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Positive Effect of Fermented Camel Milk on Liver Enzymes of Adolescents with Metabolic Syndrome: a Double Blind, Randomized, Cross-over Trial

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ABSTRACT

Background: Metabolic syndrome (MetS) has several health consequences. Liver enzymes elevation is among them. **Aim:** This study aimed to assess the effects of fermented Camel milk (FCM), as a functional food and dairy, on some features of MetS in adolescents including liver enzymes status, serum lipids and anthropometric measures. **Methods:** Overweight/obese adolescents with MetS were randomly assigned to FCM 250 cc per day for 8 weeks, a 4-week washout, and then to diluted Cow's yogurt (DCY) 250 cc per day for 8 weeks, or the reverse sequence. Anthropometric measures, liver enzymes and serum lipids were measured just before and after each one of the four periods. A three-day food record and physical activity questionnaire were completed before each period. Statistical analyses were done using Minitab and SPSS soft-wares considering the significance level of 0.05. **Results:** Twenty-four participants with a mean age (SD) of 13.77 (1.87) years (range: 10.45-16.25) (58% girls) completed the study. It resulted significant mean reduction of aspartate aminotransferase (AST) (-3.75 U/L [95% CI: -7.06; -0.43]; p=0.042) and alanine aminotransferase (ALT) (-2.54 U/L [95% CI: -3.33; -2.24], and p=0.006) and AST/ALT ratio (-0.16 U/L [95% CI: -0.28; -0.05]; p= 0.029) by FCM consumption in comparison to DCY. Non-significant favorable effects on anthropometric measures and serum lipids were seen as well. **Conclusion:** According to the observed favorable effects of fermented camel milk on liver enzymes, its consumption may be considered as a functional food supplement in related circumstances.

Keywords: Metabolic syndrome, Liver enzyme, adolescent, chronic disease, lipids, camel milk.

1. INTRODUCTION

Metabolic syndrome (MetS), the clustering of some cardio-metabolic risk factors, is seen in children and adolescents with a prevalence rate up to 19.2% (1). European adolescents study has shown that a higher cardiovascular health is associated with lower alanine aminotransferase (ALT) and healthier hepatic profile (2). Strong relation of elevated ALT and aspartate aminotransferase (AST) levels with most cardio-metabolic risk factors is documented in adolescents at national level (3). Changes in liver function tests are observed in obese patients with MetS, especially in ALT level. Moreover, insulin resistance, one of the suggested pathogenic mechanisms of MetS, is shown to be an independent influencing phenomenon on liver function tests in obese youth (4). Healthier life style in terms of diets and physical activity improves MetS components (5). Observational studies conclude that consumption of milk, yogurt, and other dairy products, might be associated with a reduced risk of weight gain and obesity as well as of cardiovascular disease, and these findings are, in part, supported by randomized trials (6). Camel milk consumption has been advised for hyperglycemia control by some traditional medical disciplines. It has been studied in a number of animal and human diabetic populations and some favorable results are described (7-9). Camel milk supplementation ameliorated serum biochemical and immunohistochemical measurements that altered after diabetes mellitus induction in albino rats (7). In some societies who consume camel milk as a major dietary resource, diabetes mellitus prevalence is reported to be very low (8). In type-2 diabetes mellitus patients, camel milk has reduced glycemic indices such as FBS, HbA_1C Insulin resistance, etc. as well (9).

The overall composition of camel milk is responsible for its benefits, and some effects might be attributed to the insulin content and its probable influences on insulin resistance/sensitivity. The insulin in camel milk may be present in nanoparticles that could pass the gastric acid, or it may contain 'insulin-like' small molecules (10). A review study showed that camel milk is highly nutritious and safe for consumption by children (11). In all parts of the world where camel lives, its milk is traditionally fermented in order to be more sustainable, nutritious and health-promoting and to have better digestibility of the milk proteins. Fermentation is the process of converting carbohydrates to acid by using bacteria (probiotics) (12). Chal or Shubat is the homemade fermented camel milk (FCM) by a semi-continuous or fed-batch fermentation process in Turkey, Kazakhstan and Turkmenistan (12) and by Turkmens in Iran. For the first time a kind of pasteurized FCM similar to Chal has been produced industrially in Iran, which was used in this study. It is reported that pasteurization does not affect the chemical composition of camel milk (13).

