ORIGINAL ARTICLE

Effect of fermented camel milk on glucose metabolism, insulin resistance, and inflammatory biomarkers of adolescents with metabolic syndrome: A double-blind, randomized, crossover trial

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Background: This study, for the first time, aimed to assess the effects of fermented camel milk (FCM) on glycemic and inflammatory parameters related to metabolic syndrome (MetS), an aggregation of cardiometabolic risk factors, in adolescents. **Materials and Methods:** In a double-blind, randomized crossover trial, overweight/obese adolescents (fulfilling MetS criteria, aged 11–18 years) were randomly assigned to receive FCM 250 cc per day for an 8-week period, a 4-week washout, and then diluted cow's yogurt (DCY) 250 cc/day for another 8-week period, or the reverse sequence. Fasting blood sugar (FBS), fasting insulin, insulin resistance by three equations, incretin hormone glucose-dependent insulinotropic peptide (GIP), and glucagon-like peptide-1 (GLP1) as well as inflammatory markers such as interleukin 6 (IL6) and tumor necrosis factor-alpha (TNF-α) were measured before and after each of the four periods. A 3-day food record and physical activity questionnaire were completed before each period. Statistical analyses were done using Minitab and SPSS software considering the significance level of 0.05. **Results:** Twenty-four participants with a mean (standard deviation) age of 13.77 (1.87) years (range: 10.45–16.25 years) (58% girls) completed the study. It resulted in nonsignificant mean reduction in IL6 (−18.28 pg/mL [95% confidence interval [CI]: −47.48; 10.90]; $P = 0.20$) and nonsignificant increase in glucose metabolizing hormones such as GIP (683.10 pg/mL [95% CI: −457.84; 1824.0]; $P = 0.22$) and GLP1 (6.98 pg/mL [95% CI: −66.61; 80.57]; $P = 0.84$) by FCM consumption in comparison to DCY. Nonsignificant decrease was observed in TNF-α in the first periods of the study. The changes of FBS, fasting insulin, and insulin resistance indices were not statistically significant as well. **Conclusion:** According to preliminary positive influences of FCM on inflammatory markers, and findings related to glucose metabolism, we suggest conducting further studies on its clinical impacts.

Key words: Adolescents, chronic disease, fermented camel milk, glucose metabolism, incretin hormones, inflammation, metabolic syndrome

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INTRODUCTION

Metabolic syndrome (MetS), the aggregation of major cardiometabolic risk factors, becomes prevalent in pediatric population.[1] Chronic diseases might develop later in life as a consequence of MetS.[2,3] MetS is correlated with insulin resistance, low-grade systemic inflammation, and elevated levels of inflammatory biomarkers.[4-7]

Metabolic disorders track from childhood to adulthood.[8] Modifying the metabolic risk at youth may return the

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individual to a nearly no-risk level.[9] Healthy changes in lifestyle and diet (as using functional foods and bioactive nutrients) are recommended as strategies to combat MetS.[10-11]

Camel milk has been used as a traditional medicinal food for controlling diabetes mellitus (DM). In studies on rats, camel milk or its extracts have ameliorated biochemical and histochemical disturbances originating from DM induction.[12-15] Moreover, it has shown antidiabetic effects in human studies, as found by a systematic review.[16] Structural studies have suggested that in this kind of milk, insulin may be present in nanoparticles capable of transporting it into the bloodstream. More probably, this milk may contain "insulin-like" molecules with similar interactions with its receptor.[17] Camel milk is traditionally fermented in order to be more sustainable, nutritious, and health promoting. Chal or Shubat is the homemade fermented camel milk (FCM) in Turkey, Kazakhstan, and Turkmenistan,[18] and by Turkmens in Iran. In Iran, a kind of pasteurized FCM similar to Chal has been produced industrially which was used in this study considering its pasteurization.

There is evidence for reduction of cardiovascular risk by fermented dairy consumption.[19] However, limited experience exists on the association between fermented dairy intake and MetS as a whole. Moreover, 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 of FCM on anthropometric, inflammatory, and metabolic features of MetS in adolescents.

MATERIALS AND METHODS

Methods are discussed elsewhere which present other findings of the study.[20,21] Here, we explain methods in more detail.

