, insulin resistance; LDL-C, low density lipoproteins cholesterol; LPS, lipopolysaccharide; MSG, monosodium glutamate; NAFLD, non-alcoholic fatty liver disease; PPAR, peroxisome proliferator-activated receptor; SCFA, short-chain fatty acids; TC, total cholesterol; TG, triglycerides; SREBP1c, sterol regulatory element-binding protein 1c; TGF-β, transforming growth factor β; VAT, visceral adipose tissue; WPI, whey protein isolate; ZO, zonula occludens

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1. Introduction

Obesity is currently recognized as a condition that belongs to the most wide-spread medical and social problem, playing an outstanding role in human pathology development. Obesity is known to reduce human reproductive potential by having a negative impact on the general human condition, working ability, and patients' quality of life.

Without any exaggeration, obesity can be characterized as a global scale epidemic; the number of people that are overweight, among both the adult and child population is getting ever greater. According to the data of the World Health Organization, 1.9 billion humans of 18 years and above are overweight, 600 million of them are obese patients [43]. During the period between 1980 and 2014, the prevalence of obesity in developed countries has drastically increased among the adult population (from 15% up to 33%). The proportion of overweight children has increased from 6% up to 19% [43]. Both overweightness and obesity promote the rise of metabolic syndromes such as type 2 diabetes and cardiovascular conditions, leading to early disability and significant life span shortening [34].

Development of obesity is associated with the disturbance of the organism's energetic balance including regulation of intake of nutritious substances and energy expenditures. Different weight-regulating mechanisms including peripheral and central signals of hunger and satiation and complex response of the gastrointestinal tract organs following food intake are subjects of numerous scientific studies [17]. Besides genetic susceptibility, environmental conditions and human life style as well as gut microbiota play a very important role in these processes [18].

The problem of the effect of probiotics on lipid metabolism and obesity is a burning issue that is currently being discussed in scientific literature [17,16,27]. Gut microbiota may be considered as an ecological factor modulating obesity development. The use of a high-fat diet (HFD) in mice has been shown to significantly change the intestine microbiota composition, demonstrating the decrease of *lacto-* and *bifidobacteria* levels which are responsible for many positive physiological processes, e.g. improving the barrier function of the intestinal mucosa [27]. The microbiome analysis of mice with genetically-determined obesity demonstrates a drastic quantitative decrease of *Bacteroidetes* representatives, the proportion of *Firmicutes* becoming, on the contrary, increased [25]. Similar changes have also been found in humans: intestines of 12 obese patients contained decreased quantities of *Bacteroidetes* and higher contents of *Firmicutes* compared to control, lean persons [26].

Prebiotics and probiotics are now of great interest [13]; they have been shown to change the microbiota components and to influence food digestion, regulating the appetite and body weight [17,39]. Our choice of probiotic for the present study is based on a previously realized comparative experimental investigation of different probiotic strains intended for treatment and prevention of non-alcoholic fatty liver disease (NAFLD) and obesity [19,40]. Administration to animals of poly-probiotic mixtures containing both live and lyophilized strains beginning from the rats' infancy led to significant decreases of total and visceral adipose tissue (VAT) weight, steatosis and liver lobular inflammation, increased insulin sensitivity and induced hypolipidemic effect; all these processes assured a preventive defense from the NAFLD development in animals with experimental monosodium glutamate (MSG) induced obesity [19,40]. Furthermore, more pronounced changes were seen following administration of a probiotic mixture containing mostly live strains compared to lyophilized ones. Our data also suggest failure of preventive action concerning obesity and NAFLD development in cases when monostrain lyophilized probiotics have been used [19].

Nutraceuticals are substrates-pharmaceuticals supporting normal functional activity of intestinal mucosa. They are able to realize immunomodulating, cytoprotective, and antioxidant functions, to take part in metabolic processes, to deliver energetic and plastic materials to epitheliocytes. This is turn influences hormone metabolism. Nutraceuticals are, in particular, short-chain and polyunsaturated fatty acids belonging to omega-3 and omega-6, sorbents. It is worth using nutraceuticals together with probiotics, as then it is possible to simultaneously improve mucosa cytoprotection and to restore its symbiosis with intestine physiological microflora.

This study aims to determine whether probiotics plus nutraceuticals, such as smectite or omega-3, are superior to probiotic alone on the MSG-induced obesity model in rats.

2. Materials and methods

2.1. *Experiment design*

Seven animals were maintained in each polycarbonate cage for rats and mice; the cages were with galvanized steel covers and contained *ad libitum* drinking water in glass bottles. The animals were fed by standard chow delivered by the firm ≪REZON-1≫ (Ukraine).

All the animals selected for experiments were examined by a vet, their acclimatization was carried out during the first five days; the animals were then randomly divided into groups and labeled with numbers.

