Arh. farm. 2024; 74: 391 – 409 *Review article/Pregledni rad*

Effects of probiotic intervention on obesity-related miRNAs

Nina Okuka,[1*] Berit Hippe,[2] Brižita Đorđević,[3] Nevena Ivanović[3]

[1]University of Banja Luka, Faculty of Medicine, Department of Bromatology, 78000 Banja Luka, Bosnia and Herzegovina

[2]University of Vienna, Department of Nutritional Science, 1090 Vienna, Austria

[3]University of Belgrade – Faculty of Pharmacy, Department of Bromatology, 11000 Belgrade, Serbia

*Corresponding author: Nina Okuka, e-mail : nina.vukicevic@med.unibl.org

Received: 24 April 2024; Revised in revised forme: 5 June 2024; Accepted: 11 June 2024

Abstract

Obesity is considered to be a chronic complex disease that increases the risk of diabetes, heart diseases and certain cancers. According to the literature, in obese people the gastrointestinal microbiota is disturbed, which could be the cause of the onset of obesity and related diseases. Moreover, there are small non-coding RNAs (microRNAs) that are disturbed in obesity, which are also considered to be a possible mechanism of probiotics' action. Certain microRNAs are involved in the development and metabolism of adipose tissue cells, as well as the secretion and action of insulin, showing that changed expression of certain miRNAs could have a significant impact on the onset and development of obesity and obesity-related diseases. Thus, miRNAs are considered to be possible markers for the diagnosis and prognosis of various metabolic diseases, and possible therapeutic targets for the treatment of obesity and related diseases. Considering the growing need of the healthcare system for nutraceuticals and dietary supplements that present effective and safe medical nutritive therapy in obese individuals, this work aimed to assess the beneficial effects of probiotics on obesity-related microRNAs. In this review, we described the role of microRNAs and probiotics in obesity and the association between probiotics and obesity-related microRNAs. Circulating miRNAs' profile in obese individuals significantly differs from that of normal-weight individuals. miRNAs such as miR-155, miR-221, miR-24-3p, and miR-181a are over-expressed, while miR-26b and 125a are under-expressed in obese patients compared to non-obese individuals. It has been found that supplementation with *Bifidobacterium*

bifidum and *Lactobacillus acidophilus* could downregulate the expression of miR-155 and miR-221, and upregulate the expression of miR-26b. Supplementation with a probiotic formulation containing *S. boulardii*, *L. plantarum 299v*, and octacosanol led to the downregulation of miR-155 and miR-24-3p expression. miR-125a and miR-181a were upregulated and downregulated, respectively, after the intervention with *L. delbrueckii* and *L. rhamnosus.* After reviewing the available literature, we can conclude that probiotics have beneficial effects on microRNAs altered in obesity, which could provide an effective strategy for the management of obesity.

Key words: obesity, microRNA, probiotics

https://doi.org/10.5937/arhfarm74-50645

Introduction

Over the past four decades, the prevalence of obesity has increased dramatically worldwide, resulting in obesity being a major contributor to the global burden of chronic disease and disability in all age groups. The World Health Organization (WHO) defines obesity as an abnormal or excessive accumulation of fat, expressed as a body mass index (BMI) of more than 30 kg/m2, which has a negative effect on all physiological functions of the body. According to the WHO report from 2022, around 60% of the European population are overweight or obese, highlighting the impact of the obesity pandemic (1).

Obesity is a complex condition with serious pathophysiological, social and psychological consequences that affects all age and socioeconomic groups. The increasing prevalence of obesity seems to be the result of the interaction of certain factors, such as environmental, behavioural, genetic, and metabolic ones. Present data suggest that the environment and behaviour play a crucial role in obesity reaching epidemic proportions (2). In recent decades, the gastrointestinal microbiota (GI microbiota) has gained significant attention, as it has a significant impact on the beginning and development of obesity and related diseases. The GI microbiota represents a complex ecosystem of bacterial cells that inhabit the gastrointestinal tract (GIT) of the host and contribute to its health through their structural function (regulating the growth and differentiation of GIT cells, regulating the permeability of tight junctions). The cells also have a metabolic function (production of vitamins, short-chain fatty acids (SCFA) production), a protective function (lowering of pH by SCFA synthesis, competition with pathogens for nutrients and binding sites) and an endocrine function (synthesis of hormones and neurotransmitters) (3).

In contrast to healthy people, a disturbed balance in the gut microbiota composition, modified metabolic activity and the distribution of intestinal bacteria (dysbiosis) are observed in obese people, which in turn could be one of the causes for an increase in the body mass index (BMI) and thus for the development of obesity (4, 5). As a result of dysbiosis, there is a change in the production of various metabolites (SCFA, indole derivatives, trimethylamine) by the microbiota, leading to a disruption of homeostasis in a direction favorable for the emergence of an inflammatory response, oxidative stress and metabolic dysfunction of the host (6). Studies investigating the association between obesity and composition of the gut microbiota showed that, compared to normal-weight individuals, an increased number of bacteria of the Firmicutes strain was present, while the Bacteroidetes strain was reduced (increased F:B ratio). It is hypothesized that this composition of the GI microbiota utilizes energy from food more efficiently, providing a possible link between dysbiosis and obesity (7). Namely, the GI microbiota contributes to the increased utilization of energy from food through the production of SCFA, as a consequence of the hydrolysis and fermentation of dietary polysaccharides in the colon and cecum. Studies in animals and humans showed higher SCFA production in the obese phenotype, compared with the normal body weight phenotype (8). A decrease in body

weight leads to a change in the GI microbiota towards an increase in Bacteroidetes and a decrease in Firmicutes bacteria (decreased F:B ratio) (9).

In recent years, literature data have supported the idea that a new approach in the treatment of obesity could be aimed at manipulating the composition of the microbiota or correcting dysbiosis, i.e. using probiotics as a safe and effective dietary intervention in the treatment of obesity (5). Numerous mechanisms have been proposed by which probiotics may have beneficial effects in obesity, e.g. regulating food intake, absorption of nutrients, achieving intestinal homeostasis, etc. (10). Recently, however, efforts have been made to identify the exact molecular mechanisms underlying the beneficial effects of probiotics in obesity. Indeed, the results of various studies suggest that epigenetic influences may be mediators of the interaction between host, microbiome and probiotics. MicroRNAs (miRNAs), short non-coding RNA molecules, have been identified as important molecular mediators in the interaction between host, microbiome and probiotics. Some of the proposed molecular mechanisms by which probiotics exert their effect on the host are epigenetic DNA methylation and activation of sirtuins, particularly SIRT1 (11, 12).