Study design and registration

This randomized controlled clinical trial was conducted as a "two treatments – two periods" (2 × 2) crossover, double-blind study between October 2016 and June 2017. The study was scientifically approved by the institutional review board and ethically by regional bioethics committee of Isfahan University of Medical Sciences (IUMS) (approval code: 193059). The trial was registered in Iranian Registry of Clinical Trials, which is one of the primary registries of clinical trials of the World Health Organization, with the identification number of IRCT201508081202N2.

Participants and setting

Participants were 11 to 18-year-old adolescents with MetS. MetS was defined as having at least three of the following criteria as described by de Ferranti *et al*.[22] (fasting blood sugar [FBS] based on the International Diabetes Federation): (1) Fasting triglycerides ≥1.1 mmol/L (100 mg/dL); (2) high-density lipoprotein <1.3 mmol/L (50 mg/dL), except in boys aged 15–19 years, in whom the cut point was <1.2 mmol/L (45 mg/dL); (3) fasting glucose ≥5.6 mmol/L (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 sampled consecutively from March 2016 to December 2016 through screening the overweight/obese adolescents, referred/recalled to the clinic of the Child and Growth Development Research Center (in Amin hospital, IUMS), and the private practice offices of the study's principal investigators. Inclusion criteria were as follows: Iranian, 11–18 years old, having MetS, healthy otherwise, not using supplements or chemical or herbal medications or under any special dietary regimens or physical activity programs for recent 4 weeks, no smoking, no history of allergies to dairies, and signing completely 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, and not willing or not being able to continue.

Sample size

Sample size determination was done based on statistical power = 80% and type one error rate = 5% for detecting at least effect size of = 1 for Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) as the study's main outcome (according to Agrawal *et al*.[23]). It was calculated to be 22.

Randomization, concealment, and treatment allocation

The biostatistician supervisor, who was not familiar with participants and aware of their condition, in another place, 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 blocks of size 2, based on age group and sex. The random assignment list was sent to the researcher in Amin-hospital clinic in the commence day of the trial. Participants' houses were scattered around the city and they were not in contact with each other routinely.

Intervention and blinding

Adolescents eligible and willing to participate were recalled by phone to the Amin-hospital clinic on a predetermined day on October, 2016. The researcher assigned the adolescents in the two abovementioned sequences based on the randomization list. The study conduct and necessary instructions were explained again and informed consent forms were completed. The researcher and adolescents knew that participants may receive FCM (doogh-e-shotor)

or diluted cow's yogurt (DCY) (usual 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 8 consecutive weeks in one sequence and the same amount and duration of consumption of type B in the other one. After a washout period of 4 weeks,[22] the participants were crossed between dairies and each participant consumed the other type of dairy for another 8 weeks. According to the study design, the only person who knew the exact content of bottles labeled as A or B was the factory manager. Every participant took 4–6 sealed-door 1 L 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). Both were sour in taste. 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* and *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 researcher was in contact with the factory manager during the entire study duration, requested the amount of dairy needed for every 2–4 weeks, received the transported bottles, 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 a healthy lifestyle, and we gave them a pamphlet explaining about proper diet and physical activity habits.

Table 1: Dietary constituents of two types of dairy products consumed by the intervention and control groups

Constituent	FCM	DCY
Total protein (g/100 g)	5.42	5.2
Total fat (g/100 g)	1.67	0.45
Saturated fat (g/100 g fat)	58.3	56.8
Trans fat (g/100 g fat)	3.6	0.47
Total carbohydrate (g/100 g)	6.11	6.08
Fat-free solids (g/100 g)	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=Diluted cow's yogurt