The rats were divided into 5 groups, each group containing 15 animals (n = 75). Group 1 control rats were administered neonatally with hypertonic saline (1.25 mg/g body weight per day, control group) subcutaneously (s.c.) at 2nd, 4th, 6th, 8th and 10th postnatal days. To obtain an experimental obesity model, all newborn rats except control were injected with monosodium glutamate - MSG (4 mg/g body weight per day) at 2–10th days of life to induced obesity [24,20,21]. Were standardized six pups per mother to ensure better lactation. After the weaning one-month aged MSG-treated animals were randomly divided into 4 groups, treated and untreated with probiotics/nutraceuticals. From weaning (30 days) to 120 days of age, rats had free access to standard rodent chow (PurinaW) and water during the entire experimental period.

The animals of group II (MSG-obesity) received water (2.5 ml/kg) (intra-gastric administration). During the experiment, groups III-V received only 2.5 ml/kg of an aqueous solution either live probiotic mixture (group III) or its combination with nutraceuticals (groups IV–V). Group III rats received the multi-probiotic "Symbiter" (2.5 mg/kg) (O.D. Prolisok", Ukraine). This preparation biomass contained 14 live probiotic strains [*Lactobacillus* + *Lactococcus* (6×10^{10} CFU/g), *Bifidobacterium* (1×10^{10} CFU/g), *Propionibacterium* (3×10^{10} CFU/g, *Acetobacter* (1×10^{6} CFU/g)]. Animal groups IV (Symbiter-Smectite) and V (Symbiter-Omega) received complex mixtures including in addition, except probiotics, smectite gel (200 mg); group V rats received also flax oil (250 mg) and wheat germ oil (250 mg), the concentration of omega-3 unsaturated fatty acids being 0.5–5%. Alpha-linoleic acid contained in these oils is a substrate for docosahexaenoic and eicosapentaenoic acids. A dose of probiotic bacteria preparations for these groups (10 cm^3) contained 2×10^{10} CFU. The study design is presented in Fig. 1.

2.2. Anthropometric measurements and obesity parameter assessment

The treatment with probiotics and nutraceuticals was started at the age of one month and was performed for 3 months, each course lasting 2 two weeks courses (i.e., 1 course per month). During the experiment, the animals were observed daily to register their status as well as their foraging and water consumption; the animal body mass was determined once weekly.

At 4 months, analysis of body weight changes was made for all rats. Twenty four hours before the beginning of the experiment, the animals were not fed using the water *ad libitum*. All the equipment used for this study was subjected to metrological control. The Lee index was used to determine the state of obesity; it was calculated from the formula: cubic root of the body weight (g)/naso-anal length (cm). The obesity state was confirmed in animals with the Lee index value above 0.300 [4].

2.3. Sample collection and blood biochemistry analysis

The animals were killed using cervical dislocation or lethal narcosis (urethane) doses (Sigma-ALDRICH, Inc., USA). Blood samples were then taken for biochemical studies, the visceral fat was removed and weighted.

Serum levels of total cholesterol (TC), triglycerides (TG), and high density lipoproteins cholesterol (HDL-C) were determined by enzymatic spectrophotometry using biochemical kits of Pointe Scientific Inc. (USA).

Serum levels of low density lipoproteins cholesterol (LDL-C) (mM/l) were calculated using Friedewald's formula:

$$\mathrm{LDL-C} = \mathrm{TC} - (\mathrm{HDL-C} + \mathrm{TG}/2.22).$$

The cholesterol concentration contained by lipoproteins of very low density (VLDL) was calculated from the ratio TG/2.22.

