

Probiotic camel milk powder improves glycemic control, dyslipidemia, adipose tissue and skeletal muscle function in T2DM patients: a randomized trial

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Abstract

Background: Due to the close association between gut microbiota and diabetes, probiotic dairy products draw a lot of attention in the development of functional foods with anti-diabetic activity.

Methods: 28 type II diabetic patients twice a day received 10 grams of camel milk powder supplemented with BBA6 and camel milk powder (control) over a total of 4 weeks.

Results: After the intervention, there was a significant decrease in fasting blood glucose, serum content of total cholesterol, the cardiovascular risk index (TC/HDL-C), the pro-inflammatory cytokines (IL-6, MCP-1) and adipokines (adiponectin, resistin, lipocalin-2, adipsin). Myokines (irisin, osteocrin) increased significantly, indicating possible improvement in skeletal muscle function. Gut microbiota analysis suggested a significant enrichment in unclassified_f__Sphingomonadaceae and depletion in Eggerthella after the 4-week intervention with the probiotic camel milk powder, there were also elevated fecal concentrations of elevated fecal concentrations of proline, uracil and galactinol accompanied with a decreased norleucine, glycerol, sedoheptulose, palmitic acid, 5-aminovaleric acid, inositol and γ -aminobutyric acid.

Conclusion: Dietary supplement with 10 grams of probiotic camel milk powder twice a day for a consecutive 4 weeks can significantly decrease fasting blood glucose of type 2 diabetic patients. This functional food also improves dyslipidemia, inflammation and functions of adipocytes and skeletal muscle, indicating the possibility of probiotic camel milk powder as a dietary treatment that target metabolic syndrome such as diabetes.

Trial registration: ClinicalTrials, NCT04296825, Registered 5th March 20206 - Retrospectively registered, <https://www.clinicaltrials.gov/ct2/show/NCT04296825>.

Background

Diabetes is a serious, long-term condition that occurs when the body cannot effectively produce or use insulin. Type 2 diabetes accounts for around 90% of diabetes worldwide, which can be effectively prevented and managed through adoption of healthy lifestyles, especially a healthy diet (1). As a result, there were many studies focused on the evaluation of the hypoglycemic activity of functional foods or ingredients, which can be mainly divided into polyphenol-enriched plant-based foods (2–5) and proteins or peptides (6–9). Meanwhile, since emerging evidence about the close relationship between gut microbiota with diabetes (10, 11), probiotics is used as a new effective therapeutic strategy in preventing and management of diabetes (12, 13). Besides supplement with the strains itself (14–16), probiotic dairy products such as probiotic soy milk (17) and probiotic-fermented milk (6, 18, 19) were also found to improve the glycemic control of T2DM patients.

Compared with other milk, there were several studies reported a profound anti-diabetic activity of camel milk both in patients with type 1 (20–26) and type 2 diabetes (27–30). However, camel mainly lives in the

desert areas of Africa/Middle East or the cooler dry areas of Asia (31), leading to the unavailability of fresh camel milk for people lived in other areas. Moreover, all the exiting clinical trials were based on fresh camel milk. Dairy products are important vectors for the delivery of probiotics to humans, therefore, our lab develop a probiotic camel milk powder product, and in the present study, we evaluate its effect on blood glucose, lipid profile, inflammatory cytokines, myokines and adipokines in T2DM patients.

Methods And Material

Trial design and sample size

This was a randomized, parallel, double-blind trial in type 2 diabetic patients, conducted in Beijing Chinese Medicine Hospital Pinggu Hospital. This study met the CONSORT criteria as recommended elsewhere (32). The study was approved by the local ethics committee of China Agricultural University (CAUHR-2018026), and registered at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04296825) (NCT04296825).

The estimate sample size of 20 was calculated using the parallel clinical trial formula, assuming an alpha error of 0.05 and a power of 80%. Supposing an estimated 10% dropout rate, there were 22~23 patients for each group (45 patients in total).

