

The effects of kumiss supplementation on body weight, food intake, nesfatin-1 and irisin levels in BALB/C mice

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ABSTRACT

The aim of this study was to investigate the plasma and tissue levels of nesfatin-1 and irisin hormones, which were discovered in recent years and are associated with endocrine and metabolic functions, in kumiss-supplemented mice. Sixteen BALB/C male mice were divided into two groups as control and kumiss groups. During the experiment, the kumiss was added to the drinking water of mice at a ratio of 1:1 to obtain a daily 2×10^8 cfu/mL bacterial colony, and was given once a day orally for 20 weeks. The weights and food intake of the animals were monitored during the experiment. The nesfatin-1 and irisin levels in plasma and tissue samples were determined using ELISA kits. Kumiss supplementation reduced the live weight for 2-12 weeks ($P < 0.05$). However, no significant difference was observed after the 12th week. The feed consumption of the kumiss group was lower at the beginning and the 10th week, and at the end, compared to the control group ($P < 0.05$). The plasma levels of nesfatin-1 and irisin ($P < 0.001$) decreased while the liver levels increased ($P < 0.05$ and $P < 0.001$, respectively). The results indicate that plasma and liver levels of nesfatin-1 and irisin are regulated by diet and are effective in weight loss and food intake.

Key words: kumiss (koumiss); weight loss; food intake; irisin; nesfatin-1

Introduction

Kumiss (also known as kumiss, kumis, kymis, kymmyz) is a traditional fermented drink made from mare's milk, which is commonly preferred in Central Asia. Mare's milk is important for nutrition, with a high amount of polyunsaturated fatty acids, low cholesterol content and different protein structure. The beneficial effects of the kumiss on health are due to the lactic acid bacteria and yeast contents, which are two different types of microorganisms, and their biological activities. Compared to other fermented milk products, such as yogurt and kefir,

kumiss has a higher alcohol and lactic acid content. The consumption of kumiss has been reported to have healthy effects on gastrointestinal, respiratory, immune, circulatory, cardiovascular, nervous and endocrine systems (DANOVA et al., 2005).

Nesfatin-1 was first identified in 2006 as an 82-amino-acid peptide derived from nucleobindin 2 (NUCB2), a 396-amino-acid protein exceptionally conserved across mammalian species (OH-I et al., 2006, FINELLI et al., 2014). It has been reported that nesfatin-1 is closely associated with

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food intake, energy expenditure, body weight reduction, diabetes, obesity, psychiatric disorders and neurogenic diseases (DAI et al., 2013). Irisin is a novel peptide associated with exercise secreted from skeletal muscle. Irisin, first discovered by BOSTROM et al. (2012), is produced from its precursor fibronectin type III domain containing 5 (FNDC5), which is a transmembrane protein. Irisin is believed to be promising in the treatment and monitoring of obesity, diabetes, cardiovascular and central nervous system diseases (MAHGOUB et al., 2018).

Up to our best knowledge and literature survey, there are no previous reports on the relationship between kumiss and weight loss and irisin and nesfatin-1 hormones. Only one recent study reported that an increase in hypothalamic nesfatin-1 inhibits feeding behavior and increases weight loss. In that study, it was stated that feeding behaviors were regulated through MC3/4R-ERK signalling but its mechanism was still unclear (ZHANG et al., 2019). In a study we conducted in 2019, we recently investigated the effects of kumiss on some biomolecules associated with cancer signaling pathways and antioxidant systems under chronic stress (GULMEZ and ATAKISI, 2019). In contrast to the study purpose, a decrease in the weight of the mice was observed. This result inspired the present study. The aim of this study was to investigate the plasma and tissue levels of nesfatin-1 and irisin hormones, which were discovered in recent years and are associated with endocrine and metabolic functions, in kumiss-supplemented mice. This study is also important for determining whether there is a relationship between weight loss, food intake and the tissue distributions and levels of these hormones.

Material and methods

Subjects. In the study, sixteen male Balb-c mice were used, weighing 20-25 g. Prior to the trial, permission was obtained from the Local Ethics Committee of the Animal Experiments of the Kafkas University (KAU-HADYEK / 2018-072). The housing, maintenance and experimental procedures were carried out at the Experimental Animal Unit of Kafkas University. The animals

were maintained in special cages without any contact between the groups, and all groups were fed with ad libitum-balanced diet. They were kept in a 12 hours light and 12 hours dark cycle at constant room temperature (19-21 °C).