Limited experience exists on the association between fermented dairy intake and risk of MetS; and to the best of our knowledge there is no published information about the effect of FCM consumption on MetS or its features. This study aimed to investigate the effect FCM on anthropometric, liver enzyme and metabolic features of MetS in adolescents.

2. MATERIAL AND METHODS

Study design and registration

This study is a double blind, two treatments - two periods (2×2) crossover, randomized controlled clinical trial. It was scientifically and ethically approved by review boards of Isfahan University of Medical Sciences (IUMS) (approval code: 193059). It was registered in Iranian Registry of Clinical Trials (www.irct.ir), one of the primary registries of the World Health Organization. The trial identification number is IRCT201508081202N2.

Participants

Participants were 11-18- year-old adolescents with MetS. MetS was defined according to de Ferranti et al. (14), fasting blood sugar (FBS) modified as IDF, as having at least three of these: 1) fasting triglycerides ≥ 100 mg/dL; 2) HDL<50 mg/dL, except in boys aged 15 to 19 years, in whom the cut point was<45 mg/dL; 3) fasting glucose ≥100 mg/dL; 4) waist circumference >75th percentile for age and gender; and 5) systolic blood pressure >90th percentile for gender, age, and height. They were identified through screening the overweight/obese adolescents referring to the clinic of the Child and Growth Development Research Center, IUMS, and the private practice offices of principal investigators. Inclusion criteria were as follows: being Iranian,11-18 years old, having MetS, healthy otherwise, without use of supplements or chemical or herbal medications at least 4 weeks prior to the trial commence, no smoking, not being under any dietary regimens or physical activity programs, no history of allergies to dairies, signing informed consent form by at least one parent and by adolescent. Exclusion criteria consisted of occurrence of any serious illnesses, not consuming trial products for more than three consecutive or seven interrupted days, not willing or not being able to continue.

Sample size calculation

Considering 90% statistical power, two-tailed type-one error rate as 5% for detecting at least a standardized effect size of 1 for main study outcomes. According to Agrawal et al. (9) the sample size was calculated to be 22.

Randomization and treatment allocation

The biostatistician supervisor, who was not aware of participants' condition, randomly assigned patients (1:1) to either type A dairy during period 1 followed by type B dairy during period 2 (AB sequence) or the reverse (BA sequence). Random allocation was done by randomization software using stratified, based on age group and sex, blocks of size 2.

Intervention and blinding

The eligible adolescents were recalled by phone and invited to refer to the clinic in a pre-determined day. The researcher put the adolescents in the two above mentioned sequences based on the randomization list. Necessary instructions was explained again and informed consent forms were completed. The researcher and adolescents knew that participants may receive FCM or diluted cow's yogurt (DCY) (Doogh) but no one knew which of the A and B products is FCM or DCY. The intervention was consumption of 250 ml of type A dairy /day (at evening for better tolerability) for eight consecutive weeks in one sequence and the same amount and duration of consumption of type B in the other one. After a wash out period of 4 weeks (9), the participants were crossed between dairies and each participant consumed the other type of dairy for another 8 weeks. FCM was the test and DCY was the control product as an assumption. Every participant took 4-6 sealed door 1-liter bottles to home for the first 16-24 days and was supplied continuously thereafter. Both products were purchased from one factory located in a northern Iranian city, manufactured under maximally similar conditions as possible, were completely alike in physical appearance and opaque bottling, differing only in their labels (A or B). Their dietary constituents which were determined blindly by private and university food analysis laboratories are reported in Table 1. The fermentation process of DCY was done by ordinary yogurt bacteria (Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus). Then yogurt was diluted by water and adding some salt to produce DCY. Lactobacilli were used as part of the production of FCM.