Measurements

All the following measurements were done in the week prior to, as well as the week after each of the two periods of two sequences (four measurements for every adolescent). Anthropometric and blood pressure measurements were done in Amin-hospital clinic and in the same weeks as mentioned above (before and after each period); the participants referred, after overnight fasting, to a specific clinical laboratory who were collaborated in order to take their blood samples and measure FBS. Serums were then kept frozen at −20°C to measure inflammatory biomarkers such as interleukin 6 (IL6) and tumor necrosis factor-alpha (TNF-α), incretin hormones such as glucagon-like peptide-1 (GLP1) and glucose-dependent insulinotropic peptide (GIP), serum free fatty acids (FFAs), and insulin (Ins) simultaneously after completion of the study. Insulin resistance was calculated by three formulas as follows: HOMA-IR = (fasting plasma glucose level [mg/dL] × fasting insulin level [μunit/mL])/405,[24] Quantitative Insulin Sensitivity Check Index (QUICKI) = 1/(log fasting insulin level [μunit/mL] + log fasting plasma glucose level [mg/dL]),[24] and adipose tissue insulin resistance (Adipo-IR) = FFAs (mmol/L) × insulin (pmol/L).[7] FBS was measured by Roche–Hitachi 911 Chemistry Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Insulin was measured by LIAISON insulin assay (chemiluminescence immunoassay method, DiaSorin kit, Italy). Other biomarkers were measured using ELISA method by Stat Fax 4700 ELISA reader (Awareness Technology, Palm city, USA). Human GIP, GLP1, FFA, and TNF-α ELISA kits were purchased from Eastbiopharm Co., Ltd (Hangzhou, China) and human IL6 ELISA kit from Boster Biological Technology Co., Ltd (Pleasanton, USA). None of the laboratory staff were aware of the kind of dairy used by the referring participant.

A standard 3-day food record (one holiday and two usual days) was completed by participants at the last week before each period in each sequence. The nutritional data of these records were calculated manually based on standard tables and transformed to participants' intakes by Nutritionist 4 software (N-Squared Computing firm, ©1994) modified for Iranian foods.

A transculturally adapted physical activity scale[25] was completed at the same time. The mean score of ten items of this scale (ranging 1–5) was considered as the physical activity score of participants.

Completed questionnaires were taken from participants in the Amin-hospital clinic or when delivering dairy to them at their homes.

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 or standard deviation (SD) and categorical data as frequency (percentage). Intervention, time, and carryover effects were evaluated by specific statistical approach for analysis of 2 × 2 crossover design using R (3.3.3) free software (R: A Language and Environment for Statistical Computing, R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2017, https://www.R-project.org). Baseline characteristics were compared using (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, Illinois, USA).

RESULTS

Overall, 37 eligible adolescents were identified and randomly allocated into two groups. Twenty-seven of them entered the study. Three of the participants 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 an acute reaction with periorbital edema and red eyes, though not proved to be a reaction to dairy or other material by sure. This case was referred to the emergency room, treated in the presence of researcher and her family, and charges were compensated. Finally, 24 adolescents completed the crossover study (58% girls), with a mean age (SD) of 13.77 (1.87) (range, 10.45–16.25) years. More details of the study stream are presented in Figure 1. The type A dairy finally revealed to be FCM and the type B was DCY. FCM was the test and DCY was the control product as an assumption. Baseline characteristics of participants who completed the study are summarized in Table 2. As is evident, no significant difference existed between the two groups.

Table 3 demonstrates the mean treatment effects of FCM compared to DCY on inflammatory markers. IL6 was reduced nonsignificantly (−18.28 pg/mL [95% confidence interval (CI): −47.48; 10.90]; $P = 0.20$). Effect on TNF-α was accompanied by significant period effect ($P = 0.02$). As evident from Table 3, nonsignificant decrease was observed in TNF-α in the first periods of study for both products.

Furthermore, the effects of FCM compared to DCY on glucose metabolism indices and FFAs as driven by crossover analysis are reported in Table 3. Two glucose

