Effects of kefir or milk supplementation on zonulin in overweight subjects

Z. Jenko Pražnikar,¹ S. Kenig,¹ T. Vardjan,² M. Černelič Bizjak,¹ and A. Petelin^{1*}

¹Faculty of Health Sciences, University of Primorska, Polje 42, SI-6310 Izola, Slovenia

²Kele & Kele, d.o.o., Laze 22/a, SI-1370 Logatec, Slovenia

ABSTRACT

Increased intestinal permeability has been shown to be involved in several diseases associated with low-grade chronic inflammation, including obesity and metabolic syndrome. In the last decade, growing evidence shows the beneficial effects of probiotic-containing food supplementation on these conditions. In this crossover intervention study on 28 asymptomatic overweight adults, we tested the effects of a 3-wk kefir supplementation compared with a 3-wk milk supplementation on serum zonulin levels. The effects on serum glucose, triacylglycerols, low-density lipoproteins, high-density lipoproteins, total cholesterol, markers of inflammation (C-reactive protein and adiponectin), anthropometric variables, mood, and appetite were also determined. Kefir supplementation resulted in a greater improvement of serum zonulin levels ($F = 6.812$, $\eta^2 = 0.275$), whereas a significant yet similar improvement in lipid profile and serum glucose levels was found in both supplementations. Positive mood was slightly but significantly enhanced with kefir supplementation, and reduced with milk supplementation. The C-reactive protein, adiponectin, and appetite were unaffected. In conclusion, supplementation with both dairy products had health beneficial effects, but only kefir showed an effect on the intestinal barrier dysfunction marker.

Key words: kefir, low-grade chronic inflammation, intestinal permeability, overweight

INTRODUCTION

Today, we are facing a worldwide epidemic of metabolic syndrome and obesity. Metabolic syndrome is a cluster of interrelated risk factors including obesity, especially visceral obesity, impaired glucose tolerance, insulin resistance, hyperlipidemia, and hypertension (Alberti et al., 2009). These factors increase the risk of diabetes, liver dysfunction, neurodegenerative disorders,

and cardiovascular diseases (Zimmet and Alberti, 1997; Scherer and Hill, 2016), which are the leading cause of death globally [World Health Organization; [https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-\(cvds\)](https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds))].

It has been shown that the development of metabolic syndrome and related chronic disorders may be explained by chronic low-grade systemic inflammation with progressive immune cell infiltration into adipose tissue, which is recognized in obesity (Ouchi et al., 2011). Recent studies suggest that gut microbiota dysbiosis may contribute importantly to such a state (Everard and Cani, 2013; Festi et al., 2014; Boulangé et al., 2016; Gérard, 2016) through several mechanisms, including impairment of intestinal wall integrity, harvest of energy, or a disturbed mucosal immune system (Arrieta et al., 2006; Chassaing and Gewirtz, 2014). The upregulation of zonulin, which regulates intestinal permeability by modulating intracellular tight junctions and is commonly used as a serum marker of compromised intestinal wall integrity (Wang et al., 2000), has been associated with these conditions (Sapone et al., 2006; Moreno-Navarrete et al., 2012; Zak-Golab et al., 2013; Jayashree et al., 2014; Genser et al., 2018).

In recent years, interest has increased in using probiotics and prebiotics as a complementary or adjuvant therapy in the treatment of certain diseases and mood disorders (Ostadrahimi et al., 2015; Rios et al., 2017; Ejtahed et al., 2019). Kefir, a fermented dairy product produced through the symbiotic fermentation of milk by lactic acid bacteria and yeast, is of interest due to its suggested beneficial properties on health, including control of plasma glucose, and antihypertensive, anti-inflammatory, anti-allergenic, antioxidant, antibacterial, and hypocholesterolaemic effects (reviewed in Rosa et al., 2017; reviewed in Pimenta et al., 2018). Milk supplementation also had beneficial effects on body fat mass (Li et al., 2018) and an inverse association with insulin resistance syndrome was observed (Pereira et al., 2002).