The only person who knew the exact content of bottles labeled as A or B was the factory manager. Participants' houses were scattered around the city and they were not in contact with each other. The researcher was in contact with the factory manager in all the study duration, provided the dairy needed for every 2-4 weeks, and delivered them to participants at their homes or at the clinic. The adolescents were asked to bring back the empty bottles.

All participants were encouraged to have healthy lifestyle, and we gave them a pamphlet explaining about proper diet and physical activity habits.

Measurements

All following measurements were done in the week prior to, as well as the week after each period (4 measurements for every adolescent). Anthropometric measures consisted of height, weight, waist circumference (WC) and hip circumference (HC). Weigh was measured with less clothing by a calibrated scale (QF-2003A; China) to the nearest 0.2 Kg and

Constituent	FCM	DCY
Total protein (g/100g)	5.42	5.2
Total fat (g/100g)	1.67	0.45
Saturated fat (g/100g fat)	58.3	56.8
Trans fat (g/100g fat)	3.6	0.47
Total carbohydrate (g/100g)	6.11	6.08
Fat free solids (g/100g)	5.84	5.45
Iron (mg/kg)	0.0083	0.0083
Potassium (mg/kg)	11.9	12.1
Sodium (mg/kg)	2500	3000
Magnesium (mg/kg)	88.2	87.8
Calcium (mg/kg)	927.8	1270
Vitamin C (mg/kg	2.27	2.27
Vitamin D (mg/kg	1.15	Under 1
FCM: fermented camel milk, DCY: liquid cow's yogurt		

Table 1. Dietary constituents of two types of dairies consumed by the intervention and control groups

height, waist and hip were measured with a standard strip meter to the nearest 0.5 centimeters; measurement of barefoot height was done in standing position adjacent to a straight wall. Body mass index (BMI) was calculated as weight (Kg) divided by height squared (m^2). Before and after each period, the subjects referred after overnight fasting to a specific clinical laboratory who was collaborated in order to take their blood samples and prepare serums for measuring total cholesterol (CHOL), low density lipoprotein - cholesterol (LDL-C), high density lipoprotein - cholesterol (HDL-C), triglycerides (TG), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Serums then were kept frozen at -20 centigrade. Lipid profile and liver function tests were measured by Roche - Hitachi 911 Chemistry Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). None of the laboratory staff were aware of the kind of dairy used by referring participant. A three - day - food record (one holiday and two usual days) was completed by participants at the week prior to each period. The nutritional data of these records were calculated manually based on standard tables and transformed to participants' intakes by Nutritionist 4 software modified for Iranian foods (15). A transcultural adapted physical activity scale (16) was completed at the same times. The mean score of ten items of this scale (ranging 1-5) was considered as the physical activity score of participants.

Statistical analysis

Normality of continuous data was evaluated using Kolmogorov-Smirnov test and Q-Q plot. Right skewed data were subjected to logarithmic transformation. Continuous data were reported as mean ± standard error (SE) or standard deviation (SD) and categorical data as frequency (percentage). Intervention, time and carry over effects were evaluated using specific statistical approach for analysis 2×2 cross-over design using Minitab statistical software (Version 16).

3. RESULTS

Twenty-seven eligible adolescents entered the study. Three of them discontinued the trial, and were excluded from the study, first due to the taste of dairy, second due to painful constipation and third due to a concomitant allergic reaction featuring periorbital edema and red eyes, though not proved to be a reaction to dairy or other material by sure. The third one was referred to emergency room, treated in the presence of researcher and her family and charges were compensated. More details of study flow are presented in Figure 1.

Baseline characteristics of participants who completed the study are summarized in Table 2. Overall, 24 adolescents completed the study (58% girls); with mean age (standard deviation, SD) of 13.77 (1.87) (range 10.45-16.25) years. The type A dairy finally revealed to be FCM and the type B was DCY. Statistical tests showed that participants had no significant difference in baseline characteristics (Table 2).