Participants

A total of 45 type 2 diabetic patients were recruited from subjects attending to the clinic of Beijing Chinese Medicine Hospital Pinggu Hospital. Inclusion criteria were age 35–68 years, absence of gastrointestinal disease, and willingness to abstain from intake of all kinds of other milk, probiotic food and fermented dairy products during the study but otherwise stick to previous eating habits. Exclusion criteria were pregnancy or lactating in women, cancer, allergy or intolerance to camel milk or cow milk. These criteria were verified during an inclusion visit that included a physical medical examination, dietary and physical activity assessments, standard anthropometrics and fasting glycaemia, insulin and lipid profile were evaluated. After the verification, 40 subjects were eligible for participating in the study. Study procedure was explained for participants, and all participants provided written informed consent.

Randomization, blinding and intervention protocol

The 40 participants were randomly divided into two groups (20 individuals in each group): camel milk powder containing BBA6 at a dose of 2×10^{10} viable cells and camel milk powder as control. Both powders were packaged in the same bags (10 gram each bag) and taken twice daily after breakfast and dinner respectively for 4 weeks. All the samples were provided by Xinjiang Jintuo Co., Ltd. (Xinjiang, China) and packaged by Sanhe Fucheng Biological Technology Co. Ltd (Langfang, China). The nutritional contents of camel milk powder used in this study is detailed in Supplementary Table S1. Patients were given sufficient supplies of the two products at the beginning of the intervention.

All participants were asked to maintain their previous diet except all kinds of other milk, probiotic food and fermented dairy products, physical activity, and medications during the study. During the study, participants underwent interviews regarding adverse effects, symptoms, or changes in quality of life and dietary every week. The allocation groups were unrevealed to the participants as well as to researchers who delivered probiotic camel milk or camel milk alone, or to who conducted the weekly follow-ups.

Blood sample collection

Blood samples were collected twice at the beginning (W0) and the end (W4) of the study, respectively. On the day of blood sample collection, patients came to the hospital without breakfast and after the collection of the fasting blood samples, they were given the same breakfast and the 2 h postprandial blood samples were collected after 2 hours of the first bite of breakfast.

Human peripheral blood was collected in Vacutainer tubes and (Cat # 368921, BD Biosciences) and Vacutainer heparin tubes (Cat # 367886, BD Biosciences), respectively. Blood samples were centrifuged at 1500 ×g for 30 min at room temperature and samples in for fasting glycaemia, 2 hour postprandial glycaemia, insulin, uric acid and lipid measurements within 1 h after blood collection, and serum samples in Vacutainer heparin tubes were carefully removed, aliquoted, snap-frozen in liquid nitrogen, and stored in aliquots at -80°C until further analysis.

Clinical measurements

Serum insulin were measured using the Architect i2000SR analyzer (Abbott Diagnostics, Abbott Park, IL), blood glucose, content of total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) were measured using a Roche cobas ® e 411 analyzer (Roche, Hvidovre, Denmark) according to the manufactures' protocol by the certified core clinical laboratory at the Beijing Chinese Medicine Hospital Pinggu Hospital.

Cytokines and hormones assays

For determination of inflammation cytokines [tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1)], myokines [fibroblast growth factor-21 (FGF-21), irisin, osteocrin, osteonectin) and adipokines (adiponectin, resistin, lipocalin-2, adiponin), human cytokine immunobead panels (Milliplex, Millipore Saint Charles, MO, USA, Cat # MHEMAG-34K, HMYOMAG-56K and HADK1MAG-61K-04) coupled with a multiplex assay (involving xMAP technology, Luminex) were used according to the manufactures' protocol.