Manipulation of gut microbiota homeostasis with the consumption of probiotics may be a profitable strategy to prevent or attenuate several metabolic complica-

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*Corresponding author: ana.petelin@upr.si

Table 1. Nutritional values of kefir and milk

Nutritional value (100 g)	Kefir	Milk
Energy value (kJ, kcal)	57, 239	64, 266
Fat content (g)	3.2	3.5
SFA (g)	2.4	2.3
Carbohydrates (g)	3.9	4.7
Sugars (g)	3.9	4.7
Proteins (g)	3.2	3.3
Salt (g)	0.1	0.12
Calcium (g)	0.12	0.12

tions (Pimenta et al., 2018). Dietary modifications are known to be most efficient in patients at the early stage of the development of metabolic syndrome components, for example, in mild hypercholesterolemia, mildly increased glucose, or overweight, when these are still likely reversible. Therefore, the aim of our study was to investigate the effects of traditional kefir in comparison to milk intake in asymptomatic overweight adults on serum zonulin levels. In addition, we followed inflammatory markers, lipid profile, serum glucose levels, anthropometric variables, and mood.

MATERIALS AND METHODS

Kefir and Milk Characteristics

The kefir used in our study was produced by Kele & Kele d. o. o., Logatec, Slovenia. The exact lactobacilli and yeast composition was shown to be very stable over time (Vardjan et al., 2013; Vardjan et al., 2018) and was reported in detail along with physical and chemical properties, such as water and DM content and pH value

(Vardjan et al., 2018). Briefly, close to 90% of bacterial isolates were genus *Lactobacillus* (*Lactobacillus parakefiri*, *Lactobacillus kefiri*, and *Lactobacillus kefirano-faciens* ssp. *kefirgranum*), and the remaining isolates were cocci. *Kluyveromyces marxianus*, *Kazachstania exigua*, and *Rhodospiridium kratochvilovae* were the predominant yeast species. The water content was around 80%, pH was 4.03, and the exact nutritional value is reported in Table 1. Milk produced by Ljubljanske mlekarne (Ljubljana, Slovenia) with the same fat content was used, and its nutritional values are in Table 1.

Study Design

This study was conducted at the University of Primorska, Faculty of Health Sciences, from April to June in 2018. The crossover intervention study lasted 8 wk and was organized into 2 different phases. A blinded investigator randomly allocated the participant to 1 of the 2 groups by a computer program. Fourteen participants consumed 300 mL of kefir per day in the first 21-d intervention period, whereas 14 participants consumed 300 mL of milk per day first. During the second intervention period, participants consumed the alternate drink. Both kefir and milk were packed in 300-g pots. The study was randomized but unblinded for participants and clinical investigators. The basic composition of both products was similar (Table 1). The two 21-d intervention periods were separated by a 7-d washout period, during which the participants abstained from eating dairy products. Participants were asked to consume the milk or kefir drink at breakfast time daily. At the end of each intervention or washout

Figure 1. Flow diagram of participants throughout the study.

period, anthropometric measurements were performed and serum samples were collected (Figure 1). Compliance to the treatment was monitored by interviewing the participants during visits.

Throughout the study, the participants were encouraged to avoid any alterations to their normal diet or exercise but were not allowed to consume additional prebiotics or probiotics in any form, or drink milk in the kefir intervention period. A 24-h recall was administered after the washout period and 3 times during consumption of the dairy products to assess the food intake during the study. An expert dietitian collected an in-person list of all foods and beverages, using food models and standardized version of 5-pass method developed by the US Department of Agriculture. Open Platform for Clinical Nutrition, accessible through the website <http://opkp.si/>, was used to calculate energy and dietary intake.

Study Participants

Fifty-five Caucasian healthy overweight participants aged between 30 and 60 years were screened for enrolment in the study. Eligible participants had to meet the following criteria: age between 30 and 60 yr, body mass index (BMI) between 25 to 29.9 kg/m², no body mass change exceeding 3% within the last 3 mo, no acute or chronic diseases, no gastrointestinal diseases or endocrine disorders, no drug use for lipid disorders or anti-inflammatory drugs, no nutritional supplements, antibiotics, not pregnant, and not lactating. The male and female volunteers were recruited through advertisement posted in Internet forums, sent via email, and in local newspapers. Seventeen participants did not meet the inclusion criteria, and 10 participants declined to participate. A total of 28 subjects (13 males and 15 females) fulfilled the eligibility criteria to participate. Twenty-seven participants completed the study, one participant dropped out because of chickenpox (Figure 1). The study protocol was approved by the Slovenian National Medical Ethics Committee (No. 0120–206/2018/4). All participants signed their written informed consent before the beginning of the intervention.