As is demonstrated from Table 3, cross over data analysis resulted in significant reduction of AST and ALT levels by FCM consumption in comparison to DCY (p value = 0.029, mean reduction ± standard error (SE) = -3.75±1.60 U/L [95%

Baseline parameter	treat-ment	Total number of participants received treat-ments in both periods	Mean	SD	P value
Weight (Kg)	A	24	67.37	14.72	0.92
	B	24	66.60	14.86	
Body mass index (Kg/m^2)	A	24	27.05	3.98	0.83
	B	24	26.75	4.09	
Waist circumference (cm)	A	24	89.25	9.35	0.91
	B	24	89.00	9.49	
Hip circumference (cm)	A	24	101.50	9.73	0.99
	B	24	101.46	9.33	
Triglycerides (mg/dl)	A	24	119.17	55.86	0.90
	B	24	115.13	42.96	
Total cholesterol (mg/dl)	A	24	152.71	30.21	0.77
	B	24	148.54	25.72	
High density lipo-protein cholesterol (HDL-C) (mg/dl)	A	24	41.08	6.77	0.86
	B	24	40.42	7.60	
Non-HDL Cholesterol (mg/dl)	A	24	111.63	27.81	0.78
	B	24	108.13	23.50	
Total cholesterol / HDL-C	A	24	3.76	0.72	0.96
	B	24	3.75	0.74	
Low density lipo-protein –cholesterol (LDL-C) (mg/dl)	A	24	85.13	22.93	0.70
	B	24	81.75	19.97	
Aspartate amino-transferase (AST) (U/L)	A	24	17.50	11.11	0.86
	B	24	16.13	8.99	
Alanine aminotrans-ferase (ALT) (U/L)	A	24	19.50	18.07	0.98
	B	24	19.33	16.21	
Physical activity score	A	24	2.15	0.67	0.76
	B	24	2.09	0.69	
Energy intake (Kcalories/d)	A	22	1667.47	342.32	0.59
	B	23	1735.54	487.27	
Protein intake (g/d)	A	22	78.64	24.93	0.96
	B	23	78.31	21.49	
Carbohydrate intake (g/d)	A	22	228.69	49.80	0.81
	B	23	224.81	61.73	
Fat intake (g/d)	A	22	53.87	21.82	0.30
	B	23	62.70	33.17	
Calcium intake (mg/d)	A	22	1194.18	347.62	0.05
	B	23	1007.37	286.32	
Vitamin D intake (microg/d)	A	22	1.00	1.15	0.67
	B	23	1.16	1.40	
Total fiber intake(g/d)	A	22	17.26	6.49	0.32
	B	23	19.60	8.98217	
P values derived from independent samples t- test.					

Table 2. Baseline characteristics of total participants in the intervention and control (A and B) groups (participants in both periods

Outcome Variable	Mean diff (SD) of seq.1, FCM period	Mean diff (SD) of seq.1, DCY period	Mean diff (SD) of seq.2, DCY period	Mean diff (SD) of seq.2, FCM period	Mean of Treatment effect [95%CI]	SE treatment effect	P values		
							Carry over effect	Treatment effect	Period effect
Aspartate aminotransferase (AST) (U/L)	-0.08(2.23)	1.75(4.04)	4.66(7.07)	-1.00(4.97)	-3.75 [-7.06; -0.43]	1.60	0.41	0.02*	0.24
Alanine aminotransferase (ALT) (U/L)	-1.91(2.39)	1.16(4.30)	3.83(3.48)	-1.00(5.37)	-2.54 [-3.33; -2.24]	1.24	0.10	0.04*	0.34
AST/ALT ratio	-0.12(0.15	0.02(0.13)	0.19(0.36)	0.01(0.17)	-0.16 [-0.28; -0.05]	0.05	0.05	0.006*	0.79
*significant at p< 0.05, ¥ significant carry-over effect, SD: standard deviation, SE: standard error, CI: confidence interval, seq.: sequence.									