Fecal sample collection

Fecal samples were collected in the morning of the day of blood collection by patients themselves at home. Before defecating waterproof paper was first put into closetool to keep feces away from liquids, then a portion of feces was put into sterile tubes containing RNAlater (Qiagen, Hilden, Germany) and the other portion was put into empty sterile tubes. Some fecal samples were collected the day before blood collection due to a higher defecation frequency. Fecal samples were brought to hospital in ice boxes and then stored at -80°C .

Gut microbiota analysis

DNA was extracted from fecal samples using the phenol-chloroform extraction method (33) and quantified using a NanoDrop spectrophotometer (OneC, Thermo Fisher Scientific, Waltham, MA, USA) and stored at -80°C until further analysis. DNA was amplified using the universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCT AAT-3') to target the V3-V4 region of bacterial 16S rRNA. The resulting 468-bp-sized products were assessed, quantified, pooled and sequenced on an Illumina Miseq PE300 platform (Illumina, San Diego, CA, USA) at Shanghai Majorbio Bio-pharm Technology Co. Ltd. (Shanghai, China) using a paired-end sequencing strategy. Raw data were spliced, filtered and then used to select the operational taxonomic units (OTUs) with USEARCH software (version 7.0) and a default cutoff of 97% sequence similarity.

OTUs were further subjected to the Ribosomal Database Project classifier software for taxonomic identification with an 80% confidence threshold at the phylum, class, order, family, genus and species levels. Further analysis such as ANOSIM/Adonis tests, principal coordinates analysis, abundance heatmap and differences in gut microbiome composition were analyzed on the free online platform of Majorbio I-Sanger Cloud Platform (<https://cloud.majorbio.com/>) using weighted unifrac distance matrices.

Fecal metabolomics analysis

Fifty milligrams of feces were mixed with 40 μL internal standard (0.3 mg/mL, 2-chloro-L-phenylalanine dissolved in methanol) and ultrasonically extracted with 360 μL methanol then with 200 μL chloroform and 400 μL ddH₂O in ice bath for 30 min, respectively. After extraction, samples were centrifuged at 12000 rpm at 4 $^{\circ}\text{C}$ for 10 min. Then, 400 μL supernatant was volatilized and oximated with 80 μL methoxyamine hydrochloride in pyridine (15 mg/mL) and shaking them for 90 min at 37 $^{\circ}\text{C}$, after which they were trimethylsilylated by adding 30 μL BSTFA (containing 1% TMCS) and 20 μL n-hexane incubating for 1 h at 70 $^{\circ}\text{C}$. After left at room temperature for 30 min, samples were then subjected to GC-MS analysis.

Metabolic profiling of fecal samples was acquired by an Agilent 7890 A/5975C GC-MS (Agilent Technologies, Santa Clara, CA, USA) using a HP-5MS fused silica capillary column (30 m \times 0.25mm \times 0.25 μm , Agilent J&W Scientific, Folsom, CA, USA). 1 μL sample was injected in a non-split mode. The

injector, ion source and quadrupole rod temperatures were 260 °C, 230 °C and 150 °C respectively. High-purity helium (>99.999%) was used as the carrier gas with a flow rate of 1.0 mL/min. The GC oven temperature program consisted of 60 °C for 2 min, after which the temperature ramped to 310 °C at 8 °C/min, and held steady for 6 min. Mass spectra were acquired and the mass scan range was set at m/z 50–600. Fecal samples were analyzed randomly.

Raw GC-MS mass spectra were converted to CDF format files by ChemStation (version E.02.02.1431, Agilent, CA, USA) and subsequently preprocessed using Chroma TOF (version 4.34, LECO, St Joseph, MI), including raw signal extraction, data baseline filtering, peak identification, and integration. After alignment with the statistical comparison component, the “.CSV” file was obtained with three-dimension data sets including sample information, retention time, the mass-to-charge ratio and peak intensity. Identification of metabolites was conducted using the Automatic Mass Spectral Deconvolution and Identification System, which was searched against commercially available databases such as the National Institute of Standards and Technology and Fiehn libraries. The signal integration area of each metabolite was normalized to the internal standard (2-chloro-L-phenylalanine) for each sample.