Probiotics and obesity

Although changes in lifestyle remain the primary strategy in the treatment of obesity and accompanying comorbidities, previous studies support the idea that one of the targets of precise nutrition in the treatment of obesity should be the microbiota, implying the importance of probiotics' use in order to treat the dysbiosis characteristic of obese people (13). Precision nutrition is defined as "a methodology to integrate genetic, metabolic and environmental information at scale, which can utilize high-throughput metabolomics, metagenomic and epigenetic approaches" (14). In addition to personalized nutritional recommendations based on microbiota analyses, personalized administration of probiotics is also a possible approach. According to the consensus adopted in 2011 by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (15). The exact mechanism by which probiotics may prevent obesity or have a role in the obesity treatment is not yet fully understood, but altering the composition of the gut microbiota to achieve eubiosis is thought to be one of the key mechanisms of action (Figure 1). The use of certain probiotic strains, such as certain *Lactobacillus spp* and *Saccharomyces boulardii* in the gut can lead to an increased number of *Bacteroidetes*, and a decreased number of *Firmicutes* phylum bacteria (16). Given that the intestinal epithelial barrier is disrupted in obese people (17), it is important to mention that the intake of probiotics leads to the establishment of eubiosis, which is necessary to sustain the intestinal barrier integrity and mucus density (18). In addition, probiotics, by strengthening intercellular "tight junctions" and promoting mucus secretion, strengthen the intestinal mucosal barrier. Literature data suggest that the intake of certain probiotic strains can lead to a reduction in the BMI, body fat mass

and waist circumference. This is attributed to their ability to reduce lipid absorption (by hydrolyzing bile salts), reduce leptin synthesis, stimulate brown adipose tissue thermogenesis, increase fasting-induced adipocyte factor (FIAF) activity, alter the activity in the appetite centres (synthesis of butyrate, which increases the feeling of satiety or regulates hormone production in the gastrointestinal tract, which is responsible for the development of a feeling of satiety) (19-22). The beneficial effects of probiotic microorganisms can also be explained by their immunomodulatory effects (20). Additionally, probiotics may have effects on epigenetic markers associated with obesity. Namely, recently probiotics have been discussed with regard to whether they have any effects on miRNAs disturbed in obesity and obesity-associated comorbidities. For example, Wang et al. reported that *Lactiplantibacillus plantarum 299v* showed a beneficial effect on intestinal function by changing cytokine levels through miRNA expression (23). Therefore, the aim of the present narrative review was to summarize the available data of probiotics effect on miRNA expression in obese individuals (Figure 2).

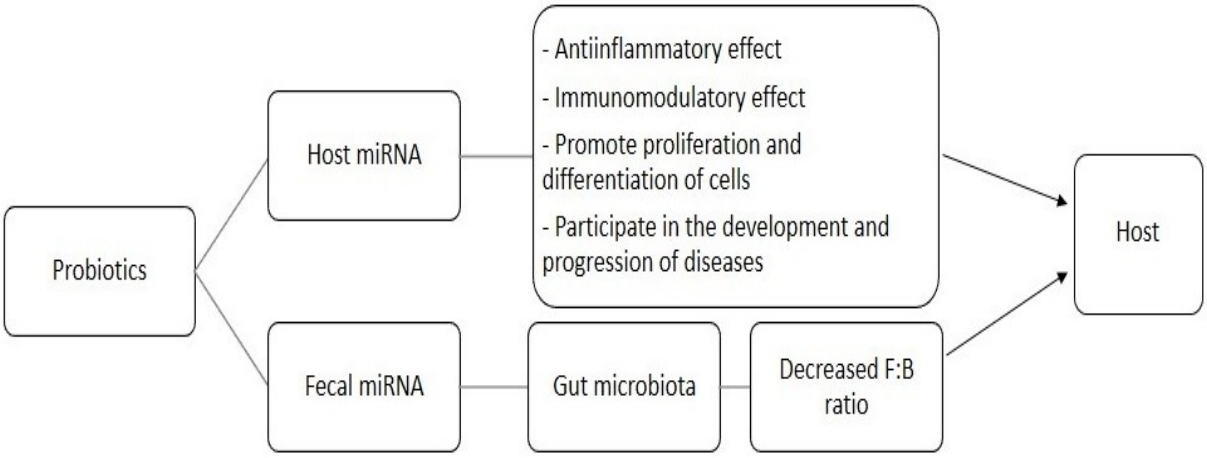

Figure 1. Potential miRNA-based molecular mechanism of probiotics action (according to (24))

Slika 1. Mogući molekularni mehanizam delovanja probiotika zasnovan na miRNK (prema (24))

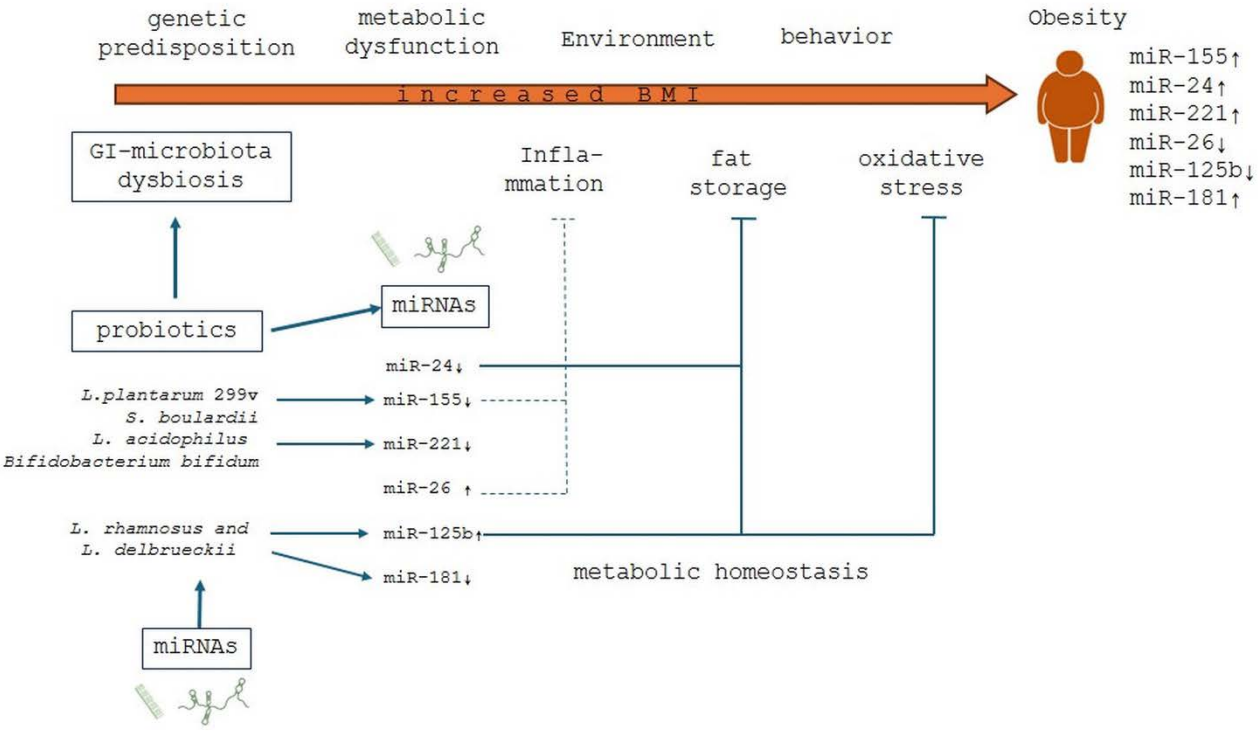

Figure 2. Probiotic's effect on miRNAs expression in obese individuals
Slika 2. Efekat probiotika na ekspresiju miRNK kod gojaznih osoba