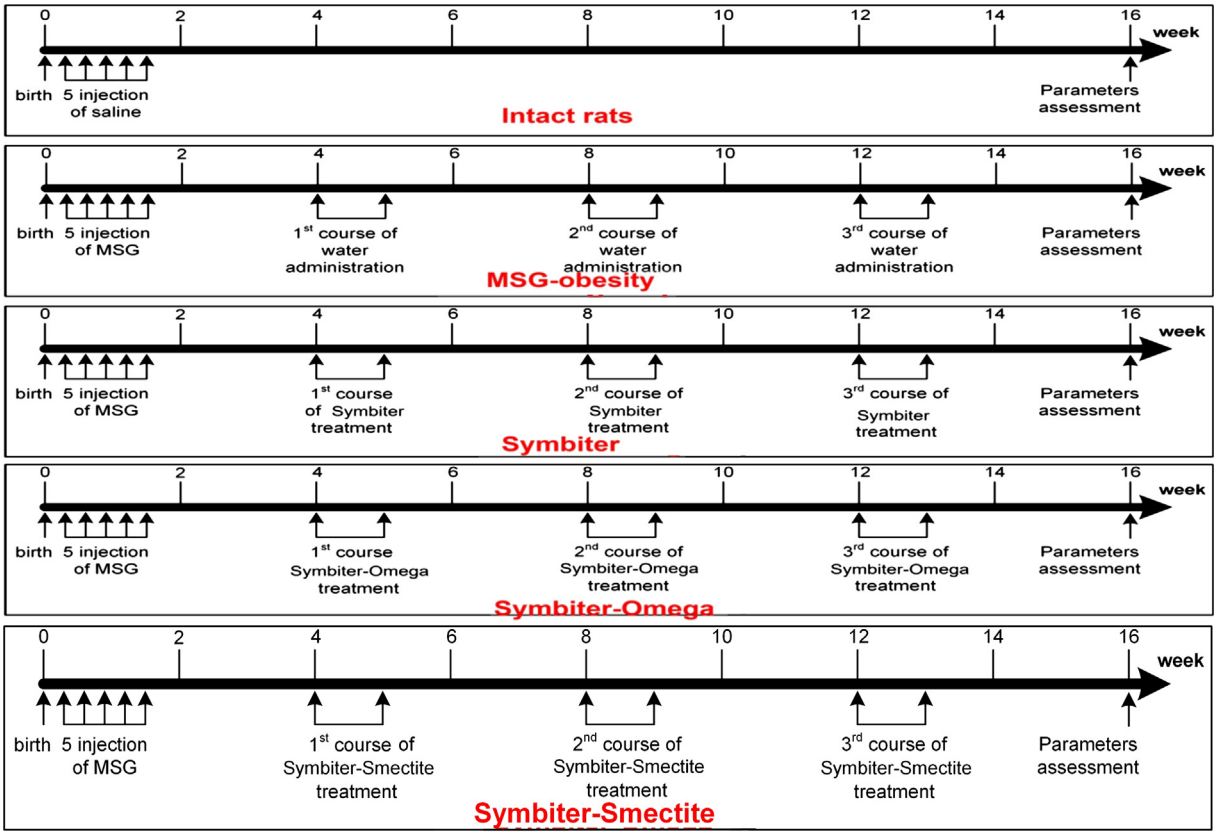

Fig. 1 – The design of the study. Five arrows with the corresponding signs demonstrate experimental groups of animals and the manipulation within these groups.

The fasting glucose levels were determined by Trinder's colorimeter method using an "Exan" apparatus.

The insulin resistance (IR) level was assessed using a structural mathematical model based on fasting glucose and insulin determination – HOMA (homeostasis model assessment), the HOMA index being calculated from the formula:

$$\text{HOMA} - \text{IR} = \text{immunoreactive insulin } (\mu\text{U/ml}) \times \text{fasting plasma glucose}/22.5.$$

2.4. *Cytokines measurement*

The contents of interleukins (ILs) 1β, 4, 10, 12B p40, interferon (INF) -γ, and transforming growth factor (TGF)-β in rat serum were measured by an ELISA approach using specific mono- and polyclonal antibodies (Sigma) to these proteins. Monoclonal antibodies to ILs 4, 10, and TGF-β were mice-produced. Polyclonal antibodies to IL-12B p40 were produced in rabbits and polyclonal antibodies to IL-1β and INF-γ were of goat origin. Studied molecules were immobilized in 96-well plates with a sorption surface. Then enzyme-labeled primary and secondary antibodies were added to wells and addition of the substrate led to a colored reaction. The solution optical density in each well after the substrate addition is proportional to the content of cytokines studied. The content was expressed as absorbance units of optical density.

2.5. *Statistical analysis*

Statistical analyses were carried out using the standard program package SPSS version 20.0 and GraphPad Prism version 6.0. Quantitative changes were presented as mean values ± standard deviations (M ± SD), qualitative values were given as%. For difference evaluation of the two quantitative parameters the Student's *t*-test for independent samples was used; in cases of three or more parameters, one-way ANOVA was taken using the Tuckey test for paired comparisons and its non-parametric analogue – Kruskal-Wallis H-test. The criterion χ^2 was taken for the analysis of qualitative variables. The inter-group difference was thought to be statistically significant at the p value below 0.05. Designations of significant inter-group differences on diagrams were made using letters *a*, *b*, *c*, *d*. The same letter over two diagram columns indicates the absence of differences between two corresponding data groups. Different letters over two diagram columns reflect significant differences between two corresponding data groups.

3. Results

Fig. 2 demonstrates the dynamics of body weight gain by animals of different experimental groups (n = 15). According to the data presented, in 30 days post experiment beginning, the lowest body mass was registered in control rats as compared to other groups, the data being statistically significant ($p < 0.005$). Later the experimental rats showed similar body mass data; at 60 and 90 days we did not find any significant body weight differences in all the experimental groups. However, at 120 days the MSG-obesity rats presented the highest