The normalized data were transformed using SIMCA-P 14.0 software (Umetrics AB, Umea, Sweden) for principal component analysis and partial least Squares-discriminant analysis (PLS-DA). The variable importance in projection (VIP) values of all the metabolites from the PLS-DA model was taken as criteria to find the variable importance of differential metabolites, and variables with a VIP >1.0 and a p-value < 0.05 were considered relevant for group discrimination. The statistical significance between two groups was evaluated by a univariate Student’s t-test.

Statistical analysis

Data entry was performed twice by two separate persons. Differences between W0 and W4 of each group were evaluated by paired two-tailed Student’s t-tests using GraphPad Prism version 7.0 software (San Diego, CA, USA). Differences between the two groups at the same time point (W0 or W4) were compared by unpaired two-tailed Student’s t-tests using GraphPad Prism. Statistical significance was evaluated at an alpha level of 0.05.

Results And Discussion

Study population

As shown in Figure 1, of the 40 participants that were randomized, 5 persons did not come to pick up the intervention products, 2 persons were lost to follow-up due to go out for travel and 5 poor compliance (took other dairy products and probiotics). At the end of the 4-week intervention, 28 participants completed the experiment and subjected to the analysis, 14 received camel milk powder supplemented with BBA6 (CA) and 14 received camel milk powder (C). None of the participants reported any adverse

effects including gastrointestinal disorders. Baseline comparison showed no significant differences in blood glucose, insulin and lipid profiles between different groups ($p > 0.05$, Table 1).

Changes in glycemic indices and serum insulin

Fasting blood glucose, 2 h postprandial blood glucose and fasting serum insulin of patients before and after 4-week intervention were shown in Figure 2. At baseline, there were both no significant differences between the four groups ($p > 0.05$). The hypoglycemic effect of camel milk has been proved in type I (20–26) and type II diabetic patients (27–30), in this study, patients in the CA group exhibited a significant decrease in fasting blood glucose after the intervention ($p = 0.0458$, Figure 2A) and a more effective hypoglycemic activity than camel milk powder alone ($p = 0.0441$, Figure 2B). However, there were no significant changes in 2 h postprandial blood glucose of patients either before and after the intervention (Figure 2C) or between the two groups (Figure 2D).

Serum content of insulin was also not affected (Figure 2E, $p > 0.05$) and the intervention did not improve the insulin resistance of the patients (Figure 2F, $p > 0.05$). Previous studies about camel milk found a consistent unchanged insulin level in type I diabetic patients (20–27), but in type II diabetic patients, the existing results were inconsistent (20, 31, 34) which may be due to the complicate mechanism in type II diabetes mellitus.

Changes in lipid profile and cardiovascular risk

The relationship between diabetes and atherosclerotic cardiovascular disease are well established, with a significantly elevated risk for cardiovascular disease in diabetic patients (35), therefore we also measured serum content of TC, TG and the indicators of vascular risk (LDL/HDL cholesterol ratio and TC/HDL-C, Figure 3). Previous clinical studies seldomly reported the effects of camel milk on lipid profile, although animal studies reported a consistent decrease in TC (27, 28, 36). In the limited studies in type II diabetic patients, one study (27) was in accordance with ours while another one reported that there were no changes in lipid profile (29). As we can see from Figure 3, both at baseline (W0) and post-intervention (W4), there were no significant differences between the two groups (CA-W0 vs C-W0 and CA-W4 vs C-W4, $p > 0.05$), whereas after the intervention, TC content of patients in CA and C group decreased compared with values at baseline (CA-W0 vs CA-W4, $p = 0.0697$ and C-W0 vs C-W4, $p = 0.0225$, Figure 3A). Furthermore, although there was no change in TG (Figure 3B) and the decreased TC in group CA was nonsignificant ($p = 0.0697$, Figure 3A), intervention of CA resulted a significant decrease in the ratio of TC and HDL-C (TC/HDL-C, $p = 0.0364$, Figure 3D), indicating a decreased vascular risk.