Sample Size

Sample size was calculated based on Zak-Golaž et al. (2013) study. Assuming type I error probability $\alpha = 0.05$, 26 participants were estimated to provide 80% power with a 2-sided test to detect a 15% decrease in serum zonulin levels after kefir intervention. In addition, assuming 10% dropout during the study, we increased the number of participants to 29.

Outcome Variables

The primary objective of this crossover interventional study was to investigate the effects of traditional kefir in comparison to milk intake in asymptomatic overweight adults on serum zonulin levels. In addition, we followed inflammatory marker C-reactive protein (CRP) and anti-inflammatory adiponectin, triacylglycerols (TAG), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), serum glucose levels, anthropometric variables [weight, fat free mass, fat (%), muscle mass, visceral fat], and mood.

Anthropometric Measurements

Anthropometric variables were measured following an overnight fast between 7 a.m. and 8 a.m. in standardized conditions by the same examiner. Body weight was measured in light clothing without shoes to the nearest 0.1 kg and height to the nearest 0.1 cm, using a Leicester Height Measure (Invicta Plastics Limited, Oadby, UK). The BMI was calculated as weight (kg) divided by height (m) squared. Body composition (total percentage of body fat and fat-free mass) was assessed using bioelectrical impedance analysis by Tanita MC-980MA (Maeno-cho, Japan) and dedicated software (GMON Pro-Tanita).

Blood Measurements

Blood samples were taken on d 7, 28, 35, and 56. The 8-mL samples of venous blood were collected from the median cubital vein in the morning following an overnight fast in vacuum test tubes. Serum aliquots were stored at -80°C . Serum concentrations of glucose, TAG, total cholesterol, LDL, HDL, and CRP were measured with Cobas c111 analyzer (Roche, Basel, Switzerland). In addition, serum concentrations of adiponectin and zonulin were measured in duplicate on a microplate reader (Tecan, Männedorf, Switzerland) using human ELISA Kits for adiponectin (BioVendor, Prague, Czech Republic) and zonulin (MyBioSource Inc., San Diego, CA). Assay sensitivity was 10 pg/mL for adiponectin and 0.5 ng/mL for zonulin.

Mood Measure

Mood was measured using the positive and negative affect schedule questionnaire (Watson et al., 1988), which has acceptably high α reliabilities, ranging from 0.86 to 0.90. It is a 20-item scale. Ten items assessed positive affect (e.g., excited, active, enthusiastic), and 10 items measured negative affect (e.g., irritable, distressed, nervous); high positive affect is a state of high

Table 2. Baseline characteristics of participants¹

Item	Mean $\pm$ SD
Characteristic ²	
Sex [female/male (%)]	15/13 (53.6/46.4)
Age (yr)	45.8 $\pm$ 8.4
BMI (kg/m ²)	29.1 $\pm$ 4.6
Weight (kg)	89.7 $\pm$ 19.6
Biochemical variable ³	
Fasting glucose (mmol/L)	6.5 $\pm$ 1.1
TAG (mmol/L)	1.3 $\pm$ 0.6
TC (mmol/L)	5.8 $\pm$ 1.8
HDL cholesterol (mmol/L)	1.7 $\pm$ 0.4
LDL cholesterol (mmol/L)	3.9 $\pm$ 1.7
CRP ⁴ (mg/L)	1.99 $\pm$ 1.19 (1.54; 0.4–4.54)

¹Values are presented as means $\pm$ SD.²BMI = body mass index.³TC = total cholesterol; CRP = C-reactive protein; HDL = high-density lipoproteins; LDL = low-density lipoproteins; TAG = triacylglycerols.⁴CRP value is presented as mean $\pm$ SD and as median (minimum and maximum) values.

energy, full concentration, and pleasurable engagement, whereas low positive affect is characterized by sadness and lethargy. Subjects were instructed to respond to the positive and negative affect schedule questionnaire on the basis of how they felt at that moment and indicate “to what extent do you feel this way right now?” Their answers ranged from 5 (extremely) to 1 (very slightly or not at all).