MicroRNAs biogenesis and rolls, linkage with obesity and probiotic usage

Recent studies have proposed miRNAs as a possible molecular mechanism of probiotics action (24). miRNAs are short, non-coding RNAs with 19 to 25 nucleotide bases, which are thought to play an important role in host gene expression processes and a functional role in shaping the gut microbiota (24).

MicroRNAs biogenesis: Primary transcripts of miRNA (pri-miRNA) are transcribed from genomic DNA by RNA polymerase II, and have hairpin structure (25, 26). In the nucleus, pri-miRNA is processed by RNase III-type endonuclease Drosha-DGCR8 to a miRNA precursor (pre-miRNA). After their biogenesis in the nucleus, pre-miRNAs are transported into the cytoplasm by exportin-5 and processed by by RNase III Dicer into mature miRNAs duplex. One mature miRNA is degraded, while the other one loads to an Argonaute 2 protein, forming a miRNA-induced silencing complex (miRISC). Via miRISC, depending on the “seed sequence” placed in 2-8 nucleotide positions of a miRNA, miRNAs most often bind to the 3’-untranslated region (3' UTR) of the messenger RNA (mRNA). In most cases, the interaction between miRNA-mRNA results in translational inhibition and/or mRNA degradation (27, 26). It has been known that, besides 3’ UTR, miRNAs could

bind to other mRNA regions, such as promoter region with an inducing effect on transcription, or coding sequence with a silencing effect on gene expression (27), while binding to 5' UTR could cause the activation or repression of translation (28). Thus, miRNAs are considered essential elements of the epigenetic machinery of the cell, which contributes to the regulation of many biological processes.

By controlling metabolic pathways, miRNAs are also important for the regulation of metabolic and energy homeostasis (29). Circulating miRNAs can be secreted by various tissues, including adipose tissue, and affect the metabolic profiles of distant organs, facilitating metabolic organ crosstalk (30). Namely, the results of recent studies have shown that miRNAs play a key role in numerous physiological processes, including cell proliferation, apoptosis, tissue differentiation, immune system development and immune responses, etc., and that there is a correlation between miRNA expression and various metabolic parameters (BMI, adipogenesis, glycemia, leptinemia). Certain miRNAs are involved in the regulation of adipose tissue development and metabolism, as well as in the secretion and action of insulin, which is why it is thought that their imbalance could play a role in the development of obesity and its associated metabolic complications (31). Indeed, literature data indicate that there is a significant difference in the expression of certain miRNAs between obese and non-obese omental fat and that their expression in the blood of obese individuals correlates significantly with glycosylated hemoglobin, leptin, body mass index and blood glucose levels (32). In addition, the profile of circulating miRNAs in individuals with obesity and metabolic disease differs from that of lean individuals (30). For all these reasons, there is speculation about the possible role of miRNA as biomarkers for the diagnosis or prognosis of various metabolic diseases, as well as promising new therapeutic targets for the treatment of obesity and related diseases (30, 31).

Many studies and a large amount of data show that miRNA expression can be directly regulated by diet, lifestyle habits, and various nutritional interventions (26, 33). Moreover, there is evidence that miRNAs are not only synthesized endogenously, but can also be ingested via food, and that food components alter the expression of endogenous miRNA genes. In addition, diet also alters the expression of endogenous miRNA genes, further reinforcing the effects of diet-miRNA interactions in gene regulation. For example, dietary components and certain dietary patterns can modulate miRNA profiles in serum, which in turn influences biological processes. In addition, a number of studies have reported that endogenously synthesized miRNAs can influence the composition of the gut microbiota by modulating the expression of genes that influence microbial growth. Conversely, the gut microbiota can regulate the miRNA expression of host cells, such as intestinal epithelial cells, whereby the bidirectional interaction between host miRNAs and gut microbiota may exert an influence on intestinal permeability. In addition, dietary plant miRNAs may be involved in creating different microenvironments in the gut to which microorganisms adapt to optimize their resources and thrive (34, 35).

The modulation of the gut microbiota by miRNAs is still an under-researched area. Understanding the communication between the host, miRNAs and microbiota is crucial

for the development of the field of miRNA-based therapies (36). For example, a recent study identified a synthetic miRNA that can specifically modulate the microbiome and alleviate inflammatory autoimmune diseases (37).

Table I Effects of probiotic intervention on miRNA expression

Tabela I Efekti upotrebe probiotika na ekspresiju miRNK

Dietary intervention	miRNA	Effect
Bifidobacterium bifidum and *Lactobacillus acidophilus*	miR-155	downregulated
	miR-221	downregulated
	miR-26b	upregulated
S. boulardii, L. plantarum 299v, and octacosanol	miR-155	downregulated
	miR-24-3p	downregulated
L. delbrueckii and *L. rhamnosus*	miR-125a	upregulated
	miR-181a	downregulated

miRNA-155

Adipose tissue, considered to be an endocrine organ where triglycerides are stored, is also considered to have a very important role in the regulation of metabolism, energy homeostasis, and inflammatory processes (38). An excessive amount of white adipose tissue present in obesity causes adipocyte dysfunction and consequently inflammatory responses and metabolic disorders. In obese individuals, adipose tissue production of anti-inflammatory adipokines is reduced, while the production of adipokines with pro-inflammatory action is elevated, causing the state of chronic inflammation (39). Moreover, these adipocytes increase the production of chemokines and cause monocytes migration to adipose tissue and their maturation into pro-inflammatory macrophages (40). Thus, low-grade chronic inflammation very often accompanies obesity, with macrophages in adipose tissue playing a crucial role in the synthesis of certain pro-inflammatory miRNAs and pro-inflammatory cytokines (41). Levels of miR-155 are dysregulated in certain diseases associated with obesity, such as atherosclerosis, metabolic syndrome, and type 2 diabetes (T2D) (42-44). Karkeni et al. showed an overexpression of miR-155 in the subcutaneous adipose tissue of obese patients compared to lean controls (44). They noticed that TNF-α, via nuclear factor-κB (NF- κB), leads to the upregulation of miR-155 expression in adipose tissue. Furthermore, Trygesstad et al. demonstrated increased miR-155 expression in obese individuals compared to the normal-weight group in isolated adipose tissue cells and blood samples (41). The mechanism by which miR-155 affects atherosclerosis, inflammation, obesity and diabetes-related comorbidities remains unclear and controversial. According to *in vivo*,