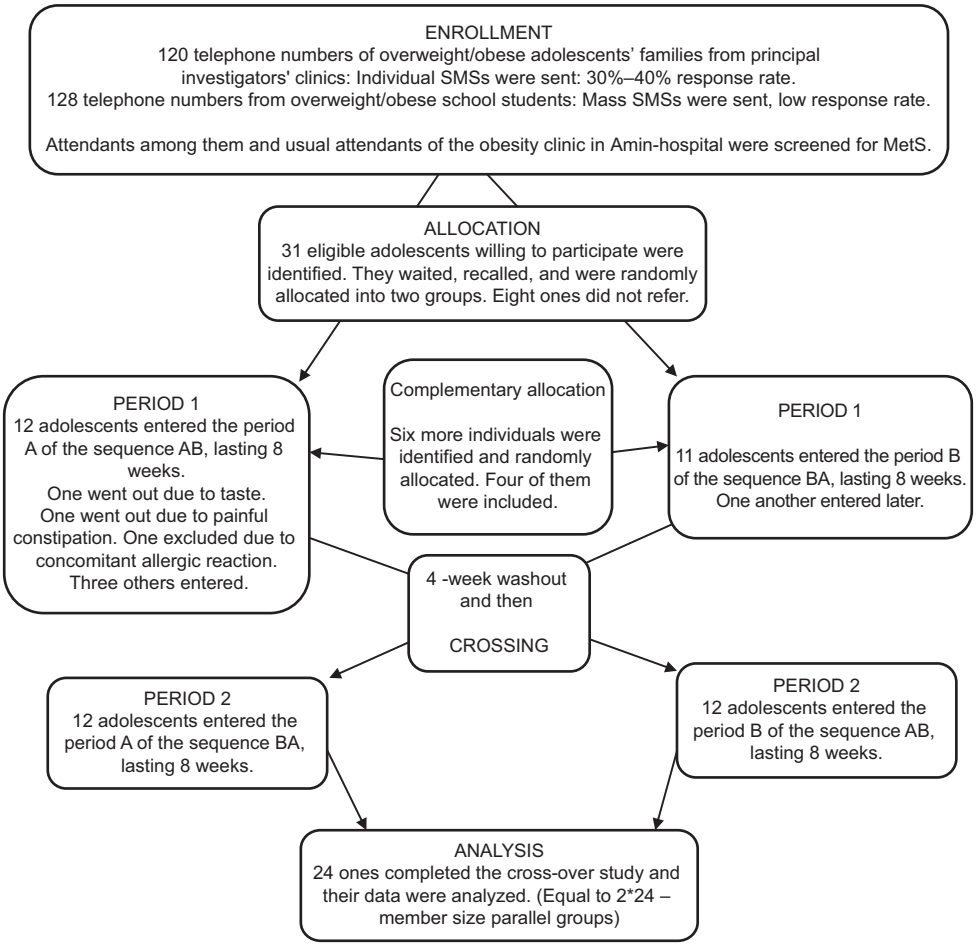

Figure 1: Flowchart of the trial

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

Baseline parameters	Treatment	Total number of participants received treatments in both periods	Mean	SD	*P*
Body mass index (kg/m^2)	A	24	27.05	3.98	0.83
	B	24	26.75	4.09	
FBS (mg/dl)	A	24	89.83	7.14	0.53
	B	24	89.21	8.64	
Fasting serum insulin (pmol/L)	A	24	16.90	7.84	0.31
	B	24	15.42	8.03	
QUICKI-IR	A	24	0.32	0.02	0.09
	B	24	0.33	0.03	
ADPIO-IR (mmol/L.pmol/L)	A	24	16.78	10.13	0.80
	B	24	18.42	13.76	
HOMA-IR GLP1	A	24	3.78	1.85	0.21
	B	24	3.34	1.69	
GLP1 (pg/ml)	A	24	183.46	132.28	0.26
	B	24	151.59	104.63	
GIP (pg/ml)	A	24	1137.14	1344.23	0.49
	B	24	1291.40	1540.38	
Serum FFA (nmol/ml)	A	24	1021.65	511.46	0.29
	B	24	1224.18	834.28	
IL6 (pg/ml)	A	24	32.59	74.72	0.57
	B	24	17.05	31.58	
TNFα (ng/L)	A	24	171.17	251.88	0.69
	B	24	149.53	185.33	
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 values derived from independent sample's *t*-test. SD=Standard deviation; QUICKI=Quantitative Insulin Sensitivity Check Index; HOMA-IR=Homeostasis Model Assessment of Insulin Resistance; ADPIO-IR=Adipose tissue-insulin resistance; FBS=Fasting blood sugar; IL6=Interleukin 6; TNFα=Tumor necrosis factor-alpha; GLP1=Glucagon-like peptide-1; GIP=Glucose-dependent insulinotropic peptide; FFA=Free fatty acids

metabolizing hormones (incretins) showed nonsignificant increase (GLP1: 6.98 pg/mL [95% CI: −66.61; 80.57]; $P = 0.84$) and GIP: 683.10 pg/mL [95% CI: −457.84; 1824.0]; $P = 0.22$). Fasting serum insulin showed nonsignificant increase as well (1.14 pmol/L [95% CI: −1.58; 3.87]; $P = 0.39$). FFAs decreased by FCM nonsignificantly (−13.99 nmol/mL [95% CI: −375.68; 347.70]; $P = 0.93$). Changes of FBS (0.79 mg/dL [95% CI: −2.80; 4.38]; $P = 0.65$) and the three insulin resistance/sensitivity indices (HOMA-IR, QUICKI, and Adipo-IR) were nonsignificant.