Appetite Measure

The visual analog scale for appetite measurement, satiety, and desire to eat consisted of 4 scales (100 mm lines) anchoring extreme appetite perceptions on both ends of each line (e.g., not at all hungry to very hungry; Sepple and Read, 1989). Subjects were requested to make a vertical mark on a line that best matched how they were feeling in the last few days. This scale was anchored at 0 (not at all hungry) to 7 (very hungry). Each score was determined by measuring the distance from the left side of the line to the mark. Average subjective appetite value was calculated as the average of the measured distances for all visual scales.

Statistics

We applied per protocol analysis. Throughout the study, only one participant dropped out and all participants reported to be compliant to the intervention. Statistical analysis was performed using SPSS version 23.0 (IBM Corp., Armonk, NY). The normality of variables was tested by the Shapiro-Wilk test. Before statistical analysis, obtained data that were not normally

distributed (CRP and zonulin levels) were logarithmically transformed to approximate a normal distribution for subsequent analysis. Normally distributed data are presented as the mean value with standard deviation or percentage. In addition, CRP and zonulin values are presented as the mean value with standard deviation and as median (minimum and maximum) values. The effects of the interventions within each group were analyzed using a paired sample *t*-test, whereas comparison of mean changes between the 2 groups was analyzed using an independent *t*-test. Moreover, the effects of the interventions were analyzed using an univariate analysis of covariance (ANCOVA) with the change at wk 3 from baseline as the dependent variable, adjusted to the corresponding values at baseline, and stratified for several variables (age, sex, energy intake, and anthropometric and biochemical variables at baseline). *P*-values <0.05 were considered statistically significant.

RESULTS

Baseline Characteristics of Participants and Compliance

Table 2 illustrates the baseline characteristics of participants. The study population was 46.4% male and 53.6% female with a mean age of 45.8 $\pm$ 8.4 yr. Initial BMI and weight before kefir or milk supplementation were 29.1 $\pm$ 4.6 kg/m² and 89.7 $\pm$ 19.6 kg, respectively. Most of the participants had elevated serum levels of glucose and mild to moderate hypercholesterolemia; the mean glucose, TAG, total cholesterol, HDL cholesterol, and LDL cholesterol were 6.5 $\pm$ 1.1, 1.3 $\pm$ 0.6, 5.8 $\pm$ 1.8, 1.7 $\pm$ 0.4, and 3.9 $\pm$ 1.7 mmol/L, respectively. The mean CRP was 1.99 $\pm$ 1.19 mg/L.

All participants were compliant with the study. Few participants reported gas problems, feelings of bloating, having loose stools, diarrhea, or cramping. Gastrointestinal disturbances were reported during both intervention periods. Apart from that, both products were well tolerated. Energy intake and the macronutrient composition of the diets did not change during either intervention period (Table 3).

Effects of Kefir and Milk Intake on Anthropometric and Biochemical Variables

The effects of kefir and milk consumption on anthropometric data, glucose, and lipid metabolism were evaluated (Table 4). In the present study, the paired *t*-test revealed no effects of kefir or milk supplementation on anthropometric variables. For BW, fat-free mass, percentage of fat, muscle mass, and visceral fat values,

Table 3. Characteristics of diet during kefir and milk supplementation¹

Item	Kefir intake	Milk intake	<i>P</i> -value ²
Energy value (kcal/d)	1,852 ± 704	1,906 ± 581	0.826
Proteins (g/d)	81 ± 30	91 ± 32	0.381
Carbohydrates (g/d)	187 ± 82	206 ± 81	0.554
Fat (g/d)	84 ± 42	77 ± 31	0.600
Fiber (g/d)	18.4 ± 13.6	16.9 ± 15.7	0.806
Ca (mg/d)	951 ± 229	922 ± 615	0.869

¹Values are presented as means ± SD.²*P*-value denotes differences between the 2 groups using an independent *t*-test.

no significant changes were observed within the groups in either of the 2 interventions. Similarly, using the ANCOVA model with adjustment for the corresponding values at baseline and stratified for several variables, no significant changes were found.