Experimental design. The subjects were divided into two groups: (1) control group (n = 8) and (2) experimental group (n = 8). The control group did not receive any special treatment during the experiment and normal feeding conditions were achieved. The kumiss group was given kumiss once a day orally for 20 weeks. The weights and food intake of the animals were monitored during the experiment. At the end of the experiment, the mice were sacrificed under anesthesia (combination of xylazine (15 mg/kg) and ketamine (50 mg/kg)) and the intracardiac blood samples from animals were centrifuged at 3000 rpm and +4 °C for 15 minutes to separate the plasma. The liver, kidney and brain tissues were homogenized using cold lysis buffer containing 10 µg/mL aprotinin (from 10 mg/mL stock in water; stored at -20 °C).

Preparation and application of the kumiss. The kumiss was purchased from the Alaş Kumiss Farm (Izmir, Turkey). Kumiss was used as fresh and kept at 4 °C in the refrigerator. The determination of the total number of specific live microorganisms in kumiss samples was determined according to the method proposed by KANDLER and WEISS (1986). The typical bacterial colony number of each medium was calculated as 2×10^8 cfu/mL and the mice were allowed to take 2×10^8 cfu/mL bacteria colony daily. During the experiment, the kumiss was added to the drinking water of mice at a ratio of 1:1, to obtain a daily 2×10^8 cfu/mL bacterial colony. The physical and chemical properties of the kumiss and feed are collectively presented in Table 1 and 2.

Table 1. Analysis of Kumiss

Analysis	Result of analysis
Ethyl alcohol	1.2% ± 0.07
Ash	0.43%
Total amount of oil	4% ± 0.045
Dry matter content	7.32% ± 0.17
Humidity	92.68% ± 2.13
Density	1.016 g/cm ³ ± 0.004

Table 2. Feed content

Feed content	
Raw protein	18%
Raw cellulose	11.2%
Ash	6.8%
Raw oil	4.5%
Calcium	1%
Phosphorus	0.62%
Lysine	0.98%
Methionine	0.58%
Sodium	0.24%
Energy (kcal /kg)	2650

Results

The live weights (g) of the animals were recorded for 20 weeks (Fig. 1). When live weights were examined, there were statistically significant differences between the control and kumiss groups for 2-12 weeks ($P < 0.05$). Kumiss supplementation reduced the live weight during this process. However, no significant difference was observed after the 12th week.

The mice were fed daily with an ad libitum balanced diet, and feed consumption was monitored for 20 weeks (Fig. 2). The feed consumption of both control and kumiss groups increased over time. The feed consumption in the kumiss group

Biochemical analysis. Plasma and all tissues were stored at $-45\text{ }^{\circ}\text{C}$. The nesfatin-1 and irisin levels in plasma and tissue samples were determined using commercial mouse ELISA kits. The protein concentrations of the tissue samples were analyzed by the Bradford method, using bovine serum albumin (BSA) as the standard, at 595 nm (BRADFORD, 1976).

Statistical analysis. Statistical analyses were performed in triplicate, and average values with standard deviation (mean \pm SD) are reported. The statistical significance was evaluated using the SPSS 16.0 software package (SPSS ver. 16.0 for windows professional edition). A one-way analysis of variance (ANOVA) was performed and was followed by Duncan's test to estimate the significance at the 5% probability level.

was significantly lower at the beginning (0-10th week), the 10th week and at the end (10-20th week) compared to the control group ($P < 0.05$).

The nesfatin-1 and irisin levels in the liver, kidney, brain and plasma samples were investigated (Fig. 3 and 4). The results obtained showed that there was no significant difference in the levels of irisin and nesfatin-1 in the kidney and brain tissues between the groups. However, the plasma levels of nesfatin-1 and irisin decreased ($P < 0.001$) while the liver levels increased ($P < 0.05$ and $P < 0.001$, respectively).