Changes in inflammatory cytokines

It was reported that there was a chronic inflammation in diabetes (37), and a greater antioxidant and immunomodulatory activity of camel milk protein than bovine and other whey proteins (8, 38). Previous studies found that camel milk or camel milk whey proteins was shown to reduce the proinflammatory IL-1 β , IL-6, and TNF α in diabetic rats (28, 39, 40). As shown in Figure 4, there were no significant differences in serum contents of inflammatory markers (TNF- α , IL-6, MCP-1) between groups both at baseline and post-intervention ($p > 0.05$). Within group comparisons (W0 vs W4) suggested that the decreased TNF- α ($p > 0.05$), IL-6 ($p = 0.0103$) and MCP-1 ($p = 0.0814$) contents all in CA group were more obvious than those in C group.

Changes in adipokines and myokines profile

Recent evidence has identified skeletal muscle and adipocytes as secretory organs, which communicate with each other to regulate energy homeostasis and insulin sensitivity through the cytokines called myokines and adipokines, respectively (41, 42). Therefore, we measured serum contents of adipokines (adiponectin, resistin, lipocalin-2, adiponin) and myokines (FGF-21, irisin, osteonectin, osteonectin) in patients before and after 4-week intervention, and the results were shown in Figure 5. There were no significant differences between different groups at baseline (W0) and after intervention (W4, $p > 0.05$). Intervention with camel milk powder supplemented with BBA6 significantly decreased the content of adipokines (adiponectin, resistin and lipocalin-2 and adiponin) and increased myokines (irisin and osteonectin) levels. Although increased level of adiponectin (Figure 5A) and adiponin (Figure 5D) were found to be associated with a lower risk of type 2 diabetes (43, 44) in human and improvement in pancreatic beta-cell function in mice (45, 46), respectively, we found a significant decrease in patients with a significant decrease in fasting blood glucose (group CA). The other two adipokines, resistin (Figure 5B) and lipocalin-2 (Figure 5C), which decreased significantly in patients intervened with camel milk powder alone and in combination, was reported to be good for the improving of diabetes. Elevated serum lipocalin-2 is closely and independently associated with impaired glucose regulation and type 2 diabetes in Chinese people (47), and the lipocalin-2 deficiency attenuates insulin resistance associated with obesity in mice (48). Resistin promotes insulin resistance in mice, whereas whether it does so in humans is unclear (49, 50) because it was synthesized in adipocytes in mice whereas in humans it is generated by macrophages and monocytes, but not adipocytes (51).

As for the myokines, there was a significant decrease in resistin after the intervention accompanied with the decreased fasting blood glucose, it maybe also positively correlated with the hyperglycemia. Furthermore, the significant and specific decrease in irisin ($p = 0.0079$, Figure 5F) and osteonectin ($p = 0.0033$, Figure 5G) was also an indicator for the improvement in diabetes. Circulating irisin levels were reported to be associated negatively with the risk of the metabolic syndrome in individuals from China (52), and the significant increase in patients intervened with camel milk powder supplemented with BBA6 indicated an improvement in diabetes. Osteonectin is also a regulator of bone growth as a novel vitamin D-regulated bone-specific protein (53), suggesting an enhanced effect on bone growth after supplemented with BBA6.