In the analysis of biochemical variables with a paired *t*-test, we found a statistically significant decrease in total cholesterol, LDL cholesterol, and glucose after milk supplementation, whereas kefir supplementation resulted in decreased glucose and HDL concentrations. After adjustments for the described variables (ANCOVA model), the reductions in total cholesterol ($P < 0.001$, $F = 19.480$, $\eta^2 = 0.520$ in kefir; $P = 0.007$, $F = 19.663$, $\eta^2 = 0.663$ in milk supplementation), LDL cholesterol ($P < 0.001$, $F = 18.317$, $\eta^2 = 0.504$ in kefir; $P < 0.001$, $F = 39.895$, $\eta^2 = 0.799$ in milk supplementation), and glucose ($P < 0.001$, $F = 44.690$, $\eta^2 = 0.680$ in kefir; $P = 0.006$, $F = 10.071$, $\eta^2 = 0.372$ in milk supplementation) were similar and statistically significant after both kefir and milk supplementation. Between the 2 groups, no significant differences were observed in anthropometric and biochemical variables.

Effects of Kefir and Milk Intake on CRP, Adiponectin, and Zonulin

To test the hypothesized effect of kefir and milk on intestinal permeability and chronic inflammation, we have measured serum levels of CRP, adiponectin, and zonulin. Table 5 shows that after kefir or milk consumption, no significant differences in CRP, adiponectin, and zonulin levels were detected with paired *t*-test. However, ANCOVA test with the change in each variable as the dependent variable, adjustment for baseline values, and stratification for several variables revealed a reduction in zonulin levels ($P = 0.018$, $F = 6.812$, $\eta^2 = 0.275$) only after kefir, but not after milk supplementation. Neither of the 2 interventions significantly affected CRP and adiponectin. In addition, no significant differences in inflammatory CRP levels, anti-inflammatory adiponectin, and zonulin levels were found between the 2 groups.

Table 4. Anthropometric and biochemical variables before and after kefir and milk supplementation¹

Item	Kefir intake				Milk intake			
	Before	After	<i>P</i> -value ³	<i>P</i> -value ⁴	Before	After	<i>P</i> -value ³	<i>P</i> -value ⁴
Anthropometric variable								
Weight (kg)	89.8 ± 19.8	89.6 ± 19.5	0.553	0.172	89.1 ± 19.3	89.0 ± 18.9	0.738	0.802
Fat free mass (kg)	60.6 ± 13.3	60.5 ± 14.4	0.982	0.525	57.9 ± 17.3	57.8 ± 17.0	0.835	0.950
Fat (%)	32.4 ± 7.1	32.4 ± 7.6	0.965	0.097	32.6 ± 7.5	32.5 ± 7.4	0.726	0.852
Muscle mass (kg)	57.5 ± 12.6	57.6 ± 13.7	0.949	0.795	54.9 ± 16.5	54.9 ± 16.2	0.812	0.877
Visceral fat	10.0 ± 4.8	9.8 ± 4.2	0.264	0.105	9.4 ± 4.7	9.1 ± 4.8	0.713	0.401
Biochemical variable⁵								
TC (mmol/L)	5.5 ± 1.6	5.2 ± 1.2	0.08	<0.001	5.6 ± 1.5	5.3 ± 1.2	0.025	0.887
LDL cholesterol (mmol/L)	3.7 ± 1.5	3.5 ± 1.2	0.068	<0.001	3.8 ± 1.5	3.5 ± 1.2	0.043	0.956
HDL cholesterol (mmol/L)	1.6 ± 0.5	1.5 ± 0.4	0.013	0.436	1.6 ± 0.4	1.5 ± 0.4	0.082	0.662
TAG (mmol/L)	1.3 ± 0.6	1.4 ± 0.7	0.414	0.694	1.4 ± 0.7	1.3 ± 0.7	0.816	0.478
Glucose (mmol/L)	6.3 ± 0.9	5.7 ± 0.5	0.001	<0.001	6.1 ± 1.1	5.6 ± 0.6	0.012	0.184

