Research Article:

Effect of Fermented Camel Milk on Obesity Measures and Blood Pressure of Adolescents With Metabolic Syndrome

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ABSTRACT

Background: Metabolic Syndrome (MetS) predisposes the human body to a variety of chronic diseases.

Objectives: This study, for the first time, aimed to assess the effects of Fermented Camel Milk (FCM), a functional dairy food, on the obesity measures and blood pressure of adolescents with MetS.

Methods: This was a crossover, randomized, double-blind trial. We enrolled overweight or obese adolescents, aged 11-18 years, meeting the diagnostic criteria for MetS. We randomly assigned the study participants to receive FCM 250 mL per day for 8 weeks, followed by a 4-week washout, then, consuming Diluted Cow Yogurt (DCY) 250 mL per day for 8 weeks, or the reverse sequence. General and abdominal obesity measures consisting of weight, Body Mass Index (BMI), BMI z-score, Waist Circumference (WC), Hip Circumference (HC), waist to height ratio and Systolic and Diastolic Blood Pressure (SBP, DBP) were measured before and after each of the 4 periods. A three-day food record and physical activity questionnaire were completed before each period. Statistical analyses were performed using Minitab and SPSS considering the significance level of 0.05.

Results: Twenty-four participants with the Mean±SD age of 13.77±1.87 years (age range: 10.45-16.25 years) (58% girls) completed the study. It resulted in nonsignificant mean changes (increase or decrease) in weight of -0.67 kg (95%CI: -1.97; 0.61; P=0.28), BMI (-0.10 kg/m^2, 95%CI: -0.65; 0.45; P=0.70), BMI z-score of -0.06 (95%CI: -0.33; 0.19; P=0.59), WC of -1.10 cm (95%CI: -3.22; 1.01; P=0.29), and HC of -0.12 cm (95%CI: -2.04; 1.79; P=0.89) by FCM consumption in comparison to DCY. The study also resulted in non-significant mean reduction in DBP of -4.45 mm Hg (95%CI: -10.04; 1.12; P=0.11).

Conclusions: According to some positive impacts of FCM on obesity measures and blood pressure, we suggest conducting further studies to validate the clinical impacts of fermented camel milk.

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1. Introduction

Ischemic heart disease and cerebrovascular disease have been the leading causes of global disability-adjusted life year in recent years (1). Concurrently, the metabolic risk factors, such as high Body Mass Index (BMI) and fasting plasma glucose significantly increased (2). Meanwhile, youth health has been largely neglected globally (3). Metabolic Syndrome (MetS), the aggregation of major cardiometabolic risk factors associated with excess weight (4), is prevalent in the pediatric population, predisposing them to a variety of chronic diseases. Lifestyle change, including diet, is part of the treatment plan in this regard (5). Diets with higher healthy eating indices have a protective effect on the occurrence of pediatric MetS (6).

Camel milk has been used for controlling Diabetes Mellitus (DM) by traditional medicine. Some favorable effects of camel milk are reported in a number of animal and human diabetic populations (7-13) as follows: terminating biochemical and immunohistochemical alterations after DM induction in albino rats (7), reducing daily insulin doses in type 1 DM patients (8), reducing glycemic indices in type 2 DM patients (9), modifying hyperglycaemia and oxidative damage in animal studies (10), positive effects on incretin hormones, insulin resistance, and inflammatory cytokines (11), and promoting immune functions (12) in experimental DM. Furthermore, a systematic review has documented the beneficial effects of camel milk on DM (13).

Some effects of camel milk might be attributed to its probable insulin content and its effect on insulin-related metabolic pathways. Disturbance of these pathways occurs in MetS. In this kind of milk, insulin might be present in nanometer-sized particles transporting it into the blood. In addition, it may contain insulin-like molecules which have similar interactions with its receptor (14). In all parts of the world where camel lives, its milk is traditionally fermented. Fermentation may augment its health benefits through fermenting probiotic bacteria. The homemade Fermented Camel Milk (FCM) in Turkey, Kazakhstan, and Turkmenistan (15) and by Turkmens in Iran is called "Chal" or "Shubat". In northern Iran, for the first time, a kind of pasteurized FCM similar to Chal has been industrially produced. This product was used in this study due to its pasteurization aspect. It is reported that pasteurization does not affect the chemical composition of camel milk (16).

Limited data exist on the association between fermented dairy intake and MetS. Moreover, there was no published information about the effect of fermented camel milk consumption on MetS or its features in humans. This work as part of a larger study aimed to investigate the effect of FCM on obesity and blood pressure measures of adolescents with MetS.