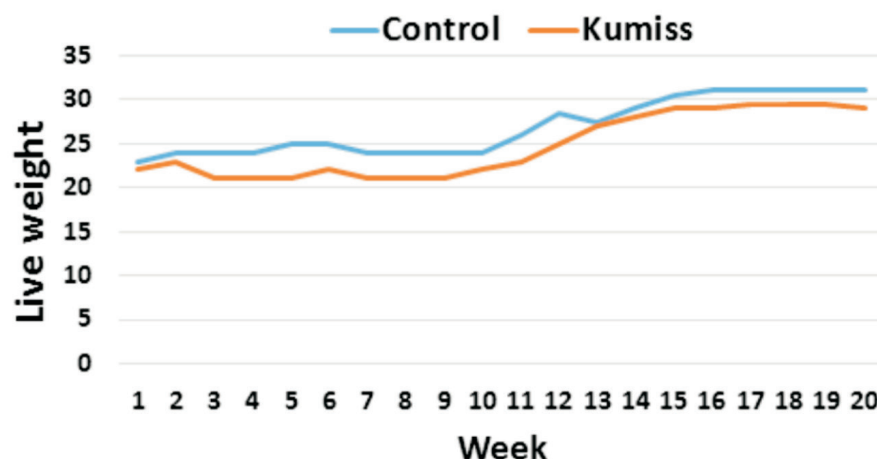


Fig. 1. Live weights (g) of mice. The live weights (g) of the animals were recorded for 20 weeks.

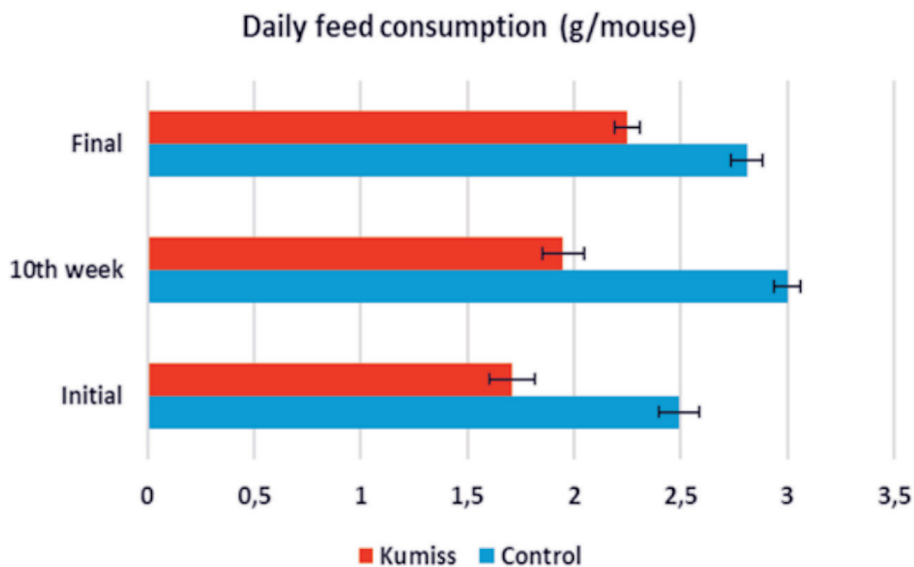


Fig. 2. Feed consumption (g) of mice. The feed consumption of mice was monitored for 20 weeks.