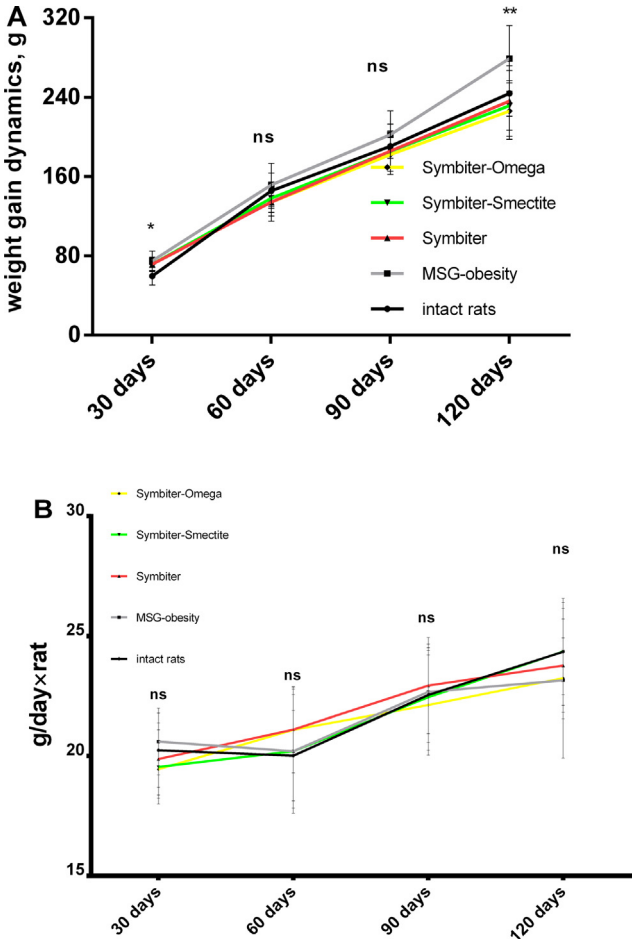

Fig. 2 – Weight gain dynamics (A) and food consumption rate (B) in experimental animals (n=15 in each group).

body weight, the data being statistically significant compared to all other groups ($p < 0.005$, Fig. 2A).

Taking into consideration the delayed dynamics of body weight gain by control animals and by rats having received the complex therapy, we did not find any statistically significant differences in the level of food consumption in any of the experimental animal groups at any time point (30–120 days, Fig. 2B).

MSG administration to neonatal animals caused stunted growth; this being one of characteristics of this experimental model. As it is seen in Fig. 3A, the animals of groups II–V demonstrate decreased naso-anal length compared to control rats (n = 15), the data being statistically significant. That is why, in spite of the absence of dynamics concerning the body weight gain as well as the absence of significant differences in animal weight on the 120th day, we stated the obesity development in 93.3% MSG-obesity animals, the data being significant higher compared to the intact ($p < 0.001$), the level of control rats obesity reaching 10%. It should be noted the probiotics administration together with nutraceuticals led to a significant lower obesity prevalence. However, the lowest obesity frequency (20%) was seen in Symbiter-Omega. The obesity prevalence in probiotic alone and Symbiter-Smectite was 33.3%, these data being insignificant compared to Symbiter-Omega ($p = 0.0649$).

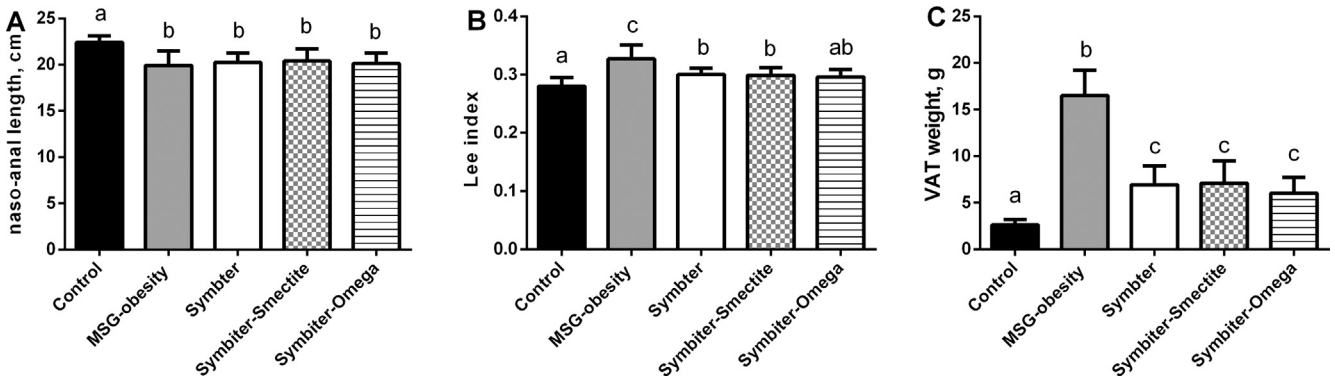

Fig. 3 – Anthropometric parameters in 4-month old rats (n=15 in each group) in the condition of MSG-induced obesity and after probiotic/nutraceuticals administration (A – naso-anal length; B – Lee index; C – visceral adipose tissue weight). Data are presented as the M±SD. One-way ANOVA with post hoc Tukeys test for multiple comparisons were performed for data analysis. [a,b,c] Values on the same row with different superscript letters show significant differences in p<0.05.

The results obtained are completely supported by Lee index value being exceeded above 0.300; such data permit to record the animals' obesity. The mean value of the Lee index in MSG-obesity rats was higher compared to control groups and all other interventional groups (Fig. 3B). The maximal preventive effect for experimental obesity development was seen following the introduction of Symbiter-Omega: we did not record significant differences of Lee index values only in this interventional group compared to control animals. Lee index values in animals of Symbiter and Symbiter-Smectite groups were statistically lower compared to the MSG-obesity group; however, no significant restoration was seen compared to the levels in control rats.