2. Methods

2.1. Study design and patients

This double-blind, two-treatment two-period (2×2) crossover and the randomized controlled clinical trial was scientifically and ethically approved (code: 193059) by Isfahan University of Medical Sciences (IUMS) and registered (ID: IRCT201508081202N2) in www.irct.ir. The study participants were adolescents with MetS, which is defined as having at least three of the following criteria according to de Ferranti et al. (17) Fasting Blood Sugar (FBS) modified based on IDF criteria, as follows: 1. Fasting triglycerides ≥1.1 mmol/L (100 mg/dL); 2. HDL<1.3 mmol/L (50 mg/dL), except in boys aged 15 to 19 years, in whom the cut-off point was <1.2 mmol/L (45 mg/dL); 3. Fasting glucose ≥5.6 mmol/L (100 mg/dL); 4. Waist Circumference (WC) >75th percentile for age and gender; and 5. Systolic blood pressure >90th percentile for gender, age, and height.

Inclusion criteria were being Iranian and 11-18 years old, being diagnosed with MetS or otherwise healthy, lack of using medications or dietary regimens at least one month before the trial, no smoking, no history of allergies to dairy, signing an informed consent by at least one parent and the adolescent participants. Exclusion criteria consisted of the occurrence of any serious illnesses, leaving trial products for more than 3 consecutive or 7 interrupted days, not willing or able to continue study participation.

The sample size was determined based on the statistical power of 80% and type I error of 5% for detecting at least a standardized effect size of 1 for Homeostatic Model Assessment Insulin Resistance (HOMA-IR) as the study's main outcome (9). It was finally calculated to be 22 patients. The study participants were consecutively recruited from March 2016 to December 2016 through screening the overweight or obese adolescents referring to the affiliated clinic of Child and Growth Development Research Center (in Amin Hospital, IUMS), and the private practice offices of the study's principal investigators. More details about the methods and procedures of the main study have been presented elsewhere (18, 19).

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2.2. Study procedure

The biostatistician supervisor, blindly and randomly assigned eligible adolescents (1:1) to either type A or type B dairies at the onset of the first period (AB sequence or BA sequence). Then, for the second period, the participants switched to alternative treatments. Random allocation list was produced by generating a list of random numbers from a uniform distribution in SPSS and applying block randomization of size 2 in Stata based on age group and gender, i.e. we conducted stratified randomization based on age and gender along with block randomization in each stratum.

After phone calls, those who referred were placed into the two groups based on the randomization list. Informed consents were signed by the study participants. The researchers were aware that the study participants might receive FCM or Diluted Cow Yogurt (DCY); however, we were blinded which of the A and B products is FCM or DCY. The intervention comprised of 250 mL of type A dairy per day use for 8 weeks in one group and 250 mL per day of type B dairy product in the other group. After a 4-week washout period (9), the groups crossed and each participant consumed the other type of dairy for another 8 weeks. FCM was the test and DCY was the control product. DCY was produced from cow milk by ordinary yogurt bacteria (Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus), then diluted by water and salt to produce DCY. Lactobacilli were used in the production of FCM as well. Dietary constituents of them are presented elsewhere (18-19).

Both products were obtained from one factory, were completely alike in physical appearance and opaque bottling, and only differed in labels as A or B. Both were sour in taste and similar in flavor. The only person who knew the exact content of the bottles was the factory manager. Participants were not close to each other. They were requested to bring back the empty bottles and were encouraged to have a healthy lifestyle. The participants were not on special dietary regimens and only followed the general healthy eating rules. We provided them a pamphlet explaining healthy diet and physical activity habits and requested them to follow those.

2.3. Measurements

Blood pressure and anthropometric measurements consisting of height, weight, BMI, BMI z-score, WC, and Hip Circumference (HC) were performed in one week prior to, and the first week after each period (4 measurements for every adolescent). They were measured according to standard protocols in the clinic of Amin Hospital. Weight was measured with least possible clothing by a calibrated scale (QF-2003A; China) to the nearest 0.2 kg and height, waist and hip were measured by a standard strip meter to the nearest 0.5 centimeters; measurement of barefoot height was done in standing position adjacent to a straight wall. BMI was calculated as weight (kg) divided by height squared (m^2).

Systolic and diastolic blood pressures were measured twice, at rest and with 10 minutes lasting interval by calibrated sphygmomanometer (Aneroid, Velcro cuff, No.500-V, ALPK2; Japan) covering two-thirds of the adolescent's arm. Mean of two measures was used in the statistical analysis. Immediately before and after each period, the subjects were referred to take some fasting laboratory tests which their results are reported elsewhere (18, 19). A standard three-day food record was completed one week prior to each period. Their nutritional data were calculated based on related tables and transformed to participants' intakes by Nutritionist 4 software modified for Persian foods nutrition. A trans-culturally adapted physical activity scale was completed at the same time.