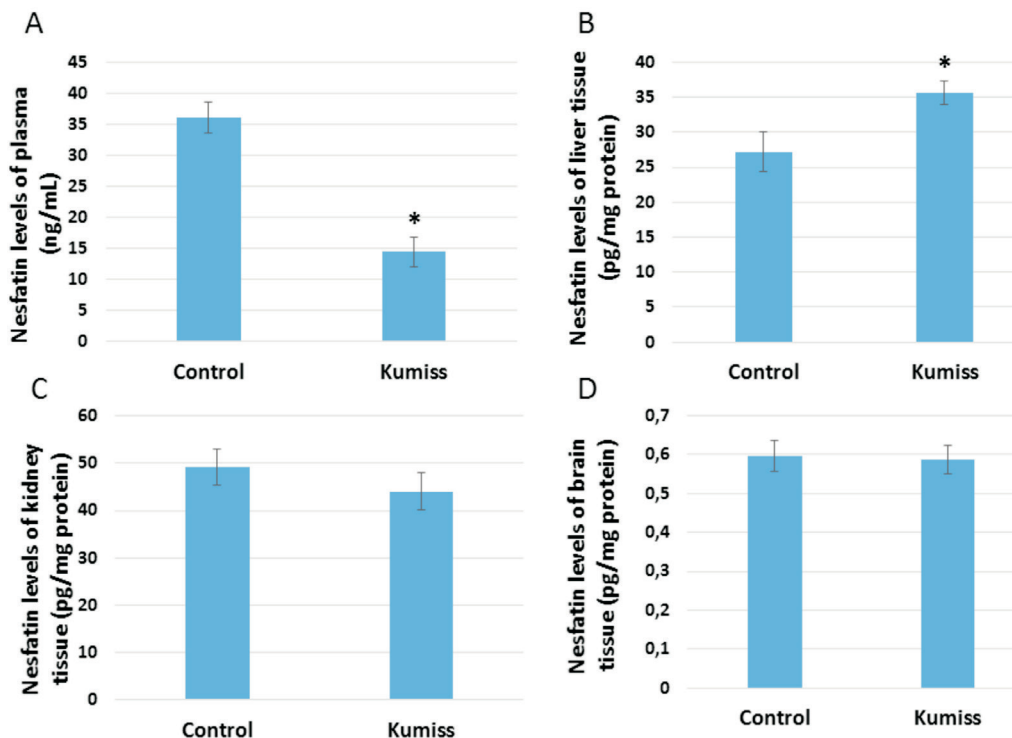


Fig. 3. Biochemical investigation results. Nesfatin-1 levels of plasma (A), liver tissue (B), kidney tissue (C) and brain tissue (D). *Compared to the control group, $P < 0.001$ (A) and $P < 0.05$ (B). The results for kidney and brain tissues are not statistically significant.

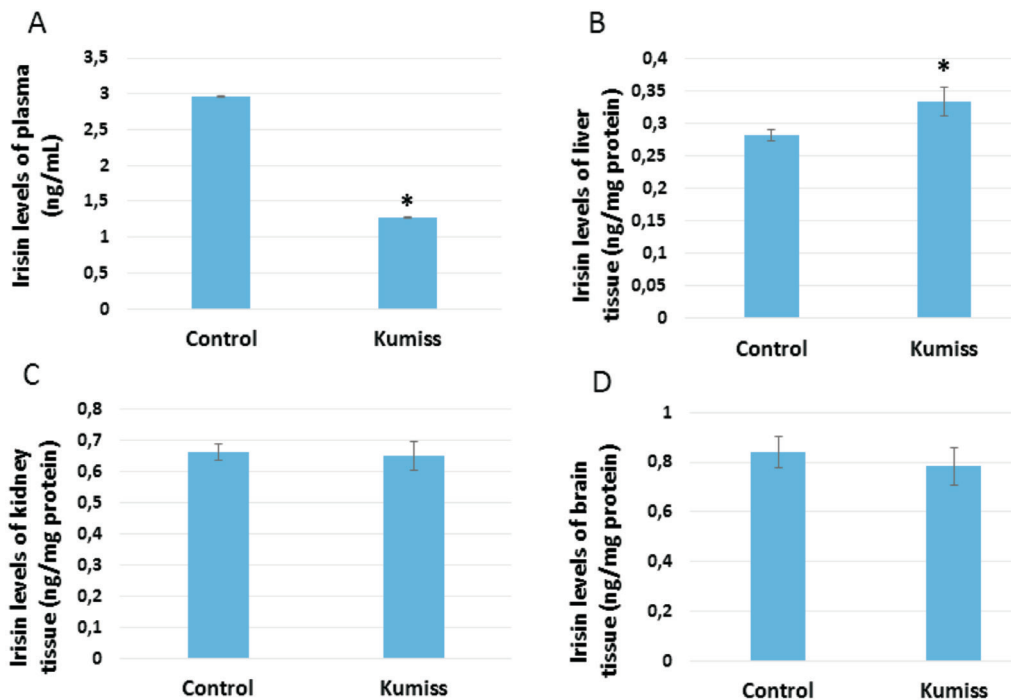


Fig. 4. Biochemical investigation results. Irisin levels of plasma (A), liver tissue (B), kidney tissue (C) and brain tissue (D). * Compared to the control group, $P < 0.001$. The results for kidney and brain tissues are not statistically significant.