in vitro and bioinformatics studies, SIRT1 has been recognized as a target gene of miR-155 action (45-47). SIRT1 has an important role in the regulation of acetylation of histones and transcription factors, such as PPAR-γ (48). Moreover, SIRT1 exerts anti-inflammatory effects by NF- κB-pathway inhibition (48). Wang et al. showed that miR-155 negatively regulates SIRT1 expression, thus promoting inflammation in the podocytes of diabetic mice (47). In addition, by stimulating monocyte recruitment and by inducing endothelial cells activation, miR-155 shows a pro-atherogenic effect (49). Increased miR-155 levels in subcutaneous adipose tissue, especially from adipose tissue macrophages, affect adipocyte metabolism by regulating *PPARG* and *GLUT4* expression, thus decreasing PPAR- γ and GLUT4 protein abundance (41).

Given the growing evidence that probiotics can modulate microbial effects on the inflammatory response by influencing miRNA expression, it has been interesting and challenging to investigate the probiotic effect on their expression. Algeri et al. investigated the effect of different probiotics on the expression of miR-155 in dinitrobenzenesulfonic acid (DNBS)-induced colitis in mice and showed that all probiotics studied exerted an anti-inflammatory effect by decreasing the expression of miR-155, which was upregulated in these mice (50). In another study investigating the connection between the occurrence of colorectal cancer and disturbed gut microbiota, it was found that some probiotic strains (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) were able to downregulate miR-155 expression and its target gene *Kirsten rat sarcoma virus* (*KRAS*) expression, indicating a significant impact of probiotics on this oncogenic miRNA and its target gene (51). A 12-week supplementation with the formulation containing octacosanol, *S. boulardii, and L. plantarum 299v* downregulated miR-155-5p expression when compared to the control group, indicating that this formulation improves the inflammatory status in obese individuals (52).

miRNA-24

Another miRNA with an important role in adipose tissue functions is miRNA-24 (53, 54). This miRNA is highly expressed in the liver and plays an important role in adipocyte functions such as insulin sensitivity and lipolysis. According to the literature, miR-24 promotes lipid accumulation in the liver and hyperlipidemia by suppressing *insulin-induced gene 1* (*Insig1*), an inhibitor of lipogenesis. Therefore, miR-24 has been identified as a target miRNA for the treatment of non-alcoholic fatty liver disease (NAFLD) and atherosclerosis (53). Jeon et al. confirmed lower levels of INSIG1 and higher levels of miR-24 in NAFLD and patients with steatohepatitis, suggesting that an interplay between these two may play a crucial role in the control of lipid homeostasis in metabolic diseases (55). In addition, miR-24 levels are elevated in abdominal adipose tissue patients with T2D obesity, and positively correlated with transcripts of secreted frizzled protein 4 (SFRP4), an antiangiogenic factor that may lead to insulin resistance and inflammation in obese individuals (54). Garavelli et al. found that circulating plasma miR-23~27~24 levels were remarkably upregulated in children with progressive type 1 diabetes (T1D), with miR-24-3p levels in children with T1D over 12 months being twice

as high as in participants with recent onset of the disease (56). Furthermore, miR-24-3p expression is elevated in children with obesity (57). Animal and human studies also reported that the levels of miR-24-3p are elevated in subjects with hypercholesterolemia (58). Thus, it is important to notice that there are some promising results suggesting that probiotics can lower miR-24-3p expression in overweight/obese individuals after a 12-week intervention with the probiotic formulation containing a combination of *S. boulardii, L. plantarum 299v*, and octacosanol (52).

miRNA-221

MiRNA-221 has been recognized as an obesity and adipogenesis-related microRNA (59). More precisely, miR-221 positively correlates with insulin, HbA1c and fasting glucose levels in obese individuals. This miRNA also downregulates *ADIPOR1* expression, which could explain its involvement in the onset of insulin resistance. Animal studies have shown that miR-221 is highly expressed in obese adipocytes (60). Interestingly, they have noticed that miR-221 decreases during the process of adipogenesis. Decreased miR-221 expression shows a significant connection with elevated expression of *TNF* (59). This was confirmed by a study in which TNF-α treatment led to decreased miR-221 expression in the adipose tissue-mesenchymal stem cells of obese women (61). Moreover, Meerson et al. have shown a positive correlation between miR-221 levels and BMI and fasting insulin levels in obese humans, suggesting that miR-221 may contribute to obesity-related insulin resistance (62). Consumption of *Bifidobacterium bifidum* and *L. acidophilus* significantly decreased the expression of miR-221 in hepatocellular carcinoma mice (63). Although the expression of miR-221 was decreased, probiotics consumption did not up-regulate PTEN, which is considered to be a tumor suppressor and a potential target of miR-221. In addition, Saffar et al. found that *S. cerevisiae* and *L. acidophilus* suppress the proliferation of colorectal cancer cells and can be used to prevent colorectal cancer (64). To date, there have been no studies investigating the effect of probiotics on miR-221 in obese individuals.

miRNA-26

The miR-26 family (miR-26a-1, miR-26a-2 and miR-26b) plays an important role in adipocyte development and physiological processes such as hepatic glucose and lipid metabolism (65-68). In addition, miR-26a is required for myogenesis, while both miR-26a/b are important in osteogenesis (68). Expression of miR-26b is decreased in visceral adipose tissue in obesity, and it is thought that this contributes to obesity-related insulin resistance via disruption of the PTEN/PI3K/AKT signaling pathway (69). Via inhibition of target gene PTEN, miR-26b modulates the activation of AKT and elevates insulin sensitivity via the PTEN/PI3K/AKT pathway. These processes are disturbed in obesity due to the down-regulated expression of this miRNA. Moreover, decreased levels of miR-26b lead to the inhibition of GLUT4 translocation, stimulated by insulin, which consequently leads to a reduced uptake of glucose in adipocytes and the development of insulin resistance in obese individuals. In addition, reduced levels of hepatic miR-26a have been found in obese mice and overweight humans (70). In a recent *in vivo* study,

loss of the miR-26 miRNA family was found to lead to an increase in adipose tissue, demonstrating its role in adipogenesis (71). The results of this study show that adipocyte progenitor cells are an important site for the action of the miR-26 family in regulating fat metabolism, i.e. that increased levels of miR-26 in adipocyte progenitor cells blocked high-fat diet-induced weight gain, adipocyte proliferation, hyperglycaemia, and hyperlipidaemia (71). It has been found that TNF-α, along with other proinflammatory proteins, downregulate the expression of hsa-miR-26b in adipocytes, suggesting that miR-26b may be an important mediator in regulating inflammation and obesity-related insulin sensitivity (72). According to the study conducted by Heydari et al., the consumption of probiotics (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) increased the expression of miR-26b in tissue and plasma samples of mice with colon cancer (51), while clinical studies did not show a statistically significant effect of probiotics on miR-26b-5p expression (52).