¹Values are presented as means ± SD.²*P*-value denotes comparison of mean changes between the 2 groups using an independent *t*-test.³*P*-value denotes differences within the 2 groups using a paired *t*-test.⁴*P*-value denotes differences within the 2 groups using univariate analysis of covariance with the change at wk 3 from baseline considered to be the dependent variable, adjusted to the corresponding values at baseline, and stratified for several variables (age, sex, energy intake, anthropometric and biochemical variables at baseline).⁵TC = total cholesterol; LDL = low-density lipoproteins; HDL = high-density lipoproteins; TAG = triacylglycerols.

Table 5. Inflammatory marker, anti-inflammatory marker, and serum zonulin before and after kefir and milk supplementation¹

Item	Kefir intake				Milk intake			
	Before	After	P-value ³	P-value ⁴	Before	After	P-value ³	P-value ⁴
Inflammatory marker								
CRP ⁵ (mg/L)	1.71 ± 1.09 (1.33; 0.28–4.24)	1.86 ± 1.48 (1.30; 0.22–5.27)	0.308	0.212	1.78 ± 1.08 (1.54; 0.27–4.54)	2.11 ± 1.66 (1.44; 0.44–5.88)	0.381	0.692
Adiponectin (µg/mL)	13.34 ± 6.30	13.51 ± 6.94	0.785	0.638	13.00 ± 6.72	12.35 ± 7.07	0.293	0.730
Marker of intestinal permeability								
Zonulin ⁵ (ng/mL)	1.09 ± 1.42 (0.65; 0.04–6.52)	0.93 ± 0.92 (0.60; 0.04–3.38)	0.610	0.018	0.87 ± 0.86 (0.55; 0.17–3.24)	0.84 ± 0.91 (0.54; 0.16–4.03)	0.876	0.762

¹Values are presented as means ± SD.²P-value denotes comparison of mean changes between the 2 groups using an independent *t*-test.³P-value denotes differences within the 2 groups using a paired *t*-test.⁴P-value denotes differences within the 2 groups using univariate analysis of covariance with the change at wk 3 from baseline considered to be the dependent variable, adjusted to the corresponding values at baseline, and stratified for several variables (age, sex, energy intake, anthropometric and biochemical variables at baseline).⁵C-reactive protein (CRP) and zonulin values are presented as means ± SD and as median (minimum and maximum) values.

Effects of Kefir and Milk Supplementation on Mood and Appetite

Table 6 shows that 8 wk of kefir supplementation enhanced ($P = 0.033$) the positive affect or mood, whereas negative mood was slightly but not significantly reduced. On the contrary, we observed reduced ($P = 0.032$) positive mood after milk supplementation. In addition, we detected differences ($P = 0.014$) in positive affect or mood also between the 2 groups. Self-reported appetite perceptions were reduced after 8 wk of kefir supplementation, but not after milk supplementation. However, the differences were not significant.

DISCUSSION

To our knowledge, this is the first study conducted to establish whether a 3-wk-long kefir or milk supplementation influences serum levels of zonulin in asymptomatic overweight healthy adults. The CRP, adiponectin, lipid profile, serum glucose levels, and anthropometric variables were also investigated. We show that kefir supplementation causes a greater improvement in serum zonulin levels compared with milk supplementation, whereas the improvements in lipid profile and serum glucose levels were similar in both interventions.

Ingestion of probiotics has been found to be a useful therapeutic strategy to prevent or attenuate several diseases, mainly through reestablishment of gut microbiota. This is one potential explanation for the observed zonulin decrease in the intervention with kefir supplementation. Several studies support this hypothesis. Kefir had a positive effect on the abundance of several bacterial species in the gut of rats (Gao et al., 2019), and supplementation with *Lactobacillus kefirianofaciens*, the most abundant bacteria in our kefir (Vardjan et al., 2013), markedly increased the diversity of gut bacteria in mice (Sun et al., 2019). *Bifidobacteria*, a group of bacteria also possibly present in kefir has been shown to improve mucosal barrier function (Griffiths et al., 2004). An additional reason why zonulin downregulation could be attributed to the probiotic nature of kefir is the fact that with milk supplementation we did not observe such a difference. A few studies investigating effects of (nonfermented and nonsupplemented) milk reported changes in the relative abundance of gut bacteria but not increased diversity (Norris et al., 2016; Li et al., 2018). Furthermore, a similar outcome as in our study was observed also using different probiotics; in an 8-wk intervention with a probiotic food supplement containing a mixture of 9 bacterial strains (van Hemert et al., 2013), zonulin decreased significantly. The utility of zonulin as a marker is currently under debate because contradicting results regarding its as-