Discussion

The aim of this study was to investigate the effects of kumiss on weight loss, food intake, plasma and tissue nesfatin-1 and irisin levels. Probiotics have physiological functions that regulate the intestinal microbiota, and contribute to the health of the host environment. Backslopping fermentation technology has been the main method for manufacturing kumiss. However, the products obtained by this method have some deficiencies in consistency, especially with regard to microbiological diversity and physicochemical and nutritional characteristics (LI et al., 2019). The kumiss microbiota was very rich in *Lactococcus* and *Streptococcus*, particularly *Lactobacillus helveticus* and *Lactobacillus kefirifaciens* (YAO et al., 2017). It is thought that beneficial effects of kumiss are related to the kumiss metabolites as well as the rich bacteria content. The production mechanisms of these metabolites are unclear, but some result directly from the activity of the kumiss microbiota. *Lactobacillus* species produces hexanoic acid (WALSH et al., 2016), pyroglutamic acid (KANJAN and HONGPATTARAKERE, 2016), L-glutamic

acid (ZAREIAN et al., 2012) and pyridoxine (OGURO et al., 2017). Some important dipeptides are also formed in the fermentation process, such as methionylasparagine, prolylasparagine and lysylvaline, but also biotin, 2'-deoxy5'-adenylic acid, S-adenosyl-L-methionine, carnosine and lysophosphatidylinositol (HOU et al., 2019).

In experimental studies, *Lactobacillus* and *Bifidobacterium* bacteria have been reported to have beneficial effects on metabolic syndrome, such as weight loss, fat reduction in the internal organs and glucose tolerance (LE CHATELIER and NIELSEN 2013). In most cases, weight loss occurs in coordination with thermogenic and lipolytic responses by stimulating the sympathetic nervous system (KARIMI et al., 2015). Probiotic strains, *Lactobacillus gasseri* BNR17, have demonstrated the ability to prevent increase in adipocyte tissue, the main source of leptin and adiponectin, and thus limit leptin secretion (KANG et al., 2013). AN et al. (2011) found that body and fat weights were reduced in mice fed a diet supplemented with lactic acid bacteria supplement (*B. pseudocatenulatum* SPM 1204, *B. longum* SPM 1205, and *B. longum* SPM

1207, 10^8 – 10^9 CFU/0.2 mL, daily) for 5 weeks. *Lactobacillus gasseri* BNR17 (final, concentration of 10^9 CFU/0.5 mL, twice a day) administered to female Sprague-Dawley rats for 8 weeks was found to reduce both weight gain (g/day) and final weight (g) compared to the control (KANG et al., 2010). In another study, male C57BL/6 mice were given low (1×10^7 CFU) and high dose (1×10^9 CFU) *Lactobacillus plantarum* LG4 with a high fat (HF) diet for 12 weeks. Both HG+low (16.62 ± 3.71) and HG+high (14.08 ± 6.1) doses of *Lactobacillus plantarum* LG4 groups were reported to reduce weight gain compared to the HG group (22.62 ± 1.24). In addition, when compared with the HG and control groups, it was determined that food intake decreased in *Lactobacillus plantarum* LG4 treated groups (PARK et al., 2014). But in another study, there was no difference in the weights of male C57BL/6 mice treated with *L. acidophilus* NCDC 13 (5×10^7 – 9×10^7 CFU/mL, daily, for 8 weeks) and male-female Sprague Dawley albino rats treated with *Lactobacillus plantarum* LS/07 and *Lactobacillus plantarum* Biocenol LP9 (3×10^9 CFU, daily, for 10 weeks) compared to the control and high fat diet groups (ARORA et al., 2012; SALAJ et al., 2013). In the present study, when the live weight of the animals in the groups was examined, it was observed that the live weights of the kumiss group decreased significantly over 2-12 weeks ($P < 0.05$). When the control and kumiss groups were compared, no significant difference was observed in the live weights after 12 weeks. The effect of kumiss on the decrease in live weight may be an important result of the multifaceted effect of the bacteria or other components available it contains, and its regulation of the whole metabolism.

Nesfatin-1, a hormone described by OH-I et al. (2006), suppresses food intake. This hormone is formed by proteolysis of the nucleobindin 2 protein (NUCB2). Nesfatin-1, also referred to as anorexigenic hormone, is associated with diabetes, obesity, anorexia nervosa, psychiatric disorders and neurogenic diseases, and its levels are mostly found in the central nervous system, as well as in the pancreas, testis, adipose tissue, pituitary gland, and stomach (OH-I et al., 2006; DAI et al., 2013). In one study, it was reported that fasting increases ghrelin