Other results of the study are presented elsewhere.[20,21]

DISCUSSION

This trial resulted in beneficial but nonsignificant effects of FCM on inflammatory markers, serum FFAs, and incretin hormones of adolescents with MetS. Meanwhile, a nonsignificant increase was documented in fasting insulin and insulin resistance. The rise of FBS was <1 mg/dL by average, nearly equal to no change. This study used a special type of dairy, FCM, which to the best of our knowledge has not been previously studied in clinical trials.

Table 3: Comparative effects of fermented camel milk and diluted cow's yogurt on inflammatory markers and glucose metabolism indices of study participants

Outcome variable	Mean difference (SD) of sequence 1, FCM period	Mean difference (SD) of sequence 1, DCY period	Mean difference (SD) of sequence 2, DCY period	Mean difference (SD) of sequence 2, FCM period	Mean of treatment effect (95% CI)	SE treatment effect	*P*		
							Carry over effect	Treatment effect	Period effect
IL6 (pg/ml)	−1.57 (3.78)	1.96 (3.53)	−0.93 (9.71)	−33.97 (96.18)	−18.28 (−47.48; 10.90)	14.07	0.21	0.20	0.30
TNFα (ng/L)	−43.10 (92.62)	25.15 (63.01)	−48.64 (156.76)	33.79 (128.93)	7.08 (−58.82; 73.00)	31.78	0.96	0.82	0.02*
FBS (mg/dL)	4.83 (8.03)	0.75 (6.10)	2.91 (9.69)	0.41 (3.94)	0.79 (−2.80; 4.38)	1.73	0.64	0.65	0.07
Fasting serum insulin (pmol/L)	1.83 (3.95)	2.41 (7.82)	−3.25 (8.65)	−0.37 (7.11)	1.14 (−1.58; 3.87)	1.31	0.14	0.39	0.20
QUICKI	−0.005 (0.015)	−0.006 (0.036)	0.020 (0.059)	−4.625 (0.014)	−0.009 (−0.029; 0.010)	0.009	0.17	0.32	0.28
HOMA-IR	0.54 (0.83)	0.60 (1.66)	−0.58 (2.05)	−0.01 (1.69)	0.25 (−0.40; 0.91)	0.31	0.14	0.42	0.33
GLP1 (pg/ml)	−11.12 (94.71)	17.68 (79.06)	30.76 (76.18)	73.54 (153.21)	6.98 (−66.61; 80.57)	35.48	0.05	0.84	0.32
GIP (pg/ml)	221.42 (635.14)	176.6 (853.98)	−861.94 (1936.9)	459.43 (2784.1)	683.10 (−457.84; 1824.0)	550.15	0.40	0.22	0.25
Serum FFA (nmol/ml)	−82.25 (247.14)	92.84 (255.15)	158.95 (984.96)	306.06 (490.87)	−13.99 (−375.68; 347.70)	174.40	0.16	0.93	0.36
ADPIO-IR (mmol/L pmol/L)	0.20 (3.47)	0.54 (11.61)	−3.81 (21.61)	6.31 (10.58)	4.89 (−3.28; 13.06)	3.94	0.82	0.22	0.19

*Significant at the level of 0.05. SD=Standard deviation; SE=Standard error; CI=Confidence interval; QUICKI=Quantitative Insulin Sensitivity Check Index; HOMA-IR=Homeostasis Model Assessment of Insulin Resistance; ADPIO-IR=Adipose tissue-insulin resistance; FBS=Fasting blood sugar; IL6=Interleukin 6; TNFα=Tumor necrosis factor-alpha; GLP1=Glucagon-like peptide-1; GIP=Glucose-dependent insulinotropic peptide; FCM=Fermented camel milk; DCY=Diluted cow's yogurt

