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Original article

Effect of fermented food consumption on biochemical parameters and adipokines levels

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Summary. *Background/aims:* The aim of this study was to determine how fermented food consumption affected the presence of changes in Adipokines levels such as apelin-13, leptin and adiponectin, besides some biochemical parameters. *Methods:* For this purpose, 23 healthy women and men were selected. Blood samples taken from 12 female and 11 male whose ages ranged between 19 and 25 years before and after kefir consumption, and apelin-13, leptin and adiponectin were analyzed from adipokines besides routine biochemical parameters. *Results:* The decrease in LDL cholesterol levels which are an indicator of endogenous cholesterol production was found significant (p <0,05), similarly decrease in triglyceride levels and MDA levels which are defined as oxidant stress indicators were significant, when adipokines were examined, it was determined that there was no significant change in adiponectin and a significant increase in apelin-13 and leptin levels from the point of kefir and yoghurt use. *Conclusions:* It was considered that the presence of beneficial effects on health of kefir as fermented product was revealed. It is thought that this work will be supported by extensive studies to be carried out in the future.

Keywords: Kefir, health, apeline-13, leptin, adiponectin, oxidative stress

Introduction

Fermentation is one of the oldest and most economical technic used for food production and preservation that continued for centuries (1). Lactic acid bac- teria (LAB) and yeasts constitute an important group among the various microorganisms involved in food fermentations. LAB produce lactic acid by using the carbohydrate substrate present in the medium. These microorganisms ensure product safety by producing lactic acid as well as some other antimicrobial active substances (2-4). Yoghurt is foremost among the most consumed of fermented foods. Kefir is also a fermented milk drink that is sour, frothy and light alcoholic milk produced with acidic and alcoholic fermentation (5). Predominant part of the microorganisms found in kefir is constituted by Lactobacilli group, the remaining part is comprised of Lactococci and yeast (6-11). Kefir might contain other defined and undefined organisms, kefir with probiotic properties is stated to be beneficial for health. Yeasts and LAB in kefir increase total effect by adhering to epithelial cells, at the same time lactic acid enhances gastrointestinal tolerance (12-13).

In recent years, fermented foods consisting LAB and yeasts are defined as products that could be a potential alternative medicine element for people which contain essential amino acids, bionutrients and some important bioactive or health promoting compounds and possess functional, therapeutic, antioxidant, antimicrobial, probiotic properties and cholesterol lowering effects (14-15). The results of several clinical studies show that a regular consumption of selected probiotics may reduce the concentration of serum cholesterol, especially of LDL (16). Probiotic bacteria are species that called active microorganisms isolated

from human and animal gastrointestinal tract and affect health and physiology positively (17-21).

In human, leptin, adiponcetin, apelin are adipose tissue markers. Leptin is secreted from adipose tissue and becomes active via leptin receptors on target cells (22). Leptins act like GABAergic neurons in hypothalamus and have effect on loss of appetite, feeling of satiety and play a significant role in preserving lipid balance by enhancing fatty acid oxidation via increasing the mitochondria amount in adipocytes (23). Leptins also play important roles in increase in insulin sensitivity, blood glucose uptake, dispose of excessive sodium, decrease of vascular tone via regulation of cardiac contractility (24).

Adiponectin is also an adipokine. It was shown that adiponectin is present mostly in adipocy- tes and considered to have insulin sensitivity enhancer, anti-inflammatory, antiapoptotic properties. In addi- tion to these also adiponectin stimulates weight loss by increasing energy expenditure in human brain (25).

Apelin is an adipokine in peptide structure that is released from white adipose tissue and ligand to G-protein receptors. It has bioactive forms with 36, 17, 13 amino acids, apelin-36, apelin-17, apelin- 13 respectively. Apelin functions by acting on the periphery and central nervous system. It decreases blood pressure by regulating the cardiac contractility, as well as by acting on the body fluid balance (24). Thus, adipokines are thought to have favorable effects on normal weight and body balance.

In this study, it was aimed to investigate the presence of changes in peptides levels such as apelin-13, leptin and adiponectin, as well as some biochemical parameters used for metabolism in human consuming fermented food within a certain period of time.

Methods

Subjects

Study population consisted of 23 people (12 female and 11 male) who declared that they were healthy and without any illness, drug, alcohol and smoking consumptions. The age range varied between 19-25 years of the group participating. The research group consumed 200 mL of kefir for ninety days three times a week and 200 mL yoghurt per day. Venous blood samples were taken from the anterior cubital vein at pre-post consumption of fermented foods. The approval was obtained from Kahramanmaraş Sütçü Imam University Faculty of Medicine Ethics Committee (2016/4—4) prior to the study. It was also stated that the cases consisting of the study group read the informed voluntary consent form and can participate in the study if they are willing.

Anthropometric parameters

Body mass index (BMI) was calculated before and after kefir and yoghurt consumption by measuring height and weight. Table 1 also shows the BMI values.