MSG administration to neonatal animals led to considerable VAT accumulation, the fat quantity becoming 6-fold higher compared to control rats (Fig. 3C). In cases of administration of probiotic strains and their combinations with nutriceuticals (groups III–V) we found a statistically significant decrease of VAT weight in all groups compared to MSG-obesity ($p < 0.001$). Although we did not find any significant inter-group differences concerning visceral obesity decrease, the lowest VAT mass was seen in the Symbiter-Omega group.

The analysis of glucose metabolism parameters demonstrates that following neonatal MSG administration, the IR formation is detected accompanied by metabolic damage mentioned above. In MSG-obesity rats, we found significant increase of HOMA-IR (4.02 ± 1.28 vs 1.63 ± 0.74, $p < 0.001$), serum insulin concentration (17.3 ± 5.39 vs 7.87 ± 2.82 μU/ml, $p < 0.001$), and serum glucose levels (5.24 ± 0.72 vs 4.59 ± 0.54 mM/l, $p = 0.042$) compared to control animals (Fig. 4A–C). Having carried out the complex therapy, we observed a tendency for glycemia levels to decrease, the data obtained being however, statistically non-significant compared both to MSG-obesity and controls (Fig. 4A). On the other hand, following the therapy, we found a statistically significant decrease of insulin levels and HOMA-IR values in all interventional groups compared to MSG-obesity; the level of adiponectin, a well-known leading regulative substance promoting the insulin sensitivity of peripheral tissues, became higher (Fig. 4B–D). However, the complex analysis of parameter changes in glucose metabolism proves the IR to be significantly improved only due to the use of Symbiter-Omega complex compared to other interventional groups. This was because there was no statistically significant difference found for group V only compared to control animals (Fig. 4B–D).

It is generally accepted that there are some marked lipid metabolism changes associated with obesity: blood levels of TG, TC, and LDL-C increase, the HDL-C level becoming lower. The administration of probiotic strains and their combinations with nutraceuticals shows a marked impact on lipid metabolism in experimental animals; however, we found some peculiarities depending on the composition (Fig. 5). For instance, we did not find statistically significant differences concerning the hypolipidemic effect expression concerning TC and LDL-C levels for all interventional groups (Fig. 5B and C). The TC concentration (Fig. 5B) and LDL concentration (Fig. 5C) in interventional groups were almost on the same level and significantly decreased by 12–15% and by 15–20%, respectively, compared to the MSG-obesity. No statistically significant differences were found in any group compared to control rats.

The most marked hypolipidemic impact on the TG level was registered in Symbiter-Omega (group V) rats. The use of such combinations led to a significant decrease of TG levels by 36.4% ($p < 0.001$) compared to MSG-obesity, the lipid concentration having been practically decreased to normal levels seen in rats; such a decrease was accompanied by the absence of significant differences between different animal groups (Fig. 5A). The significant decrease of TG levels was also seen in other interventional groups; however, it was not significant compared to the control group (Fig. 5A).

A significant increase of HDL levels [by 23.3 ($p = 0.006$) and by 21.5% ($p = 0.023$), respectively], was shown only following the use of probiotics (group III) and their combination with omega-3 (group V) compared to MSG-obesity (Fig. 5D). In the Symbiter-Smectite group (group IV) we observed only a tendency for HDL levels to increase by 14.2% ($p = 0.186$) compared to the MSG-obesity (Fig. 5D). However, in spite of moderate efficacy, no biochemical parameters of lipid metabolism were increased to the control group levels.

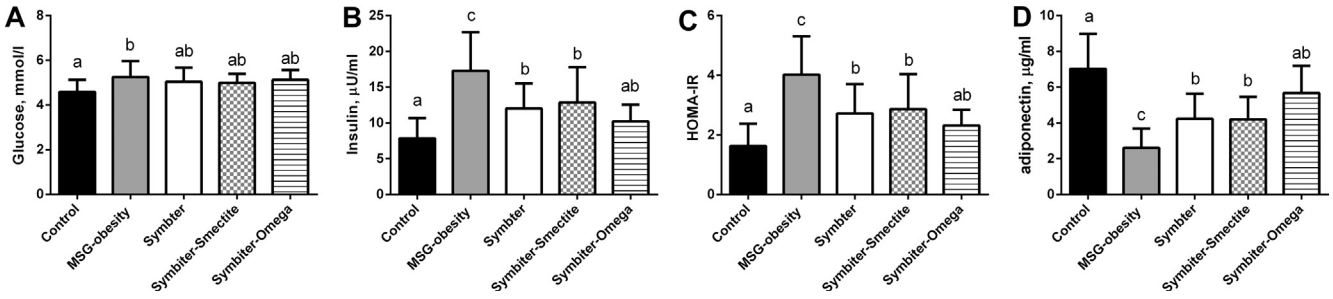

Fig. 4 – Glucose metabolism parameters in 4-month old rats (n=15 in each group) in the condition of MSG-induced obesity and after probiotic/nutraceuticals administration (A – glucose; B – insulin; C – HOMA-IR; D – adiponectin). Data are presented as the M±SD. One-way ANOVA with post hoc Tukeys test for multiple comparisons were performed for data analysis. [a,b,c] Values on the same row with different superscript letters show significant differences in p<0.05.