miRNA-125

miR-125b-5p also has an important role in adipogenesis. According to the literature, its expression is decreased in obese subjects (73). miR-125b-5p inhibits matrix metalloproteinase11 (MMP11), an enzyme that negatively regulates adipogenesis (74). Furthermore, miR-125b-5p reduces triglyceride accumulation in adipocytes. Additionally, by targeting the mRNA of the Stearoyl-CoA Desaturase 1 (*SCD-1*) gene, miR-125b affects the synthesis of triglycerides (73). This miRNA is highly expressed in preadipocytes and has a protective function against oxidative stress (75). Some studies also suggest that miR-125-b might be involved in insulin resistance development, as reduced circulating miR-125b levels were found in T2D patients (76). In addition, weight loss by bariatric surgery led to a downregulation of miR-125b in morbidly obese patients (77). Moreover, decreased levels of miR-125a-5p have been found in T2D patients, causing hyperglycemia and hyperlipidemia (78). Signal transducer and activator of transcription 3 (STAT3), an important regulator of many cell processes, has been reported as the target gene of miR-125a-5p (78). The expression of miR-125a-5p is negatively correlated with STAT3 expression in T2D mice, and elevated STAT3 levels are considered to be connected with the development of insulin resistance in T2D. Therefore, miR-125a-5p is very important in glucose and lipid metabolism due to its involvement in hepatic glycogen synthesis, gluconeogenesis, and lipogenesis. An *in vitro* study using peripheral blood mononuclear cells from patients diagnosed with lupus treated with *L. delbrueckii* and *L. rhamnosus* resulted in the upregulation of miR-125a expression (79). Although this study does not refer to obese patients, the observed effect of probiotics on the immune response is very important, as the immune response is also impaired in obese people.

miRNA-181

The miR-181 family is a very important miRNA family for the control of metabolism and inflammatory pathways (80). Although the mechanism of this miRNA's action still remains unclear, it has been considered that a dysregulated miR-181-PTEN

axis could be involved in the development of metabolic syndrome (80). Moreover, miR-181 affects the immune response via regulation of T and B-cell differentiation (81), and its expression is positively correlated with inflammatory diseases. Virtue et al. found the miR-181 family expression significantly elevated in the white adipose tissue of obese mice and humans, and concluded that a disturbed microbiota-miR-181 axis plays an important role in obesity development (82). An *in vitro* study showed that supplementation with *L. delbrueckii* and *L. rhamnosus* downregulates the expression of miR-181a in PBMCs isolated from systemic lupus erythematosus patients, suggesting that probiotics could modulate the immune response via this miRNA (81). As far as we know, there have been no studies investigating the effect of probiotics on this microRNA in obese subjects.

Conclusions

Obesity is a widespread disease in modern society, and it is very often associated with various diseases such as T2D, heart disease, and dyslipidemia. Although lifestyle intervention is essential for obesity treatment, it is necessary to understand epigenetic traits in the onset of obesity to make new strategies for the management of obesity. Probiotics, besides their beneficial effects on dysbiosis, have a beneficial effect on the expression of obesity-related microRNAs. Thus, we find probiotics to be useful as adjuvant therapy for obesity treatment.

Acknowledgements

This research was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia through two Grant Agreements with University of Belgrade-Faculty of Pharmacy No 451-03-65/2024-03/ 200161 and No 451-03-66/2024-03/ 200161.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.“

Author contributions

Nina Okuka: Investigation, Writing - original draft; Berit Hippe: Visualization, Data curation; Brižita Đorđević: Writing - review & editing; Nevena Ivanovic: Conceptualization, Supervision, Writing - review & editing;

References

1. WHO European regional obesity report 2022: World Health Organization. Regional Office for Europe; 2022.
2. Haththotuwa RN, Wijeyaratne CN, Senarath U. Worldwide epidemic of obesity. In: Mahmood TA, Arulkumaran S, Chervenak FA, editors. Obesity and obstetrics. Elsevier; 2020; p. 3-8.
3. Michaličkova D. Uticaj suplementacije sojem Lactobacillus helveticus L10 na markere imunskog i oksidativnog statusa vrhunskih sportista [dissertation]. [Beograd]: Univerzitet u Beogradu; 2017.
4. DeGruttola AK, Low D, Mizoguchi A, Mizoguchi E. Current understanding of dysbiosis in disease in human and animal models. Inflamm Bowel Dis. 2016;22(5):1137-50.
5. Geng J, Ni Q, Sun W, Li L, Feng X. The links between gut microbiota and obesity and obesity related diseases. Biomed Pharmacother. 2022;147:112678.
6. Graham C, Mullen A, Whelan K. Obesity and the gastrointestinal microbiota: a review of associations and mechanisms. Nutr Rev. 2015;73(6):376-85.
7. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027-31.
8. Blaut M. Gut microbiota and energy balance: role in obesity. Proc Nutr Soc. 2015;74(3):227-34.
9. Amabebe E, Robert FO, Agbalalah T, Orubu ES. Microbial dysbiosis-induced obesity: role of gut microbiota in homoeostasis of energy metabolism. Br J Nutr. 2020;123(10):1127-37.
10. Cerdó T, García-Santos JA, G. Bermúdez M, Campoy C. The role of probiotics and prebiotics in the prevention and treatment of obesity. Nutrients. 2019;11(3):635.
11. Vähämiko S, Laiho A, Lund R, Isolauri E, Salminen S, Laitinen K. The impact of probiotic supplementation during pregnancy on DNA methylation of obesity-related genes in mothers and their children. Eur J Nutr. 2019;58:367-77.
12. Liu T, Song X, An Y, Wu X, Zhang W, Li J, et al. Lactobacillus rhamnosus GG colonization in early life ameliorates inflammaging of offspring by activating SIRT1/AMPK/PGC-1α pathway. Oxid Med Cell Longev. 2021;2021:3328505.
13. Cani PD, Van Hul M. Novel opportunities for next-generation probiotics targeting metabolic syndrome. Curr Op Biotechnol. 2015;32:21-7.
14. Livingstone KM, Ramos-Lopez O, Perusse L, Kato H, Ordovas JM, Martínez JA. Precision nutrition: A review of current approaches and future endeavors. Trends Food Sci Technol. 2022;128:253-264.
15. Morelli L, Capurso L. FAO/WHO guidelines on probiotics: 10 years later. J Clin Gastroenterol. 2012;46:S1-S2.
16. Stojanov S, Berlec A, Štrukelj B. The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. Microorganisms. 2020;8(11):1715.
17. Portincasa P, Bonfrate L, Khalil M, Angelis MD, Calabrese FM, D'amato M, et al. Intestinal barrier and permeability in health, obesity and NAFLD. Biomedicines. 2021;10(1):83.
18. Wiciński M, Gębalski J, Gołębiewski J, Malinowski B. Probiotics for the treatment of overweight and obesity in humans—a review of clinical trials. Microorganisms. 2020;8(8):1148.
19. Arora T, Singh S, Sharma RK. Probiotics: interaction with gut microbiome and antiobesity potential. Nutrition. 2013;29(4):591-6.
20. Garcia-Gonzalez N, Battista N, Prete R, Corsetti A. Health-promoting role of Lactiplantibacillus plantarum isolated from fermented foods. Microorganisms. 2021;9(2):349.