hormone, and decreases nesfatin-1, and nesfatin-1 has anorexigenic and anti-hyperlipidemic effects (UNAL et al., 2018). High levels of nesfatin-1 are found in the hypothalamic nucleus and brainstem areas that are known to be associated with food intake and metabolism regulation. The protein expressions and mRNA of nesfatin-1 in the hypothalamic paraventricular nucleus and supraoptic nucleus was affected by energy changes after acute or chronic malnutrition (OH-I et al., 2006; BECKERS et al., 2009). Intraperitoneal nesfatin-1 injection has been reported to affect the expression of appetite factors to inhibit the food intake of Siberian sturgeon (ZHANG et al., 2018a). In another study by the same author, it was determined that fasting affects NUCB2 levels in the liver and brain. Acute and chronic nesfatin-1 injections have been reported to inhibit food intake and affect the expression of appetite factors in Siberian sturgeon (ZHANG et al., 2018b). It has been founded that an increase in food uptake suppressors (such as cholecystokinin, MSH) increases mRNA expression of NUCB2, and activates nesfatin-1-immunopositive neurons in the hypothalamus and brainstem (GOEBEL et al., 2009). The synthesis and release of nesfatin-1 are altered by relative amounts of nutrients, and this effect varies according to the amount of nutrients, the duration of treatment, the frequency, and the tissue. In the study by MOHAN et al. (2018), a fat diet inhibited nesfatin-1 while it was stimulated by a protein and carbohydrate diet. The conclusion that the nesfatin-1 level is modulated by diet is consistent with the findings of this study. In the study conducted by KUYUMCU et al. (2019) reported that there was a positive relationship between median diet and nesfatin-1 concentration, and diet and nesfatin 1 levels were independent protective variables for chronic arterial disease.

In this study, kumiss influenced plasma and tissue levels of nesfatin-1, as well as food intake, and body weight decreased. It has been determined that plasma levels of nesfatin-1 decreased while the liver levels increased ($P < 0.001$) and the kidney and brain levels did not change. Nesfatin-1 has been shown to be effective in regulating feeding habits, food intake, body weight and glucose balance in rats (RODGERS et al., 2012). Nesfatin-1 is thought

to exert an appetite-regulating effect, independent of many neurotransmitter systems, with other anorexigenic molecules, and especially through leptin or melanocortin (BECKERS et al., 2009). In a study of obese and morbidly obese individuals, a significant relationship was found between body fat percentage and circulating nesfatin-1 levels. High calorie, carbohydrate and protein intake in obese individuals has been associated with lower nesfatin-1 levels (MIRZAEI et al., 2015). FINELLI et al. (2014) reported that the data obtained in basic experiments on nesfatin-1 may be useful for the development of a new anti-obesity treatment.

Nesfatin-1, phoenixin, spexin and kisspeptin have been reported to be novel multifunctional neuropeptides of the brain, and these neuropeptides play a role in regulating nutrient uptake and anxiety responses (PAŁASZ et al., 2018). However, in this study, kumiss supplementation did not lead to a change in brain and kidney nesfatin-1 levels. When the literature was examined, a limited number of studies were found on the connection between food intake and body weight and nesfatin-1 level. We found that weight loss and a reduction in food intake was associated with plasma and tissue nesfatin-1 levels. With the effect of kumiss, nesfatin-1 may influence appetite factors to reduce food intake through various signaling pathways.

Irisin is considered as potential biomarker in metabolic syndrome and obesity, due to its ability to turn white adipose tissue to brown adipose tissue. The increase in the amount of brown fat tissue in the body provides weight control and energy balance protection (CHOI et al. 2013). In different studies, irisin has been reported to have a protective effect on hepatic metabolism by improving glucose uptake glucose tolerance, and hepatic glucose and lipid metabolism (SO and LEUNG, 2016; XIN et al., 2016). CASTILLO-QUAN (2012) was reported to lead to improvements in obesity and glucose homeostasis. Moreover, it was found that irisin increases secretion of glycerol, decreases lipid accumulation, and inhibits hepatic cholesterol synthesis (GAO et al., 2018; TANG et al., 2016). Irisin in mice has been shown to cause obesity-related insulin resistance, and a significant increase in total body energy consumption (TIMMONS et