sociation with irritable bowel syndrome and functional dyspepsia (Talley et al., 2019) were reported. A widely used ELISA kit was found to be nonspecific (Scheffler et al., 2018), which seems to be the most likely reason for the observed discrepancies.

It has been suggested that the impairment of intestinal wall integrity is an important factor contributing to the low-grade chronic inflammation in obesity (Arrieta et al., 2006). In fact, we did not find any significant improvement in CRP and adiponectin levels after kefir or milk supplementation. Increased intestinal permeability involve increasing passage of LPS into the circulation, which subsequently stimulates secretion of inflammatory cytokines by targeting specific tissues and interacting with specific receptors (Cani et al., 2008; Boroni Moreira and de Cássia Gonçalves Alfenas, 2012; Jayashree et al., 2014). The anti-inflammatory potential of kefir has been evaluated in an animal model of asthma, rats with edema, and on the intestinal mucosal immune response in mice (Rodrigues et al., 2005; Vinderola et al., 2005; Lee et al., 2007). Hadisaputro et al. (2012), Rosa et al. (2016), and Kim et al. (2017) found that kefir supplementation improved the balance between pro- and anti-inflammatory cytokines in an experimental model for diabetes, metabolic syndrome, and obesity. Mechanism explaining the anti-inflammatory properties of kefir may involve direct or indirect action on the gut microbiota. Indirect actions involve the bioactive peptides produced during fermentation, which can activate macrophages and increase phagocytosis and suppression of the Th2 immune response (Adiloğlu et al., 2013). Apart from being a probiotic, kefir contains of B complex vitamins, vitamins C, A, K, and carotene, minerals, and EAA (Otlés and Cagindi, 2003), which could all contribute to the reduced inflammation and other positive effects of kefir intake.

Some evidence indicates that kefir and milk supplementation have the ability to improve serum glucose levels in experimental diabetic and metabolic syndrome models and in diabetic humans (Hadisaputro et al., 2012; Ostadrahimi et al., 2015; El-Sayed et al., 2016; Rosa et al., 2016). Here we show that in asymptom-

atic overweight healthy adults with mild to moderate hyperglycemia, both kefir or milk consumption significantly reduced serum glucose levels. In line with some previous reports, we also found that both interventions caused a significant decrease in total cholesterol levels and LDL cholesterol levels. Indeed, various studies have concluded that milk and fermented milk products, especially kefir, are able to reduce the serum cholesterol levels in animals and humans (Wang et al., 2009; Soerensen et al., 2014; El-Sayed et al., 2016; Fathi et al., 2017). However, a few studies also indicated insignificant effects of milk and fermented milk products on lipid profile (St-Onge et al., 2002; Maki et al., 2015; Ostadrahimi et al., 2015).

Again, the beneficial effects of kefir on glucose level may be related to an alteration in the intestinal microbiota. Because reduced intestinal permeability could reduce oxidative stress and low-grade chronic inflammation (Gomes et al., 2014; Rosa et al., 2017), the function of insulin receptors could be restored, leading to a better control of blood glucose (Rosa et al., 2017). Nevertheless, many studies have demonstrated that milk, especially milk proteins and bioactive peptides, has glucose regulatory properties (reviewed in Nong-onierma and FitzGerald, 2015; El-Sayed et al., 2016). The results from El-Sayed et al. (2016) suggest that milk protein, especially milk protein hydrolysate, significantly reduces the concentration of plasma glucose and could be a good source of anti-diabetic agents. The suggested mechanism proposes an insulintropic activity, incretin secretagogue action, as well as activity on different metabolic enzymes involved in the regulation of serum glucose (Lacroix and Li-Chan, 2014). A similar influence of kefir and milk on glucose levels in the present study favors the later explanation. The intakes of carbohydrates, fats, proteins, and daily energy intake were unchanged during both interventions and therefore cannot explain the observed differences in the glucose level.