21. Mazloom Z, Yousefinejad A, Dabbaghmanesh MH. Effect of probiotics on lipid profile, glycemic control, insulin action, oxidative stress, and inflammatory markers in patients with type 2 diabetes: a clinical trial. Iran J Med Sci. 2013;38(1):38.
22. Rouxinol-Dias AL, Pinto AR, Janeiro C, Rodrigues D, Moreira M, Dias J, et al. Probiotics for the control of obesity–Its effect on weight change. Porto Biomed J. 2016;1(1):12-24.
23. Wang Q, Sun Q, Wang J, Qiu X, Qi R, Huang J. Lactobacillus plantarum 299v changes miRNA expression in the intestines of piglets and leads to downregulation of LITAF by regulating ssc-miR-450a. Probiotics Antimicrob Proteins. 2021;13(4):1093-105.
24. Zhao Y, Zeng Y, Zeng D, Wang H, Zhou M, Sun N, et al. Probiotics and MicroRNA: their roles in the host–microbe interactions. Front Microbiol. 2021;11:604462.
25. Ibarra PE, García-Solís P, Solís-Sáinz JC, Cruz-Hernández A. Expression of miRNA in obesity and insulin resistance: a review. Endokrynol Pol. 2021;72(1):73-80.
26. Iacomino G, Siani A. Role of microRNAs in obesity and obesity-related diseases. Genes Nutr. 2017;12:1-16.
27. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol. 2018;9:388354.
28. Sacco LD, Masotti A. Recent insights and novel bioinformatics tools to understand the role of microRNAs binding to 5'untranslated region. Int J Mol Sci. 2013;14(1):480-95.
29. Dumortier O, Hinault C, Van Obberghen E. MicroRNAs and metabolism crosstalk in energy homeostasis. Cell Metab. 2013;18(3):312-24.
30. Ji C, Guo X. The clinical potential of circulating microRNAs in obesity. Nat Rev Endocrinol. 2019;15(12):731-43.
31. Landrier J-F, Derghal A, Mounien L. MicroRNAs in obesity and related metabolic disorders. Cells. 2019;8(8):859.
32. Heneghan H, Miller N, McAnena O, O'brien T, Kerin M. Differential miRNA expression in omental adipose tissue and in the circulation of obese patients identifies novel metabolic biomarkers. J Clin Endocrinol Metab. 2011;96(5):E846-E50.
33. Pointner A, Krammer UD, Tomeva E, Magnet U, Hippe B, Jacob U, et al. Lifestyle-Driven Variations in Nutrimiromic MicroRNA Expression Patterns across and beyond Genders. Life. 2024;14(3):390.
34. Díez-Sainz E, Lorente-Cebrián S, Aranaz P, Riezu-Boj JI, Martínez JA, Milagro FI. Potential mechanisms linking food-derived microRNAs, gut microbiota and intestinal barrier functions in the context of nutrition and human health. Front Nutr. 2021;8:586564.
35. Jia M, He J, Bai W, Lin Q, Deng J, Li W, et al. Cross-kingdom regulation by dietary plant miRNAs: an evidence-based review with recent updates. Food Funct. 2021;12(20):9549-62.
36. del Pozo-Acebo L, López de las Hazas MC, Margollés A, Dávalos A, García-Ruiz A. Eating microRNAs: pharmacological opportunities for cross-kingdom regulation and implications in host gene and gut microbiota modulation. Br J Pharmacol. 2021;178(11):2218-45.
37. Liu S, Rezende RM, Moreira TG, Tankou SK, Cox LM, Wu M, et al. Oral administration of miR-30d from feces of MS patients suppresses MS-like symptoms in mice by expanding Akkermansia muciniphila. Cell Host Microbe. 2019;26(6):779-94.e8.

38. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. American J Clin Nutr. 2006;83(2):461S-5S.
39. Martins LM, Oliveira ARS, Cruz KJC, Torres-Leal FL, Marreiro DdN. Obesity, inflammation, and insulin resistance. Braz J Pharm Sci. 2014;50:677-92.
40. Karczewski J, Śledzińska E, Baturo A, Jończyk I, Maleszko A, Samborski P, et al. Obesity and inflammation. Eur Cytokine Netw. 2018;29:83-94.
41. Tryggestad JB, Teague AM, Sparling DP, Jiang S, Chernausek SD. Macrophage-derived microRNA-155 increases in obesity and influences adipocyte metabolism by targeting peroxisome proliferator-activated receptor gamma. Obesity. 2019;27(11):1856-64.
42. Mahdavi R, Ghorbani S, Alipoor B, Panahi G, Khodabandehloo H, Esfahani EN, et al. Decreased serum level of miR-155 is associated with obesity and its related metabolic traits. Clin Lab. 2018;64:77-84.
43. Lopez YON, Garufi G, Seyhan AA. Altered levels of circulating cytokines and microRNAs in lean and obese individuals with prediabetes and type 2 diabetes. Mol Biosyst. 2017;13(1):106-21.
44. Karkeni E, Astier J, Tourniaire F, El Abed M, Romier B, Gouranton E, et al. Obesity-associated inflammation induces microRNA-155 expression in adipocytes and adipose tissue: outcome on adipocyte function. J Clin Endocrinol Metab. 2016;101(4):1615-26.
45. Huang G, Hao F, Hu X. Downregulation of microRNA-155 stimulates sevoflurane-mediated cardioprotection against myocardial ischemia/reperfusion injury by binding to SIRT1 in mice. J Cell Biochem. 2019;120(9):15494-505.
46. Yang N, Cheng H, Mo Q, Zhou X, Xie M. miR-155-5p downregulation inhibits epithelial-to-mesenchymal transition by targeting SIRT1 in human nasal epithelial cells. Mol Med Rep. 2020;22(5):3695-704.
47. Wang X, Gao Y, Yi W, Qiao Y, Hu H, Wang Y, et al. Inhibition of miRNA-155 alleviates high glucose-induced podocyte inflammation by targeting SIRT1 in diabetic mice. J Diabetes Res. 2021;2021:1-11.
48. Zhong Y, Lee K, He JC. SIRT1 is a potential drug target for treatment of diabetic kidney disease. Front Endocrinol. 2018;9:412179.
49. Virtue A, Johnson C, Lopez-Pastraña J, Shao Y, Fu H, Li X, et al. MicroRNA-155 deficiency leads to decreased atherosclerosis, increased white adipose tissue obesity, and non-alcoholic fatty liver disease: a novel mouse model of obesity paradox. J Biol Chem. 2017;292(4):1267-87.
50. Algieri F, Garrido-Mesa J, Vezza T, Rodríguez-Sojo MJ, Rodríguez-Cabezas ME, Olivares M, et al. Intestinal anti-inflammatory effects of probiotics in DNBS-colitis via modulation of gut microbiota and microRNAs. Eur J Nutr. 2021;60:2537-51.
51. Heydari Z, Rahaie M, Alizadeh AM, Agah S, Khalighfard S, Bahmani S. Effects of Lactobacillus acidophilus and Bifidobacterium bifidum probiotics on the expression of microRNAs 135b, 26b, 18a and 155, and their involving genes in mice colon cancer. Probiotics Antimicrob Proteins. 2019;11:1155-62.
52. Okuka N, Schuh V, Krammer U, Polovina S, Sumarac-Dumanovic M, Milinkovic N, et al. Epigenetic Aspects of a New Probiotic Concept—A Pilot Study. Life. 2023;13(9):1912.
53. Ng R, Wu H, Xiao H, Chen X, Willenbring H, Steer CJ, et al. Inhibition of microRNA-24 expression in liver prevents hepatic lipid accumulation and hyperlipidemia. Hepatology. 2014;60(2):554-64.

54. Nunez Lopez YO, Garufi G, Pasarica M, Seyhan AA. Elevated and correlated expressions of miR-24, miR-30d, miR-146a, and SFRP-4 in human abdominal adipose tissue play a role in adiposity and insulin resistance. Int J Endocrinol. 2018;2018:7351902.
55. Jeon TI, Osborne TF. miRNA and cholesterol homeostasis. Biochim Biophys Acta. 2016;1861(12):2041-6.
56. Garavelli S, Bruzzaniti S, Tagliabue E, Di Silvestre D, Prattichizzo F, Mozzillo E, et al. Plasma circulating miR-23~ 27~ 24 clusters correlate with the immunometabolic derangement and predict C-peptide loss in children with type 1 diabetes. Diabetologia. 2020;63:2699-712.
57. Zhang B, Xing L, Wang B. Dysregulation of circulating miR-24-3p in children with obesity and its predictive value for metabolic syndrome. Obes Facts. 2021;14(5):456-62.
58. Ren K, Zhu X, Zheng Z, Mo Z-C, Peng X-S, Zeng Y-Z, et al. MicroRNA-24 aggravates atherosclerosis by inhibiting selective lipid uptake from HDL cholesterol via the post-transcriptional repression of scavenger receptor class B type I. Atherosclerosis. 2018;270:57-67.
59. Arner P, Kulyté A. MicroRNA regulatory networks in human adipose tissue and obesity. Nat Rev Endocrinol. 2015;11(5):276-88.
60. Xie H, Lim B, Lodish HF. MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. Diabetes. 2009;58(5):1050-7.
61. Chou W-W, Wang Y-T, Liao Y-C, Chuang S-C, Wang S-N, Juo S-HH. Decreased microRNA-221 is associated with high levels of TNF-α in human adipose tissue-derived mesenchymal stem cells from obese woman. Cell Physiol Biochem. 2013;32(1):127-37.
62. Meerson A, Traurig M, Ossowski V, Fleming J, Mullins M, Baier L. Human adipose microRNA-221 is upregulated in obesity and affects fat metabolism downstream of leptin and TNF-α. Diabetologia. 2013;56:1971-9.
63. Heydari Z, Rahaie M, Alizadeh AM. Different anti-inflammatory effects of Lactobacillus acidophilus and Bifidobactrum bifidioum in hepatocellular carcinoma cancer mouse through impact on microRNAs and their target genes. J Nutrition Intermed Metab. 2019;16:100096.
64. Saffar KN, Larypoor M, Torbati MB. Analyzing of colorectal cancerrelated genes and microRNAs expression profiles in response to probiotics Lactobacillus acidophilus and Saccharomyces cerevisiae in colon cancer cell lines. Mol Biol Rep. 2024;51(1):122.
65. Benderska N, Dittrich A-L, Knaup S, Rau TT, Neufert C, Wach S, et al. miRNA-26b overexpression in ulcerative colitis-associated carcinogenesis. Inflamm Bowel Dis. 2015;21(9):2039-51.
66. Cristóbal I, Manso R, Gónzález-Alonso P, Madoz-Gúrpide J, Rojo F, García-Foncillas J. Clinical Value of miR-26b Discriminating Ulcerative Colitis–associated Colorectal Cancer in the Subgroup of Patients with Metastatic Disease. Inflamm Bowel Dis. 2015;21(10):E24-E5.
67. Liu H, Chu W, Gong L, Gao X, Wang W. MicroRNA-26b is upregulated in a double transgenic mouse model of Alzheimer's disease and promotes the expression of amyloid-β by targeting insulin-like growth factor 1. Mol Med Rep. 2016;13(3):2809-14.
68. Karbiener M, Pisani DF, Frontini A, Oberreiter LM, Lang E, Vegiopoulos A, et al. MicroRNA-26 family is required for human adipogenesis and drives characteristics of brown adipocytes. Stem Cells. 2014;32(6):1578-90.
69. Xu G, Ji C, Song G, Zhao C, Shi C, Song L, et al. MiR-26b modulates insulin sensitivity in adipocytes by interrupting the PTEN/PI3K/AKT pathway. Int J Obes. 2015;39(10):1523-30.