Table 3. Comparative effects of Fermented camel milk and diluted cow's yogurt on liver enzymes of study participants

Outcome Variable	Mean diff (SD) of seq.1, FCM period	Mean diff (SD) of seq.1, DCY period	Mean diff (SD) of seq.2, DCY period	Mean diff (SD) of seq.2, FCM period	Mean of Treatment effect [95%CI]	SE of treatment effect	P values		
							Carry over effect	Treatment effect	Period effect
Triglycerides (mg/dl)	9.41(42.28)	11.91(39.27)	16.83(47.51)	9.25(44.34)	-5.04 [-26.19;16.11]	10.1	0.87	0.62	0.80
Total cholesterol (mg/dl)	4.91(14.43)	4.08(14.27)	5.08(15.06)	-2.58(20.70)	-3.41[-13.52; 6.69]	4.87	0.48	0.49	0.39
High density lipoprotein cholesterol (HDL-C) (mg/dl)	-1.00(4.34)	-1.58(4.90)	0.00(3.04)	-1.83(6.35)	-0.62 [-3.25; 2.00]	1.26	0.80	0.62	0.35
Non-HDL Cholesterol (mg/dl)	5.91(12.63)	5.66(12.88)	5.08(13.10)	-0.75(17.44)	-2.79 [-11.98; 6.40]	4.43	0.33	0.53	0.50
Total cholesterol / HDL-C	0.22(0.34)	0.18(0.36)	0.11(0.32)	0.09(0.48)	0.004 [-0.24; 0.25]	0.11	0.34	0.97	0.79
Low density lipoprotein –cholesterol (LDL-C) (mg/dl)	4.25(9.93)	0.83(11.11)	2.91(7.47)	-4.25(14.16)	-1.87 [-8.30; 4.55]	3.10	0.32	0.55	0.10
SD: standard deviation, SE: standard error, CI: confidence interval, seq.: sequence.									

Table 4. Comparative effects of the Fermented camel milk and diluted cow's yogurt on serum lipid levels of study participants

CI -7.06; -0.43], and p value = 0.042, mean reduction ±SE= -2.54±1.24 U/L [95% CI -3.33; -2.24], respectively). Significant simultaneous AST/ALT ratio reduction (p value = 0.006, mean reduction ±SE= -0.16±0.05 U/L [95% CI -0.28; -0.05]) implies that AST is more reduced than ALT.

Serum lipids (listed in Table 4) had non-significant decrease as well.

4. DISCUSSION

This study resulted in the favorable effects of fermented camel milk consumption on liver enzymes AST and ALT and their ratio. It showed non-significant positive influence on serum lipids as well.

The obese/overweight adolescents who meet the MetS criteria have significantly higher rates of non-alcoholic fatty liver (NAFLD) and higher liver enzymes levels compared to non-MetS ones (17, 18). Our study participants were overweight or obese with MetS and some of them showed elevated liver enzymes.

It is likely that overweight children with MetS have had increased genetic susceptibility to unhealthy diet consequences compared to their non-MetS counterparts (19). The current evidence supports the effect of dietary interventions for the management of NAFLD/ liver enzymes levels in children (19, 20). Studies on nutrition programs, Mediterranean diet and the combination of diet and exercise have provided a potential for improvement in serum ALT activity and/or liver fat content or prevention of NAFLD (21-23).

Animal studies by Hamad et al, Kim et al and Chen et al have shown the favorable effects of kinds of dairies or their derivatives on NAFLD and liver enzymes: whey (a kind of milk protein) has significantly reduced hepatic triglycerides and liver enzymes ALT and AST in rats, and kefir was reported to modulate gut microbiota and mycobiota in mice, which prevents obesity and NAFLD via promoting fatty acid oxidation, and to improve NAFLD by inhibiting the lipogenesis pathway (24-26). Nabavi and coworkers have reported that probiotic yogurt consumption improves hepatic enzymes, total cholesterol and LDL-C levels in adults with NAFLD (27). Our study findings as lowering liver enzymes (and serum lipids) are in accordance with those studies. Meantime this study has not resulted in unfavorable effects on most of the other cardio-metabolic risk factors. However, our dairy intervention results are in contrary to Manheimer and colleagues' meta-analysis findings (28). According to their work, Paleolithic diet, the ancient-time like dietary recommendation to exclude grains, dairy, and nutritional products of industry resulted in greater short-term improvements in metabolic syndrome components than did guideline-based control diets. As the authors emphasize, it is less clear whether the avoidance of whole grains and dairy products is essential for the optimal control of metabolism and future studies should examine the impact of avoiding them on health.