Several mechanisms have been proposed to justify the potential of kefir supplementation in reducing cholesterol levels. Kefir supplementation increases the pro-

Table 6. Mood (affect) and appetite after kefir and milk supplementation¹

Item	Kefir intake			Milk intake			
	Before	After	<i>P</i> -value ³	Before	After	<i>P</i> -value ³	<i>P</i> -value ²
Positive affect	33.4 ± 4.8	34.3 ± 4.3	0.033	34.2 ± 5.9	31.9 ± 6.9	0.032	0.014
Negative affect	20.1 ± 7.1	19.1 ± 8.0	0.204	18.9 ± 8.4	18.6 ± 7.5	0.726	0.658
Appetite	15.9 ± 3.9	15.5 ± 3.7	0.24	16.2 ± 1.9	16.2 ± 2.0	1.00	0.377

¹Values are presented as means ± SD.

²*P*-value denotes comparison of mean changes between the 2 groups using an independent *t*-test.

³*P*-value denotes difference within the 2 groups using a paired *t*-test.

duction of short-chain fatty acids, which are believed to reduce the production of cholesterol levels either by inhibiting hydroxymethylglutaryl CoA reductase or by redistributing plasma cholesterol to the liver (St-Onge et al., 2002; Wang et al., 2009; Arora et al., 2011). Another possible mechanism involves the production of bile salt hydrolase enzyme by bacteria and yeasts present in kefir grains, the enzyme that catalyzes the deconjugation of bile acids and increases their excretion in the feces, therefore helping to lower cholesterol levels (Liu et al., 2012). Several nutrients and bioactive compounds present in milk have been hypothesized to have desirable effects on serum lipid levels (reviewed in Nongonierma and FitzGerald, 2015). For example, high calcium intake from dairy products, present in similar amounts in kefir and milk used in our study (Table 1), has been shown to lower serum total cholesterol and LDL levels in humans (Soerensen et al., 2014), probably by increasing fecal fat excretion (Christensen et al., 2009).

In the present study, we found no significant changes in anthropometric variables after kefir and milk supplementation. The lack of any significant effect on BW is similar to previous reports (Ostadrahimi et al., 2015) and was not expected in such a short intervention.

Finally, based on our results, kefir supplementation significantly increased positive mood. Steenbergen et al. (2015) provided the first evidence that the intake of probiotics may help to reduce sad mood in healthy individuals. In a recent study, it has been also shown that kefir could be used as a diet to prevent depression, anxiety, cognitive impairment, and an available natural therapy for patients suffering from nicotine-induced anxiety and depression as seen in the animal model (Noori et al., 2014). The mechanism includes the action of tryptophan (serotonin precursor) as an essential AA abundant in kefir (Garrote et al., 2001; Otles and Cagindi, 2003). Therefore, kefir as a food supplement with a high level of tryptophan can alter serotonin level and thus can be effective in enhancing positive moods. However, further studies are warranted.

We must stress that the present study has certain limitations. The participants were allowed to remain in a “free-living” environment, so the results are based on participant’s motivation and compliance. The participants were instructed to omit dairy products other than the supplement, but were otherwise asked to keep their normal diet. A 24-h recall was checked 3 times during the kefir and milk supplementation period to control that they indeed followed the instructions. It is noteworthy, however, the participants were living in a free-living environment and we cannot fully exclude the possibility that they did not include all information. Also, the information of the exact bacterial profile of

our product is not exhaustive, which could be relevant considering the evidence that probiotic effects are species and even strain specific.

CONCLUSIONS

Our study is the first showing that kefir supplementation causes an improvement in serum zonulin levels, and positive effects, compared with milk supplementation, and yet similar improvement in lipid profile and serum glucose levels compared with milk supplementation in asymptomatic overweight adults.

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