2.4. Statistical analyses

Normality of continuous data was evaluated using the Kolmogorov-Smirnov test and Q-Q plot. Right skewed data were subjected to logarithmic transformation. Continuous data were reported as mean± SE or SD and categorical data as frequency (percentage). Intervention, time and carryover effects were evaluated by specific statistical approaches for the analysis of 2×2 cross-over design using R free statistical software (3.3.3) (R: A Language and Environment for Statistical Computing, R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2017, https://www.R-project.org). SPSS was used for other statistical analyses.

3. Results

Overall, 27 eligible adolescents were enrolled in the study. Three of the study participants were excluded from the study. Finally, 24 adolescents completed the crossover trial (58% girls); with the Mean±SD age of 13.77±1.87 (age range: 10.45-16.25) years and a body weight range of 42.1-93.4 kg (average: 66.20). More details of the study are presented in Figure 1.

The type A dairy finally was revealed to be FCM and the type B was DCY. Baseline characteristics of participants who completed the study are summarized in
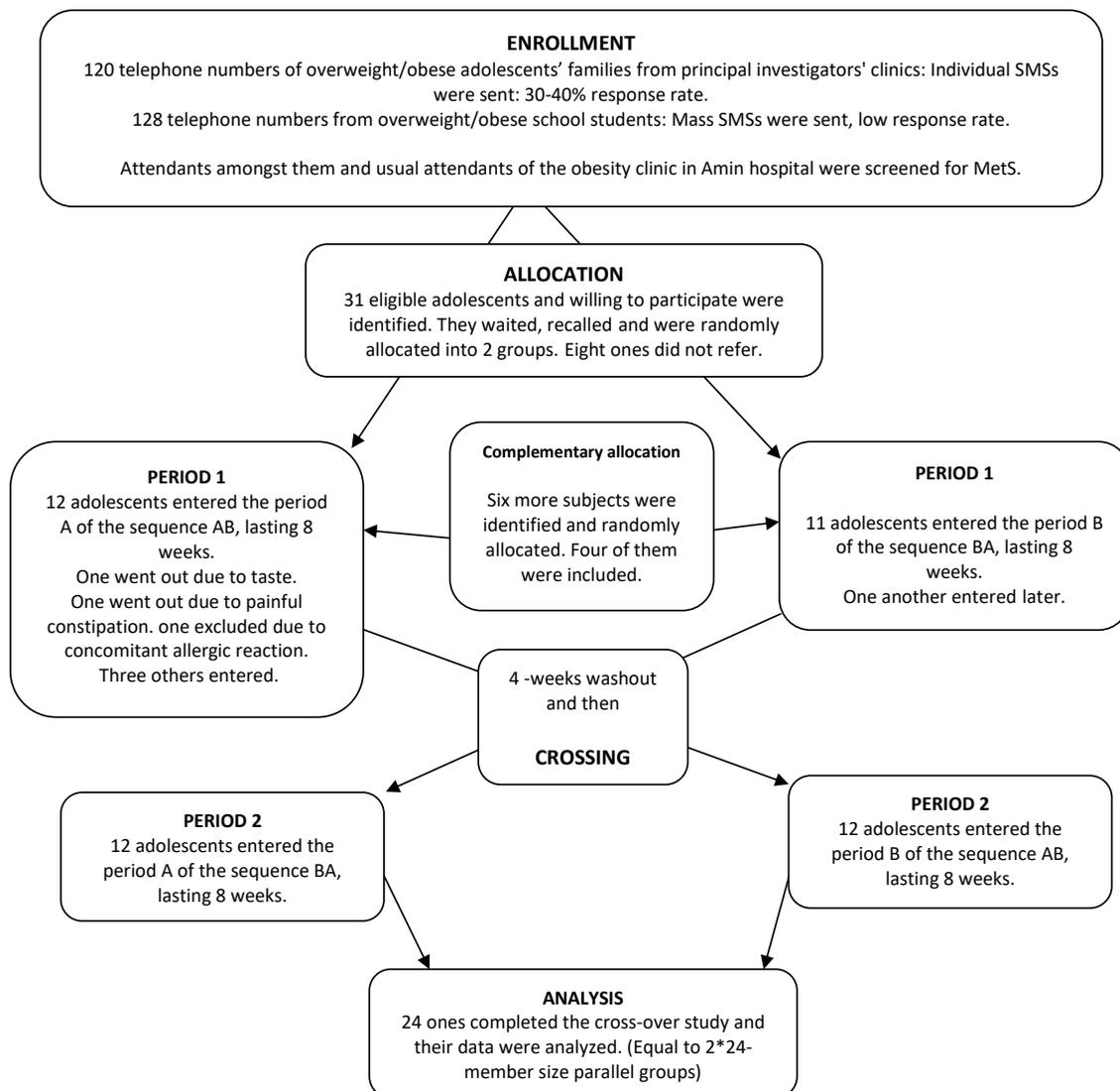
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Figure 1. Flowchart of the trial

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Table 1. No significant difference existed between the baseline characteristics of participants of two groups.