Changes in gut microbiota

More and more evidence suggest a close relationship between gut microbiota and diabetes (54) and since there is a component of probiotics in our study, we analyzed gut microbiota before and after the intervention using the two-tailed Student's t-test (Figure 6). There was a significant enrichment in the relative abundance of *unclassified_f_Sphingomonadaceae* ($p = 0.02477$) accompanied with a depletion in *Eggerthella* ($p = 0.04577$, Figure 6A) in group CA at the genus level. It was found that members of the *Eggerthella* genus possess particular pathogenic potential, and the significant decrease in its abundance after the intervention with CA may be related to the improvement in inflammation (55). There were no different genera before and after the intervention in group C. In addition, since group CA contained a dietary supplement with BBA6, we analyzed the relative abundance of *Bifidobacterium animalis* at the species level, which was enriched in group CA ($p = 0.02754$, Figure 6B). We also analyzed the different genera between group CA and C after the 4-week intervention ($p = 0.0498$, Figure 6C). There was a significant elevation in the relative abundance of *Holdemania*, which was not due to the difference between groups before the intervention (Figure 6D). It was reported that the *Holdemania* genus was found to be associated with being lean in Japanese men (56) but also correlated with clinical indicators of impaired lipid and glucose metabolism (57).

Changes in fecal metabolites

Fecal metabolites concentrations between the two groups were compared and the top ten abundant significantly changed metabolites were shown in Figure 7. We previously compared the fecal metabolites from diabetic patients intervened with camel milk and cow milk, among the top ten abundant significantly changed metabolites, six are amino acids or the metabolites of amino acids (Supplementary Figure S1A) and the other two are FFAs (Supplementary Figure S1B), indicating the anti-diabetic activity of camel milk may be related to the different amino acid composition. However, when compared fecal metabolites in group CA and C, among the top ten abundant fecal metabolites, norleucine, glycerol, sedoheptulose, palmitic acid, 5-aminovaleric acid, inositol and γ -aminobutyric acid decreased significantly, whereas proline, uracil and galactinol increased significantly in group CA ($p < 0.05$).

Diabetic individuals had elevated serum proline levels (58, 59) and our study found an elevated fecal proline concentration, but lower fecal levels of uracil was found in type 2 diabetes (60) and high-fat diet-induced pre-obese individuals (61). Besides uracil, it was also worth noting that the decreased fecal metabolites in group CA were kind of good for glycemic control or energy metabolism. For example, sedoheptulose was found to decrease serum levels of glucose, total cholesterol, TNF- α , IL-6, resistin in type 2 diabetic db/db mice (62), γ -aminobutyric acid can be used to treat diabetes due to the protective effects on β -cell survival and function (63, 64), as well as the promotion of the conversion of α -cells to β -cells (65). Supplement with norleucine in rats stimulated postprandial protein synthesis in adipose tissue, skeletal muscle, and liver (66), and inositol was shown to reduce the risk of metabolic disease in people with PCOS (67), the lysine degradation product, 5-aminovaleric acid decreases β -oxidation of fatty acids in mouse cardiomyocytes (68). Since oligosaccharides in camel milk (69) could function as prebiotics for

BBA6 and gut commensal bacteria (70), we speculated that the decreased concentrations of sedoheptulose, 5-aminovaleric acid, and γ -aminobutyric acid may be the direct effect of BBA6, because there were no significant differences between patients received camel milk powder containing BBA6 and BBA6 alone (Supplementary Figure S2B). Combined these results, it was suggested that camel milk powder supplemented with BBA6 specially decreased fecal concentrations of glycerol and inositol and increased proline and uracil levels, which was not altered by camel milk powder alone or BBA6 alone.