Available animal and human investigations on camel milk and its derivatives are mostly in diabetic subjects. Meena et al have reported that camel milk ameliorates hyperglycemia and

oxidative damage in type-1 diabetic experimental rats (29). Manaer and colleagues showed that shubat has significant hypoglycemic potential in type 2 diabetic rats and may modulate lipid metabolism and protect renal function in them (30). Agrawal and coworkers have searched the effects of camel milk consumption on patients with type 1 and 2 diabetes mellitus in multiple studies. All the studies have resulted in promising effects in terms of better glycemia control, reduction of insulin doses and controlling of microalbuminuria (9, 31-33). Ebaid et al reported that camel milk whey protein improves pancreatic cell structure and function in diabetic rats (34). Korish et al have shown that camel milk ameliorates the biochemical and cellular features of experimental NAFLD (35). Our study findings resemble to Korish et al. observations on liver enzymes and serum lipids and Manaer et al observations on serum lipids.

Kadooka and co-workers (36) and Takano et al have shown the favorable effects of fermented milk on BMI and abdominal adiposity including visceral fat (37). Andrade and colleagues also have reported that women taking fermented milk with a baseline mild hypercholesterolemia have shown a significant reduction in LDL cholesterol. HDL cholesterol was also reduced by the test product (38). Our findings, though non-significant, are in accordance with these studies in relation to obesity measures and serum lipids. Parnami et al. have also shown that Indian fermented milk fortified with probiotic bacteria and inulin improves serum lipids (39). In contrary to our findings, Ostadrahimi and co-workers reported that serum lipids levels did not show significant change with fermented milk consumption (40).

Our main intervention has been an especial kind of milk (camel milk) product. Many of our finding may be attributed to the nature of camel milk in addition to its fermented status. The concomitant decrease of serum lipids and lesser increase in weight and BMI of adolescents (though non-significant) may have mediated the favorable alterations in their liver enzymes. All of the possible effects and side effects of FCM and their explanatory mechanisms need to be investigated in future studies.

Study strengths and limitations

The study was conducted under maximally achievable blinding and randomization. Its cross-over design made the cases and controls matched. It used natural products which are domestic, can be publicly advised and added to routine dietary regimens though FCM is rather expensive. The achieved sample size may has resulted in non-significance of some observed favorable effects. We recruited as much as possible participants. Due to effects of maturity processes in adolescents, seasonal manufacturing difficulties of FCM and the issues related to laboratory kits, the time between the first and the last subject's entrance could not be more expanded. The daily amount and length of consumption of dairies are two other probable factors in this regard. However, both had been determined based on previous studies with camel milk, products sustainability, and adolescents' compliance. Our study did not include liver imaging.

5. CONCLUSION

According to the observed favorable effects of fermented camel milk on liver enzymes, it may be considered as a dietary supplement in related circumstances. Furthermore, effects on anthropometric and lipid levels should be confirmed in subsequent studies. The observed side effects were constipation and probable allergic reaction in two of participants. A third one did not like the taste.

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- **Authors contribution:** ZF had substantial contributions to conception, conduct of study, acquisition of data, drafting the article, AF had substantial contributions to design, randomization, statistical analysis and interpretation of data, revising manuscript critically for important intellectual content, MH had substantial contributions to conduct of study, revising manuscript critically for important intellectual content, RK had substantial contributions to conception, conduct of study, revising manuscript critically for important intellectual content, And all authors finally approved the manuscript and agreed to be accountable for all aspects of the work.
- **Conflict of interest:** none declared.

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