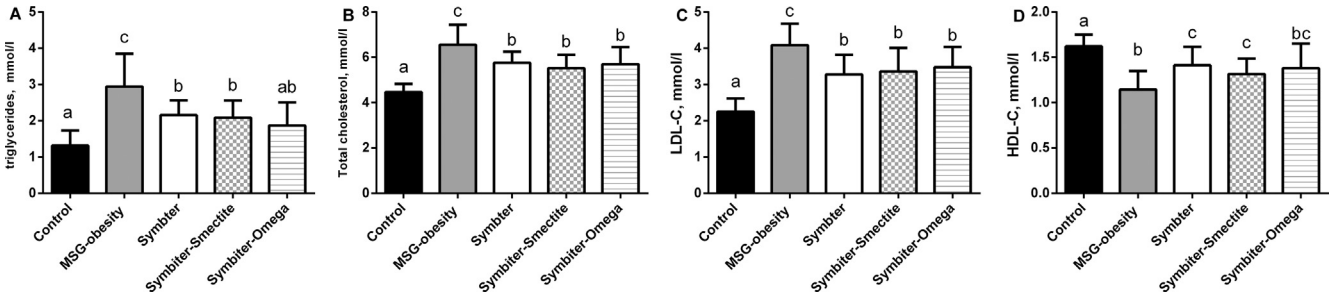

Fig. 5 – Serum lipid levels in 4-month old rats (n=15 in each group) in the condition of MSG-induced obesity and after probiotic/nutraceuticals administration (A – triglycerides; B – total cholesterol; C – LDL-cholesterol; D – HDL-cholesterol). Data are presented as the M±SD. One-way ANOVA with post hoc Tukeys test for multiple comparisons were performed for data analysis. [a,b,c] Values on the same row with different superscript letters show significant differences in p<0.05.

The analysis of the animals' immune status demonstrates the development of the chronic systemic inflammatory state in MSG-obesity conditions manifested by statistically significant increases of serum pro-inflammatory cytokines IL-1β and IL-12B p40, as well as by decrease of anti-inflammatory IL-4 and TGF-β compared to MSG-obesity rats (Fig. 6). Of note, we stated the increase of IL-10 levels contrary to other anti-inflammatory cytokines; such a fact may witness the process of compensatory activation of the anti-inflammatory system (Fig. 6F). Regarding the INF-γ level we did not find any significant differences between any of the interventional groups (Fig. 6C).

The separate use of probiotics and/or their combinations with nutraceuticals led to the anti-inflammatory system activation confirmed by significant increases (by 25–35%, $p < 0.001$) of serum IL-4 and TGF-β levels and restoring of IL-10 levels compared to MSG-obesity (Fig. 6D–F). It should be noted that therapeutic results were almost the same in all interventional groups independent of the components used; the restoration of the anti-inflammatory response was observed up to control group levels (Fig. 6).

We also found significant decreases in anti-inflammatory cytokine levels in all interventional groups compared to the MSG-obesity. The maximal decrease of IL-1β concentration was seen in the Symbiter-Smectite group (Fig. 6A), the lowest IL-12B p40 level having been found in the Symbiter-Omega group (Fig. 6B). In both groups the changes of cytokine concentrations were significantly lower compared to both MSG-obesity animals and to rats receiving probiotics alone.

4. Discussion

Several previous studies carried out using experimental obesity models with mono-strain probiotics belonging mostly to *Lactobacillus* and *Bifidobacterium* genera showed wide therapeutic possibilities of probiotic therapy leading to decreased body weight as well as to mitigation of other obesity-associated pathological conditions, such as IR, chronic systemic inflammation, hepatic steatosis [17,18,30]. Cani and Van Hul [7] analyzed the efficacy of obesity treatment using at least 15 different *Lactobacillus* strains and 3 *Bifidobacterium* ones in different protocols and experimental models. It should be noted that only 10 strains among the 18 used decreased the total body and/or VAT weight; 12 strains led to mitigation of liver and/or fat tissue inflammation when used alone. The differences seen are, most probably, due to different potential action mechanisms, the effects described should be thought to be strain-specific. The mechanisms responsible for probiotic strain therapeutic actions are the following: regulation of fat absorption and excretion [15]; increase of primary cholic acids due to bile salt hydrolase (BSH), mediating the glucose deponation, insulin synthesis, and gluconeogenesis decrease *via* the activation of the FXR receptor [37,10]; increase in the level of glucagon-like peptides