Glycemic indices

Our intervention by FCM did not change FBS. There are some reports of the effects of camel milk on glucose metabolism in patients with diabetes. Concluding from a randomized clinical trial, Agrawal *et al*. have reported significant decrease in mean blood glucose, hemoglobin A1c (HbA1c) levels, and insulin doses of patients with type-1 diabetes consuming camel milk compared to nonusers.[26,27] In their crossover study with cow milk as placebo, camel milk reduced FBS, postprandial glucose, HbA1c, and HOMA-IR in patients with type-2 diabetes significantly and no hypoglycemic effect on glucose-tolerant individuals was detected.[23] Mohamad *et al*. in their research on patients with type-1 diabetes showed significant decrease of FBS, HbA1c, and daily insulin dose by camel milk added to usual care compared to usual care alone.[28] Ejtahed *et al*. in their pilot clinical trial on twenty patients with type-2 diabetes reported no significant differences in FBS between test and control groups at the end of study.[29] Some animal studies have shown the same blood glucose reducing effect for camel milk.[13,14,30] Our study finding about FBS is not in accordance with most of the above studies on patients with diabetes and is similar to the results of studies on glucose-tolerant individuals. Our study participants were not diabetic and a few of them showed impaired fasting glucose levels between 100 and 107 mg/dL while screened for MetS. These near-normal levels have probably led to similarity of our finding on FBS to nondiabetic patients. No between-group difference of FBS observed in Ejtahed *et al*'s. study might be due to its small sample size. We may conclude that this dairy has no hypoglycemic effect on near-normal glucose metabolism.

Serum insulin

Interestingly, we found a nonsignificant elevation of fasting serum insulin by FCM consumption. It is in the same manner of the findings of Mohamad *et al*'s. study.[28] In their study, C-peptide levels were markedly higher in the camel milk consuming group. They suggested that camel milk can aid metabolic control in type-1 diabetes, at least by enhancing endogenous insulin secretion. Ejtahed *et al*. observed almost the same. Mean of insulin concentration was significantly increased in the camel milk consuming group during their study. They stated that it might be beneficial to glycemic control.[29] Elevation of serum insulin is reported in Meena *et al*'s. study on type-1 diabetic rats as well.[13] In their experiment, insulin concentration enhanced to nondiabetic levels in diabetic rats receiving camel milk, while goat, cow, and buffalo milk failed to enhance insulin. In addition, Korish *et al*. in their study on type-2 diabetic rats documented significant increase in fasting insulin by camel milk.[14] Manaer *et al*. reported significantly increased C-peptide levels in type-2 diabetic rats fed with Shubat (kind of traditional FCM). Their histological assay

showed protection of the function of pancreatic islets by Shubat.[30] Though Agrawal *et al.* reported nonsignificant insulin change, there was significant increase of plasma insulin in both groups of their study on type-1 diabetes as mentioned in Table 1.[26] In their other reports, they observed gradual increase in mean basal C-peptide in patients with type-1 diabetes consuming camel milk[27] and decrease of area under the curve (AUC) of insulin in camel milk consuming group in type-2 diabetes.[23] Based on these observations, our finding of insulin elevation might be resulted from the insulin-like properties proposed for camel milk. It might be transporting insulin into body or boosting endogenous insulin secretion. These need further research. The volume of dairy consumed daily by our study participants was less than that of most of the abovementioned human studies (250 cc vs. 500 cc). This lesser amount might has resulted in weaker effect and non-significance of our finding.

Insulin resistance

The insulin resistance as HOMA-IR was increased and insulin sensitivity as QUICKI was decreased in our study under the effect of FCM. Considering the way they are computed, (HOMA-IR is the product of fasting insulin and fasting glucose/405 and QUICKI equals 1 divided by log of fasting insulin + log of fasting glucose), and keeping in mind the nearly unchanged FBS and increased insulin levels by FCM, these findings become expected. The same might be true for the adipose tissue insulin resistance (Adipo-IR = insulin × FFA) which was increased in spite of FFAs' decrement. Adipo-IR elevation is reported in MetS (median 68.7 mmol/L. pmol/L vs. 22.9 mmol/L. pmol/L in controls) and may be a marker of adipose tissue dysregulation.[7] The abovementioned studies which have reported insulin resistance attenuation by camel milk had shown considerable decrease in FBS as well.[23,26] Our findings are in accordance with that of Ejtahed *et al*'s. pilot study.[27] They detected significant increase in HOMA-IR during the study in both of their groups. Though this finding may seem unfavorable, the true clinical implications of it as explained above warrant further research.