As detailed in Table 2, crossover analysis for anthropometric measures suggested that weight, BMI, BMI z-score, WC, HC, and WHR had a nonsignificant increase or slight decrease in the mean scores by FCM consumption in comparison to DCY. Crossover analysis for blood pressure resulted in non-significant mean score reduction in DBP by FCM consumption compared to DCY. Systolic Blood Pressure (SBP) with a mean decrease of -5.52 mmHg (95%CI: -12.82; 1.78; P=0.13) had a significant carryover effect (P=0.040). We compared the groups based on the obtained data in the first period; however, we could not find any decreasing effects on SBP.

4. Discussion

This trial resulted in beneficial but nonsignificant effects of FCM on the indices of general and abdominal obesity consisting of weight, BMI, BMI z-score, waist circumference, hip circumference, WHR, and blood pressure of adolescents with MetS. This study used a special type of dairy, FCM, which to the best of our knowledge has not been previously studied in clinical trials.

Dairy products have protein-stimulated metabolic effects which differ by their amino acid composition, the rate of absorption, and protein or food texture (20). The incorporation of functional foods and bioactive nutrients can serve as dietary treatment strategies in MetS (21). In relation to obesity measures, the favorable effects of fermented milk on BMI and abdominal adipos-

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Table 1. The baseline characteristics of all study participants in the intervention and control (A and B) groups (participants in both periods)

Baseline Parameter	Treatment	Total Number of Participants Who Received Treatments in Both Periods	Mean±SD	P
Weight, kg	A	24	67.37±14.72	0.92
	B	24	66.60±14.86	
Height, cm	A	24	157.00±8.92	0.99
	B	24	156.98±9.54	
BMI, kg/m^2	A	24	27.05±3.98	0.83
	B	24	26.75±4.09	
BMI z-score	A	24	3.54±1.85	0.79
	B	24	3.04±1.88	
Waist circumference, cm	A	24	89.25±9.35	0.91
	B	24	89.00±9.49	
Waist-to-height ratio	A	24	0.567±0.046	1.00
	B	24	0.567±0.048	
Hip circumference, cm	A	24	101.50±9.73	0.99
	B	24	101.46±9.33	
Systolic blood pressure, mm Hg	A	24	107.69±12.95	0.93
	B	24	105.13±9.81	
Diastolic blood pressure, mm Hg	A	24	67.08±6.74	0.15
	B	24	66.88±6.22	
Physical activity score	A	24	2.15±0.67	0.76
	B	24	2.09±0.69	
Energy intake, Kcal/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 derived from independent samples t-test.

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Table 2. Comparative effects of fermented camel milk and diluted cow yogurt on the anthropometric and blood pressure measures of the study participants

Outcome Variable	Mean±SD				Mean Score of Treatment Effect (95%CI)	SE of Treatment Effect	P		
	Seq.1, FCM Period	Seq.1, DCY Period	Seq.2, DCY Period	Seq.2, FCM Period			Carryover Effect	Treatment Effect	Period Effect
Weight, kg	0.62±1.74	0.48±1.61	1.88±2.35	0.38±2.87	-0.67(-1.97; 0.61)	0.62	0.38	0.28	0.20
BMI, kg/m²	-0.26±0.84	0.11±0.62	-0.05±1.09	0.12±1.18	-0.10 (-0.65; 0.45)	0.26	0.71	0.70	0.30
BMI z-score	-0.11±0.39	0.08±0.31	-0.02±0.50	0.04±0.60	-0.06 (-033; 0.19)	0.12	0.84	0.59	0.29
Waist circumference, cm	0.41±0.64	0.25±3.39	1.87±3.65	-0.50±3.63	-1.10 (-3.22; 1.01)	1.02	0.77	0.29	0.22
Waist to height ratio	-0.0008±0.0271	-0.0025±0.0217	0.0041±0.0264	-0.0033±0.0234	-0.0029 (-0.0156;0.0098)	0.0061	0.79	0.64	0.46
Hip circumference, cm	1.16±2.51	0.25±1.48	0.83±3.88	-0.33±3.52	-0.12 (-2.04; 1.79)	0.92	0.57	0.89	0.27
Diastolic blood pressure, mm Hg	-0.83±11.04	3.75±10.42	1.66±8.07	-2.66±10.52	-4.45 (-10.04; 1.12)	2.69	0.53	0.11	0.96
Systolic blood pressure, mm Hg	3.37±11.06	3.08±12.26	2.91±9.94	-8.41±11.12	-5.52 (-12.82; 1.78)	3.52	0.04*	0.13	0.11