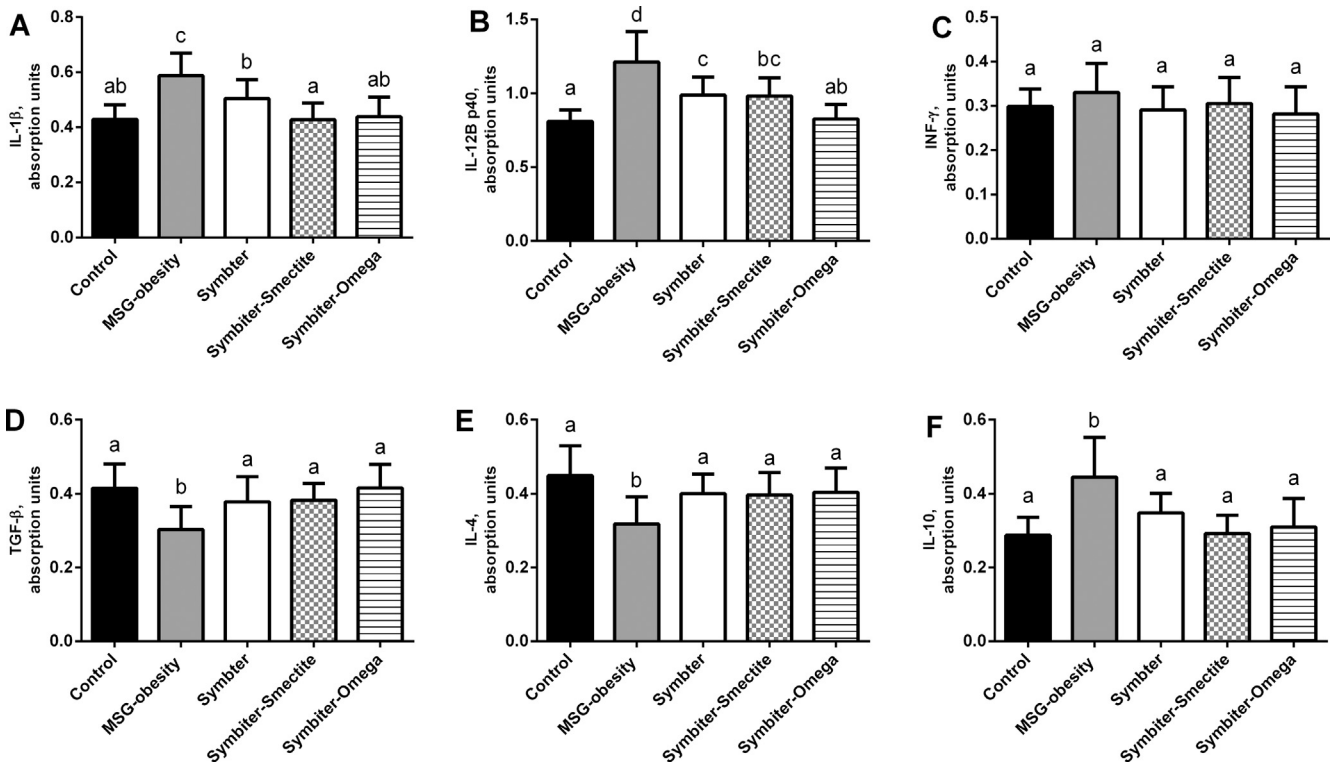

Fig. 6 – Serum pro- and anti-inflammatory cytokines levels in 4-month old rats (n=15 in each group) in the condition of MSG-induced obesity and after probiotic/nutraceuticals administration (A – IL-1β; B – IL-12B p40; C – INF-γ; D – TGF-β; E – IL-4; F – IL-10). Data are presented as the M±SD. One-way ANOVA with post hoc Tukeys test for multiple comparisons were performed for data analysis. [a,b,c] Values on the same row with different superscript letters show significant differences in $p<0.05$.

(GLP 1 and GLP 2) leads to hunger mitigation and satiation development, decreasing the energy consumption and improving the sensitivity to insulin and β-cell function [41]; regulation of gene expression (SREBP1c, ACAT, FAS, PPAR-α) lowering the lipogenesis *de novo* and accelerating fatty acid β-oxidation in the liver and/or in fat tissue [29,44]; regulation of proteins ZO-1 and ZO-2 expression [5] leading to restoration and maintenance of intestine barrier function, decrease of LPS absorption [38] and lowered ≪metabolic endotoxemia≫ [6]; increase of short chain fatty acid synthesis (SCFA), mostly butyric acids, in the large intestine leading to the mitigation of chronic systemic inflammation and releasing anti-inflammatory cytokines by fat tissue, IR decrease, improving β-cell differentiation, proliferation, and development [35].

In our previous study [19] we showed higher efficacy of live probiotic strains produced as bacterial cell suspensions in a special protective medium. Bacteria in such suspensions are biologically active and able to act in the human organism immediately following intake of the preparation [34,1].

Preparing this study we have proposed that enriching of live multi-probiotic symbioses with nutraceuticals, such as smectite and omega-3 polyunsaturated fat acids, may lead to summation of their single positive effects. Choosing the nutraceuticals for this study we have taken into consideration the facts stated below.