Biochemical analysis

Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were analyzed using Advia 1800 automated chemistry analyzer (Bayer, Germany). Adipokin (apelin-13, leptin and adiponectin) levels were determined with ELISA (enzyme-linked immunosorbent assay) method by using a commercial kit (USCN Life Science Inc.), with an automatic ELISA microplate reader (Thermo Scientific, Finland). Results were expressed as ng/ml.

Measuring SOD, MDA and NO

SOD activity was measured using the method described by Fridovich (1983). It was measured with optical density at 505 nm on spectrophotometer. SOD activity was expressed as U/ml (26). Lipid peroxidation level in plasma samples was defined by MDA measurement. MDA was measured according to the method of Ohkawa et al. (1979). Results were given as mmol/ ml. Serum NO levels were measured by the Griess method (27).

Statistical analysis

Results were given as mean ± standard deviation (SD) number, and percent. Statistical significance was accepted as $p < 0.05$. SPSS version 22 (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States) was used for statistical evaluation of the data.

Results

ments were obtained by analysis of study group's blood samples which were taken before and after consumption of kefir and yoghurt. The significance of the difference between them were shown in Table 1. Similarly SOD, MDA and NO levels are shown in Table 2, adipokines (adiponectin, apelin-13 and leptin) levels and significance scores are shown in Table 3. When the results were examined, there was no significant difference in the BMI of the individuals (Pre 24.55 ± 4.72, post 24.41 ± 4.44, p> 0.05) as a result of regular kefir and yoghurt consump- tion. At the same time, there was no significant differen- ce in total cholesterol levels whereas significant decrease in LDL cholesterol levels (Pre 98.70±30.80 mg/dL, post 83.56±34.31 mg/dL) (p <0.05), which is an indicator of endogenous cholesterol production and likewise a significant decrease in triglyceride levels were observed (Pre 127.85±57.70 mg/dL, post 94.60±33.73 mg/dL). Also MDA values (Pre 7.70±5.23 mmol/ ml post 3.46±1.49 mmol/ ml) defined as oxidant stress indicator significantly reduced (Table 2). When the adipokines in Table 3 were analyzed and examined the values from the point of before and after kefir and yoghurt use, there was no significant change in adiponectin while significant increases were observed in Apelin-13 and Leptin levels (Pre 1462.07±76,17 ng/ ml, post 2654,37±129,35 ng/ml and Pre 3138.19±778.59 pg/ml, post 4350.10±154.11 pg/ml respectively).

Table 1. BMI, serum glucose, total cholestrerol, LDL-cholesterol, HDL-cholesterol, triglyceride, AST, ALT and AST concentrations of all subjects in Pre and in Post (n=23)

	Case (Pre) Min-Max	Case (Post) Min-Max	P
BMI	24.55±4.72 (18.40-37.87)	24.41±4.44 (18.60-36.24)	0.05
Glucose$^{\gamma}$	84.70±10.45 (58.00-98.00)	85.04±11.85 (48.00-65.00)	0.906
Cholesterol$^{\gamma}$	155.35±29.99 (102.00-226.00)	154.04±26.47 (86.00-217.00)	0.761
HDL$^{\gamma}$	57.15±11.55 (41.50-83.60)	58.36±10.48 (38.30-84.40)	0.398
LDL$^{\gamma}$	98.70±30.80 (33.10-176.80)	83.56±34.31 (39.40-181.80)	0.048*
Triglyceride$^{\gamma}$	127.85±57.70 (74.00-277.00)	94.60±33.73 (54.00-197.00)	0.037*
AST$^{\delta}$	20.04±8.5 (9.00-43.00)	21.65±10.02 (14.00-54.00)	0.259
ALT$^{\delta}$	11.00±2.68 (5.00-52.00)	15.00±1.96 (7.00-95.00)	0.001*
ALP$^{\delta}$	62.35±19.38 (30.00-100.00)	68.83±23.43 (35.00-129.00)	0.074

Paired t test; Wilcoxon test; α: 0,05; Difference is statistically significant, γ: mg/dl, δ:(U/L)*

Discussion

Nowadays, nutritional problems seem to be impor tant especially in the young generation(28). Especially avoiding cooking, excessive consumption of ready-to-eat food and fast food cause obesity which is one of

Table 2. SOD, MDA and NO parameters in Pre and in Post situation (n=23)

	Case (Pre) (Min-Max)	Case (Post) (Min-Max)	P
SOD$^{\psi}$	18.07±7.51 (10.30-41.44)	19.28±6.52 (7.83-35.63)	0.782
MDA$^{\chi}$	7.70±5.23 (1.16-20.51)	3.46±1.49 (1.16-7.35)	0.001*
NO$^{\beta}$	0.11±0.06 (0.05-0.29)	0.15±0.10 (0.05-0.48)	0.103

Paired t test; Wilcoxon test; α: 0,05; Difference is statistically significant, ψ: U/ml, χ:mmol/ ml, β: nmol/ml*

Table 3. Adipokin parameters in Pre and in Postsituation (n=23)