* Significant at the level of 0.05. Abbreviations: SD: Standard Deviation; SE: Standard Error; CI: Confidence Interval, BMI: Body Mass Index

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ity, including visceral fat were reported by Kadooka et al. (22) and Takano and colleagues (23).

Agrawal et al. have revealed nonsignificant BMI decrement by camel milk in type 2 diabetes patients (9). Mudgil et al. in vitro study for the first time reported an inhibitory potential of protein hydrolysates from camel milk on diabetes and obesity intermediates (24). Our findings, though nonsignificant, are in accordance with these studies. Moreover, a longitudinal study on adolescents found no association between dairy food consumption and BMI z-score or body fat percentage in adolescents (25). Our findings may have resulted from the special properties of camel milk in comparison to cow milk. Due to the incompliance of adolescents with excess weight with restrictive dietary regimens, this finding is clinically promising.

Our intervention detected a nonsignificant decreasing effect on blood pressure. Usinger et al. meta-analysis revealed a modest overall effect of fermented milk on SBP and no effect on DBP. However, the overall conclusion of authors is that fermented milk does not effect on blood pressure (26). Ejtahed et al. study on camel milk (27), and Hove et al. (28), and Usinger et al. studies on fermented milk demonstrated no significant antihypertensive effects either (29). The small sample size of the study might have resulted in the nonsignificant decrease in blood pressure. Moreover, different types of milk used migh be the reason for the differences between our results and the other two studies. Additionally, Ayyash et al. (30) have demonstrated in vitro antihypertensive activity for FCM. Antihypertensive effect of camel milk and FCM are concluded by Khatoon et al. and Yahya et al. as well (31, 32).

In respect of the mechanism, Shori et al. review states that during milk fermentation, an excessive amount of peptides are liberated from milk proteins. Of these are ACE inhibitory and antihypertensive peptides. The concentration of ACE-inhibitory peptides appears to rely on a balance between their formation and subsequent degradation. Thus, fermented milk has potential blood pressure lowering properties (33). Our finding is in line with these studies. Although the finding is not statistically significant, it might be clinically significant due to being achieved without any medications use.

The study has maximal blinding and randomization. Its crossover design makes the cases and controls matched. The financial costs prevented further evaluating the related laboratory parameters. The accessed sample size, amount and the length of consumption have probably impacted on the non-significance of effects. These three were due to the influences of maturity processes on adolescents, seasonal manufacturing difficulties of FCM and the issues related to laboratory kits. The amount and length of consumption were determined based on previous studies with camel milk, products sustainability, sourness and probable consequent weakness of FCM and DCY consumption and adolescents' compliance.

5. Conclusion

The beneficial effects of FCM on the anthropometric measures and blood pressure of adolescents with MetS, as preliminary findings of this study, need to be explored in future investigations. Many of the present study findings may be attributed to the properties of camel milk as a special dairy and to its fermented state. Further studies are required to reveal the exact mechanisms of its action and evaluate the clinical impacts of camel milk products.

Ethical Considerations

Compliance with ethical guidelines

This double-blind, two-treatment two-period (2×2) crossover and the randomized controlled clinical trial was scientifically and ethically approved (code: 193059) by Isfahan University of Medical Sciences and registered (ID: IRCT201508081202N2) in www.irct.ir.

Authors contributions

Conceptualizatio: Zahra Fallah; Methodology: Awat Feizi, Zahra Fallah, Roya Kelishadi; Investigation: Zahra Fallah, Roya Kelishadi, Nasim Namazi, Leila Azarbayejani; Statistical supervision and analysis: Awat Feizi; Writing-original draft: Zahra Fallah, Awat Feizi, Nasim Namazi, Leila Azarbayejani; Writing-review & editing: Roya Kelishadi, Awat Feizi, Zahra Fallah; Final approval and permission to submit: All authors; Funding acquisition: Roya Kelishadi; Resources: Roya Kelishadi, Zah-

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Conflict of interest

The authors declared no conflict of interest.

Acknowledgements

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