al., 2012). In this study, as in the case of nesfatin-1, the liver levels of the irisin increased with kumiss supplementation, while kidney and brain levels did not change. UYSAL et al. (2018) suggest that the decrease in anxiolytic behavior due to regular voluntary exercise may be associated with locally produced brain irisin. It has been shown that recombinant irisin attenuated kidney damage and improved renal function in mice. Plasma levels of irisin have been reported to be regulated by diet, obesity, lipid profile, exercise, pharmacological agents and different pathological conditions (MAHGOUB et al., 2018). In a study, healthy eating habits were associated with low irisin levels, whereas a high carbohydrate diet was associated with high irisin levels. The circulating irisin concentrations are positively correlated with body mass index, fasting blood glucose and total cholesterol. It has also been reported that after weight loss due to obesity surgery, circulating irisin levels, as well as muscle FNDC5 gene expression, are significantly reduced (MORENO-NAVARRETE et al., 2013). In another study, the circulating irisin level was higher in obese individuals, and was positively associated with total bone mass density, muscle, and fat masses after surgery (LU et al., 2019).

Irisin is stimulated by exercise in mice and humans, and a slight increase in the circulating level of irisin leads to an increase in energy expenditure, with no change in movement or food intake (CASTILLO-QUAN, 2012). However, in this study, kumiss reduced both irisin plasma levels and food intake. The weight loss in mice may be a result of the influence on various metabolic processes by kumiss, particularly food intake and appetite, through the irisin and nesfatin-1 hormones. The suppression of irisin was reported to reduce food intake and a decrease in irisin modulated metabolic peptides in zebra fish. BUTT et al. (2017) suggest that irisin is an anorexigenic factor, and its effects are partially mediated by appetite-regulating factors. Irisin infusion has been shown to increase food intake and does not alter body weight in rats. It was also found to decrease serum leptin level and increase ghrelin level. In the first study, it has been suggested that irisin exercises its physiological properties through a cell surface receptor (BOSTROM et al., 2012).

Despite increasing knowledge about the circulating effects of irisin, the receptor (or receptors) that mediate these effects remains to be discovered.

Conclusion

In the present study, it was observed that the kumiss administered to mice for 5 months reduced body weight and food intake, and affected plasma and liver levels of nesfatin-1 and irisin hormones. To date, no study showing the relationship between kumiss use and nesfatin and irisin hormones has been found. The mechanism underlying reductions in body weight is not yet known, the influence of kumiss on weight loss and food intake is a result of the multifunctional effects of its components or bacteria, and regulation of the whole metabolism. Somehow, with the effect of kumiss, nesfatin-1 and irisin hormones may influence appetite factors to reduce food intake through various signaling pathways. The results indicate that irisin and nesfatin-1 levels are regulated by diet and are effective in weight loss and food intake.

Conflict of interest

All authors declare that there is no potential conflict of interest.

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GULMEZ, C., O. ATAKISI: Učinak dodatka kumisa na tjelesnu masu, unos hrane te razine nesfatina-1 i irizina u BALB/C miševa. Vet. arhiv 90, 627-636, 2020.

SAŽETAK

Cilj ovoga rada bio je istražiti razine nesfatina-1 i hormona irizina, koji su nedavno otkriveni i povezani su s endokrinim i metaboličkim funkcijama, u plazmi i tkivu miševa hranjenih dodatkom kumisa. Šesnaest BALB/C muških jedinki podijeljeno je u dvije skupine, kontrolnu i pokusnu. Za vrijeme istraživanja kumis je dodavan u vodu za piće u omjeru 1 : 1, kako bi se dnevno postiglo 2×10^8 cfu/mL bakterijskih kolonija, te je davan jedanput dnevno peroralno tijekom 20 tjedana. Praćeni su porast tjelesne mase i unos hrane. Razine nesfatina-1 i irizina u uzorcima plazme i tkiva određene su ELISA-om. Dodatak kumisa utjecao je na smanjenje tjelesne mase između 2. i 12. tjedna ($P < 0,05$). Nije uočena znakovita razlika nakon 12. tjedna. Unos hrane u pokusnoj skupini smanjen je na početku i deseti tjedan te je na kraju uspoređen s kontrolnom skupinom ($P < 0,05$). Razine nesfatina-1 i irizina u plazmi ($P < 0,001$) su se smanjile, dok su razine u jetri porasle (nesfatin-1 $P < 0,05$; irizin $P < 0,001$). Rezultati upućuju na to da su razine nesfatina-1 i irizina u plazmi i jetri regulirane prehranom i djeluju na smanjenje tjelesne mase i unos hrane.

Ključne riječi: kumis; gubitak tjelesne mase; unos hrane; irizin; nesfatin-1