	Case (Pre) Min-Max (Pre)	Case (Post) Min-Max (Post)	P
Adiponectin**	34.55±15.09 (12-62)	37.64±14.98 (16-79)	0.484
Human A13*** (Apelin)	1462.07±76,17 (672.68-2801.43)	2654,37±129,35 (731.77-4990.65)	0.007*
Leptin**	3138.19±778.59 (1141.87-3875.98)	4350.10±154.11 (1428.65-7275.50)	0.008*

Paired t test; Wilcoxon test; α:0,05; Difference is statistically significant, **: pg/ml, ***: ng/ml*

the most important problems of our age and related to diabetes, atherosclerosis, muscle joint disorders, kidney disorders, and it also brings about important life problems such as depression(29). Along with these problems, important work loss and significant expenditures for health treatment are seen as side effects. In this nutrition style, processed foods(fast food) and the additives used to extend their shelf life are known to have significant negative effects (30). It is known that processed foods (high fat, high energy-dense foods) are more absorbed in the intestines and especially those consisting of carbohydrates increase blood glucose levels excessively which triggers the excess release of insulin. As a result of this situation if continues for a long time high glucose levels cause insulin resistance leading to primarily by metabolic syndrome after that causing diabetes and cardiovascular problems (31). Kefir, and yoghurt are some of this fermented foods (32). These products contain some microorganisms which are an important group of LABs and yeasts. The health-promoting effect of the fermented functional foods arises directly through interaction with consumed microorganisms (probiotic effect) or, indirectly, as a result of action of microbial metabolites generated during fermentation process(33). Vitamins, proteins, peptides, oligosaccharides, and organic acids, including. fatty acids are involved in the most important biogenic metabolites. Probiotic bacteria can reduce the resorption in GIT by metabolizing cholesterol. Lactobacilli, bifidobacteria and other milk bacteria assimilate cholesterol, incorporate it into membranes, deconjugate and precipitate the bile acids both in vivo and in vitro were investigated in some experiments (34). Decongestant bile acids are therefore less absorbed than the conjugated forms of the intestinal lumen, because they are less soluble. As a result, more free bile acids are excreted via feces. Furthermore, free bile acids are less effective in the dissolution and reabsorption of fats in the intestine (33).

As a result of our study, significant decrease in triglyceride (26.01 %) and LDL-cholesterol (11.81 %) levels were observed during the research period. Triglycerides are storage form of the free fatty acids in adipocytes. Also excess of carbohydrate is converted into acetyl CoA and is stored as trigliseride in adipocytes. Therefore, it is possible to say that the use of degraded carbohydrates (carbohydrates are broken down by enzymes into monosaccharides i.e., mainly into glucose, fructose and galactose) by LAB and similar bacteria in intestine results in less absorption to portal system of these molecules from the intestines and therefore the blood level of this molecules are kept in the more normal limits (35). Besides increased bacterial activitiy in the large intestine results in enhanced bile acid. Decongugated bile acids are not well absorbed by the gut mucosa and are excreted. Consequently, cholesterol, being a precursor of bile acids, is utilitized to a greater extent for bile acid synthesis. LDL-cholesterol is synthesized in the liver and meets the cholesterol requirement of peripheral tissues. It seems possible to claim that kefir and yoghurt are effective in reducing the levels of LDL-cholesterol (11.81%) in this study. Agerholm-Larsen et al., (2000) reported 9% reduction of LDL-cholesterol was seen in the meta-analysis study based on six studies (36). HDL is involved in reverse cholesterol transport, movement of cholesterol back to the liver, therefore decreases oxidation of LDL cholesterol remnants in the blood (32). Another biomarker of oxidative stress is Malondialdehyde (MDA), MDA is significantly lower in persons consuming foods that contain LABs and yeasts. For this reason especially fermented foods have beneficial effects on oxidative stress(37). Thus, Lücke in 2000 and Tamang in 2007 mentioned the presence of antioxidant properties of these fermented foods (14-15). Our findings seem to support this. In our study on adipokine measurements; leptin and Apelin-13 levels were found to increase. It is known that Apelin-13 plays important roles in metabolism and foremost among them is induction of insulin release. Apelin stimulates glucose transport and its effect is additive to that of insulin. Apelin could also regulate glucose metabolism, via promoting glucose absorption by enterocytes and by increasing portal blood glucose and insulin secretion (24).

Therefore, it seems possible to mention the blood sugar lowering effect and thus reducing the synthesis of fatty acid from glucose indirectly by removing the excess glucose from circulation. Kefir and yoghurt increase apelin-13 levels it means that they also increase these effects. It is possible to indicate the fact that important effects such as the entry of blood glucose into the cell and the increase of the oxidation of fatty acids

cause the decrease in triglyceride levels increasingly, and the restriction of endogenous cholesterol (LDL) synthesis in the liver (23).

Conclusion

In conclusion, all the information obtained from these studies shows that, fermented foods especially kefir and yoghurt increase the levels of leptin and apelin13 that appear to be useful for metabolism, regulate the blood sugar and reduce the endogenous triglycerides levels in blood; both by restriction of carbohydrate absorption and by increasing the fatty acid oxidation via adipokines, in shortly it seems possible to make mention of their entirely beneficial effects. It is thought that this work will be supported by extensive studies to be carried out in the future.

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