Smectite (bentonite) is a natural loamy poly-mineral compound containing about 60–70% of montmorillonite group minerals [2]. Loamy minerals of this group are formed by extremely small particles, its capability for hydration being the highest among loamy minerals [2]. The gel smectite form is the most physiologically active, assuring excellent wrapping capability of mineral; that is why it is able to be distributed on the intestinal tract surface, improving the strength of the mucosa barrier and permitting mineral particles to interact with glycoproteins of the mucosa as well as with the microbial biolayer; leading to a cytomucoprotective therapeutic effect [42,23]. Following the mixing of probiotic biomass and smectite gel, the sorbent becomes bound to surface structures of bacterial cells and covers them with a protective layer promoting the increased probiotic biomass survival during its transit through the more aggressive gastrointestinal tract areas. The microdispersive gel structure assures this gel absorbing capacity with viruses, toxins, radionuclides, heavy metals and bacterial endotoxins without "swallowing up" normal microbiota cells and physiologically important nutrients; it improves the therapeutic possibilities of probiotic-nutraceutical preparations [11,14].

Long-chain omega-3 fatty acids belong to the family of polyunsaturated fatty acids. They are known to exert a strong positive influence on metabolism and inflammation due to their participation in the regulation of SREBP-1c and PPAR-a gene expression, promoting the fatty acid β-oxidation [33,31,22]. Taking into consideration the importance of omega-3 fatty acids as essential human diet components, their *de novo* synthesis in humans is impossible, the use of nutraceuticals was proposed for complex therapy of obesity

and obesity-associated conditions [45]. Rajkumar et al. [36], carried out a placebo-controlled double-blind study and compared the efficacy of probiotics VSL#3 and omega-3 fatty acids as mono-therapy components and in combinations in healthy volunteers with overweight. Nutraceuticals-probiotics combinations were shown to cause a more marked hsCRP decrease and improvement of insulin sensitivity compared to the action of separate probiotics. In this study, as well as in some others, micro-capsulation technology was used, the capsules included omega-3 fatty acids and probiotic bacteria [32,8]; this technology foresees the protection of bioactive non-stable food substances and prevention of their rapid degradation before the product delivery to certain areas [3]. Das et al. [9], were the first to propose co-encapsulation of probiotic bacteria and omega-3 fatty acids in the same carrier particle the idea being that such a cooperation would promote probiotic bacteria binding to the intestinal epithelium and to assure a synergetic result. Co-encapsulation technology is currently widely used in systems of pharmaceutical agent delivery; however for a long while it was impossible to unite these nutraceuticals in the same bioactive preparation because of the hydrophobic properties of omega-3 and hydrophilic properties of probiotic mass. Eratte et al. [12], were the first to co-encapsulate omega-3 fatty acids and probiotic bacteria in a single microcapsule formed by whey protein isolate (WPI)–gum Arabic (GA) complex coacervate. Tuna oil (O) and *Lactobacillus casei* 431 (P) were used as models of omega-3 and probiotic bacteria, respectively. The co-microcapsules (WPI-P-O-GA) and *L. casei* containing microcapsules (WPI-P-GA) were converted into powder using spray and freeze drying. The viability of *L. casei* was significantly higher in WPI-P-O-GA co-microcapsules than in the WPI-P-GA. In this study we have used concentrated biomass of probiotic bacteria enriched with sterile smectite gel and omega-3-polyunsaturated fatty acids contained in oils extracted from flax seeds and wheat embryos. Alpha-linoleic acid contained in these oils is a substrate for docosahexaenoic and eicosapentaenoic acids. Such a pharmaceutical form was shown to possess higher efficacy compared to microcapsules used by Eratte et al. [12]. Because of the biologically active form of the bacteria present in preparations of different concentrations; it is why these bacteria are ready to act immediately after the oral intake of the preparation. On the other hand, the presence of smectite gel is intended to promote the probiotic efficacy, the gel being in active form plays the role of active enterosorbent.

The limitation of our study may be the model of experimental obesity that we chose. This model is an early-onset obesity resulting from MSG induced lesions in arcuate nucleus to neonatal animals. This model characterized with increased fat to body weight ratio according to accumulation of VAT, severe hyperleptinemia, hyperinsulinemia and an extremely high HOMA index that pointed to development of insulin resistance [28]. Impact on these metabolic parameters by probiotic/nutraceuticals mixture was the main aim of our study. From the other hand, MSG model has a number of unique features, such as stunted growth, that may affect the results interpretation. In setting of human obesity the closest experimental model is high-fat diet induced obesity. Therefore, in future studies, it's important to implement and rechecked the data from our study on high-fat diet obesity model.

5. Conclusion

Probiotics and nutraceuticals have led to significantly lower prevalence of obesity, reduction of insulin resistance, total and VAT weight in the rat MSG obesity model. Our study has demonstrated that supplementation of probiotics with omega-3 may have the most beneficial anti-obesity properties.

Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the general ethical principles of animal experiments, approved by the First National Congress on Bioethics Ukraine (September 2001). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Taras Shevchenko National University of Kyiv (Protocol number: 2/2016). The rats were kept in collective cages in controlled conditions of temperature (22 ± 3 °C), light (12 h light/dark cycle) and relative humidity (60 ± 5%).

Author contributions

NK analyzed all data, wrote the manuscript, performed statistics, and designed the figures. TF performed the measurement of blood biochemistry analysis. NB supported the cytokines evaluation and helped with data interpretation. OT supplied basic lab equipment. TB and LO had the idea, acquired the funding, designed and supervised all experiments, checked the data, and finalized the manuscript.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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