70. Fu X, Dong B, Tian Y, Lefebvre P, Meng Z, Wang X, et al. MicroRNA-26a regulates insulin sensitivity and metabolism of glucose and lipids. J Clin Invest. 2015;125(6):2497-509.
71. Acharya A, Berry DC, Zhang H, Jiang Y, Jones BT, Hammer RE, et al. miR-26 suppresses adipocyte progenitor differentiation and fat production by targeting Fbxl19. Genes Dev. 2019;33(19-20):1367-80.
72. Xu G, Ji C, Shi C, Fu H, Zhu L, Zhu L, et al. Modulation of hsa-miR-26b levels following adipokine stimulation. Mol Biol Rep. 2013;40:3577-82.
73. Ortiz-Dosal A, Rodil-Garcia P, Salazar-Olivo LA. Circulating microRNAs in human obesity: a systematic review. Biomarkers. 2019;24(6):499-509.
74. Rockstroh D, Löffler D, Kiess W, Landgraf K, Körner A. Regulation of human adipogenesis by miR125b-5p. Adipocyte. 2016;5(3):283-97.
75. Brandao BB, Guerra BA, Mori MA. Shortcuts to a functional adipose tissue: The role of small non-coding RNAs. Redox Biol. 2017;12:82-102.
76. Ortega FJ, Mercader JM, Moreno-Navarrete JM, Rovira O, Guerra E, Esteve E, et al. Profiling of circulating microRNAs reveals common microRNAs linked to type 2 diabetes that change with insulin sensitization. Diabetes Care. 2014;37(5):1375-83.
77. Ortega FJ, Mercader JM, Catalan V, Moreno-Navarrete JM, Pueyo N, Sabater M, et al. Targeting the circulating microRNA signature of obesity. Clin Chem. 2013;59(5):781-92.
78. Xu L, Li Y, Yin L, Qi Y, Sun H, Sun P, et al. miR-125a-5p ameliorates hepatic glycolipid metabolism disorder in type 2 diabetes mellitus through targeting of STAT3. Theranostics. 2018;8(20):5593.
79. Vahidi Z, Saghi E, Mahmoudi M, RezaieYazdi Z, Esmaeili S-A, Zemorshidi F, et al. Lactobacillus rhamnosus and Lactobacillus delbrueckii Ameliorate the Expression of miR-125a and miR-146a in Systemic Lupus Erythematosus Patients. Appl Biochem Biotechnol. 2024. doi: 10.1007/s12010-023-04827-w. Epub ahead of print. PMID: 38351428.
80. Williams A, Henao-Mejia J, Harman CC, Flavell RA. miR-181 and metabolic regulation in the immune system. Cold Spring Harb Symp Quant Biol. 2013;78:223-30.
81. Vahidi Z, Samadi M, Mahmoudi M, RezaieYazdi Z, Sahebari M, Tabasi N, et al. Lactobacillus rhamnosus and Lactobacillus delbrueckii ameliorate the expression of miR-155 and miR-181a in SLE patients. J Funct Foods. 2018;48:228-33.
82. Virtue AT, McCright SJ, Wright JM, Jimenez MT, Mowel WK, Kotzin JJ, et al. The gut microbiota regulates white adipose tissue inflammation and obesity via a family of microRNAs. Sci Transl Med. 2019;11(496):eaav1892.

Efekti upotrebe probiotika na mikroRNK povezane sa gojaznošću

Nina Okuka,[1*] Berit Hippe,[2] Brižita Đorđević,[3] Nevena Ivanović[3]

[1]Univerzitet u Banjoj Luci, Medicinski fakultet, Katedra za bromatologiju, 78000 Banja Luka, Bosna i Hercegovina

[2]University of Vienna, Department of Nutritional Science, 1090 Vienna, Austria

[3]Univerzitet u Beogradu – Farmaceutski fakultet, Katedra za bromatologiju, 11000 Beograd, Srbija

*Autor za korespondenciju: Nina Okuka, e-mail: nina.vukicevic@med.unibl.org

Kratak sadržaj

Gojaznost se smatra hroničnom, kompleksnom bolešću koja povećava rizik od nastanka drugih oboljenja poput dijabetesa, kardiovaskularnih oboljenja i određenih karcinoma. Prema literaturnim podacima, kod gojaznih osoba dolazi do narušavanja gastrointestinalne mikrobiote, što nadalje može biti uzrok razvoja gojaznosti i bolesti povezanih sa gojaznošću. Dodatno, postoje male nekodirajuće RNK (mikroRNK) čije su koncentracije promenjene kod gojaznih osoba u poređenju sa osobama normalne telesne mase. Takođe, mikroRNK se smatraju mogućim mehanizmom delovanja probiotika. Određene mikroRNK se smatraju ključnim u procesima razvoja i metabolizma masnog tkiva, kao i u procesima sinteze i delovanja hormona insulina, time ukazujući na to da bi promene u ekspresiji određenih miRNK mogle imati značajan uticaj na nastanak i razvoj gojaznosti i bolesti povezanih sa gojaznošću. Zbog navedenog, miRNK se smatraju mogućim markerima za dijagnozu i praćenje metaboličkih bolesti, ali se razmatraju i kao moguća terapija u tretmanu gojaznosti i oboljenja povezanih sa gojaznošću. U svetlu rastućih potreba sistema zdravstvene zaštite u pogledu dodataka ishrani i nutraceutika koji pružaju efikasnu i bezbednu podršku medicinskoj nutritivnoj terapiji kod gojaznosti, cilj ovog rada bio je da se istraže korisni efekti probiotika na mikroRNK povezane sa gojaznošću. U ovom narativnom pregledu opisali smo ulogu probiotika i mikroRNK u gojaznosti, kao i moguću povezanost probiotika sa mikroRNK koje su izmenjene kod gojaznih ljudi. Profil cirkulišućih miRNK se značajno razlikuje kod gojaznih osoba u poređenju sa osobama normalne telesne mase. Ekspresija miRNK poput miR-155, miR-221, miR-24-3p i miR-181a je značajno povišena, dok je ekspresija miR-26b i miR-125a snižena kod gojaznih osoba u poređenju sa osobama normalne telesne mase. Suplementacija sa *Bifidobacterium bifidum* i *Lactobacillus acidophilus* probiotskim sojevima snižava ekspresiju miR-155 i miR-221, dok je ekspresija miR-26b povišena nakon suplementacije ovim sojevima. Probiotska formulacija koja sadrži kombinaciju *S. boulardii, L. plantarum 299v* i oktakozanola dovela je do smanjenja ekspresije miR-155 i miR-24-3p. Ekspresija miR-125a je

bila povećana, dok je ekspresija miR-181a bila snižena nakon suplementacije sojevima *L. delbrueckii* and *L. rhamnosus.* Nakon pregleda dostupne literature, možemo zaključiti da probiotici imaju pozitivno dejstvo na mikroRNK izmenjene kod gojaznih osoba, te da bi primena probiotika mogla biti efikasna strategija u tretmanu gojaznosti.

Ključne reči: gojaznost, miRNK, probiotici