Incretin hormones

The incretin hormones GLP-1 and GIP are released from the intestine in response to nutrient consumption. They activate insulin secretion, while GLP-1 also inhibits glucagon release and gastric emptying, attenuating postprandial glucose peak.[31] Both hormones increased nonsignificantly by FCM in our study. Manaer *et al*'s. study with Shubat (a traditional FCM) on type-2 diabetes in rats documented increased GLP-1 level by intervention. They concluded that effect of Shubat on glucose might be related to its probiotics acting through the release of GLP-1.[30] Yoo *et al*. reported that GLP-1 elevation after meal ingestion is inversely associated with MetS in patients with type-2 diabetes.[32] Our findings are in accordance with both. On the other hand, De Toro-Martin *et al.* found GIP oversecretion as an important link between catch-up growth and the development of later metabolic disturbances.[33] Normal glucose tolerance individuals with MetS compared to those without MetS have shown higher AUC for GIP and similar AUC for GLP-1 during the oral glucose tolerance test in Calanna *et al*'s. study.[34] Kiec-Klimczak *et al*. also documented stimulation of prolonged release of incretins, mainly GIP, by fat containing meals concomitant to the increase of the markers of inflammation.[35] Yamaoka *et al*. reported that GLP-1 levels are significantly increased with the accumulation of MetS components.[36] Concluding from these contraries, we propose that the nature of relation between incretins and MetS components depends on the chronicity of syndrome. Their acute relation seems to be inverse, and as the process lasts, the relation seems to become direct. Exploring the true interactions is highly valuable.

Inflammatory biomarkers

We detected nonsignificant decrease of IL6 level by FCM consumption compared to DCY. TNF-α levels showed comparable decrease between the two types of dairies in the first periods of crossover trial. Dugan *et al*. showed that TNF-α was reduced significantly only in women (not in men) with MetS who consumed low-fat dairies.[37] In Pei *et al*'s. study, low-fat yogurt compared to a nondairy control food reduced TNF-α in premenopausal women.[38] Mohamadshahi *et al*'s. trial with probiotic yogurt on patients with type-2 diabetes resulted in significant lowering of TNF-α and nonsignificant reduction of IL6 by the intervention compared to control.[39] Labonte *et al*. reported that both their control (nondairy) and dairy diets similarly reduced IL-6 concentrations in healthy adults with low-grade systemic inflammation.[40] Our findings, though nonsignificant, are in accordance with those results.

On the other hand, there were no differences in TNF-α between patients with type-2 diabetes who consumed fermented milk and those who consumed placebo in Hove *et al*'s. study.[41] Our findings differ; these differences might be due to differing populations or different dairies we used. Reduction of inflammatory markers has clinical importance regarding the probable improvement in systemic inflammation.

CONCLUSION

This trial showed beneficial effects of FCM on inflammatory markers, serum FFAs, and incretin hormones with MetS though nonsignificantly. Meanwhile, fasting insulin and indices of insulin resistance had nonsignificant rise. The increase of FBS was <1 mg/dL by average, nearly equal to

no change. These results, as preliminary findings, need to be confirmed in future studies. The accessed sample size, the daily amount, and the length of consumption of dairies are factors which may have resulted in nonsignificance of effects. Due to the influences of maturity processes on adolescents, seasonal manufacturing difficulties of FCM, and the issues related to laboratory kits, the time between the first and the last individual's entrance could not be more expanded. 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, and adolescents' compliance. Many of our findings might be attributed to the properties of camel milk regarding insulin and to its fermented state. Further studies to confirm results, to reveal the underlying mechanisms, and to find the clinical impacts of FCM are suggested.

Study strengths and limitations

The study was conducted under maximally possible blinding and randomization. Its crossover design makes the cases and controls matched. It uses natural products which are domestic, can be publicly advised, and could be added to routine dietary regimens. However, FCM is somehow expensive. Though costly, it could be rational to evaluate more related laboratory parameters including C-peptide and probiotic-related parameters. Due to nonsignificance of findings, the study represents preliminary data.

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Conflicts of interest

There are no conflicts of interest.

AUTHOR CONTRIBUTION

ZF had substantial contributions to conception, conduct of study, acquisition of data, drafting the article, AF had substantial contributions to conception, 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.

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