Conclusion

As a traditional milk in Africa, Asia and Middle East, it was believed that regular consumption of fresh camel milk may aid in prevention and control of diabetes. In this study, we evaluated the anti-diabetic activity of a probiotic camel milk product, camel milk powder supplemented with BBA6, a strain of *Bifidobacterium animalis* isolated by our lab in type II diabetic patients. It was found that a 4-week intervention of this probiotic camel milk powder can significantly decrease fasting blood glucose, serum content of TC and the cardiovascular risk (TC/HDL-C). Meanwhile, patients intervened with camel milk powder supplemented with BBA6 also exhibited a decrease in inflammatory cytokines (IL-6, MCP-1) and adipokines (adiponectin, resistin, lipocalin-2, adipsin), as well as an improvement in myokines (irisin, osteocrin). Camel milk powder containing BBA6 significantly enriched the relative abundance of *unclassified_f_Sphingomonadaceae* and depleted *Eggerthella* after the 4-week intervention, and patients in this group exhibited a gut microbiota with an enrichment in the relative abundance of *Holdemania* when compared with patients supplemented with camel milk powder alone. Furthermore, elevated fecal concentrations of proline, uracil and galactinol accompanied with a decreased norleucine, glycerol, sedoheptulose, palmitic acid, 5-aminovaleric acid, inositol and γ -aminobutyric acid was found in patients of group CA. Our study reported a significant enhanced anti-diabetic activity of camel milk powder when combined with BBA6, which can be used as a functional food in assisting treatment of type 2 diabetes. However, the improvement in 2 h postprandial blood glucose, serum insulin level was not significant. Further research is required for a less dose but longer intervention time in more subjects and tests for glucose tolerance and glucose stimulated insulin response can be took into consideration for the anti-diabetic activity.

Abbreviations

BBA6, *Bifidobacterium animalis* A6; W0, the beginning of the study; W4, the end of the study; group CA, camel milk supplemented with BBA6; group C, camel milk; TC, total cholesterol; TG, total triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; MCP-1, monocyte chemotactic protein-1; FGF-21, fibroblast growth factor-21; OTUs, operational taxonomic units; PLS-DA, squares-discriminant analysis; VIP, variable importance in projection.

Declarations

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Authors' contributions

B.F., M.Z., L.D. and F.R. designed the trial. S.G. were responsible for the analysis and production of fermented milk. F.B., M.Z., X.Z. and L.D. conducted the research-were responsible for study recruitment, screening, and delivery of interventions. F.B. did the statistical analysis. All the authors participated in data interpretation. F.B. wrote the first draft of the report, and all other authors commented on the draft and approved the final version.

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Availability of data and materials

We have made publicly and freely available without restriction the data described in the manuscript, at: https://www.dropbox.com/scl/fi/4zbnv0xhwynl9xij2ngyl/data.xlsx?cloud_editor=excel&dl=0&force_role=personal&rlkey=ju9ri88jwyadtruwlcz1t5hd

Ethics approval and consent to participate

The study was approved by the local ethics committee of China Agricultural University (CAUHR-2018026), and registered at ClinicalTrials.gov (NCT04296825).

Consent for publication

All authors approved the publication of the manuscript in the Nutrition Journal.

Competing interests

The authors declare that they have no competing interests.

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Table

Table 1. Baseline characteristics of study participants

| | Camel milk+BBA6 (CA) | Camel milk (C) |
|--|----------------------|----------------|
| N (female/male) | 14 (10/4) | 14 (8/6) |
| Age (years) | 58.36±6.25 | 57.29±7.57 |
| BMI (kg/m ²) | 26.56±4.40 | 26.56±4.40 |
| Fasting blood glucose (mmol/L) | 9.39±2.50 | 10.48±3.78 |
| 2 Hour postprandial blood glucose (mmol/L) | 14.05±3.26 | 16.19±5.28 |
| Insulin (µU/mL) | 11.69±11.12 | 11.01±7.11 |
| TG (mmol/L) | 5.20±1.50 | 5.10±1.00 |
| TC (mmol/L) | 1.77±0.81 | 1.14±0.14 |
| HDL-C (mmol/L) | 1.24±0.26 | 1.22±0.26 |
| LDL-C (mmol/L) | 3.27±1.25 | 3.33±0.86 |
| TC/HDL-C | 4.29±1.31 | 4.47±0.77 |

Note: Data were analyzed for normal distribution by SPSS and expressed as mean \pm S.D.

Figures

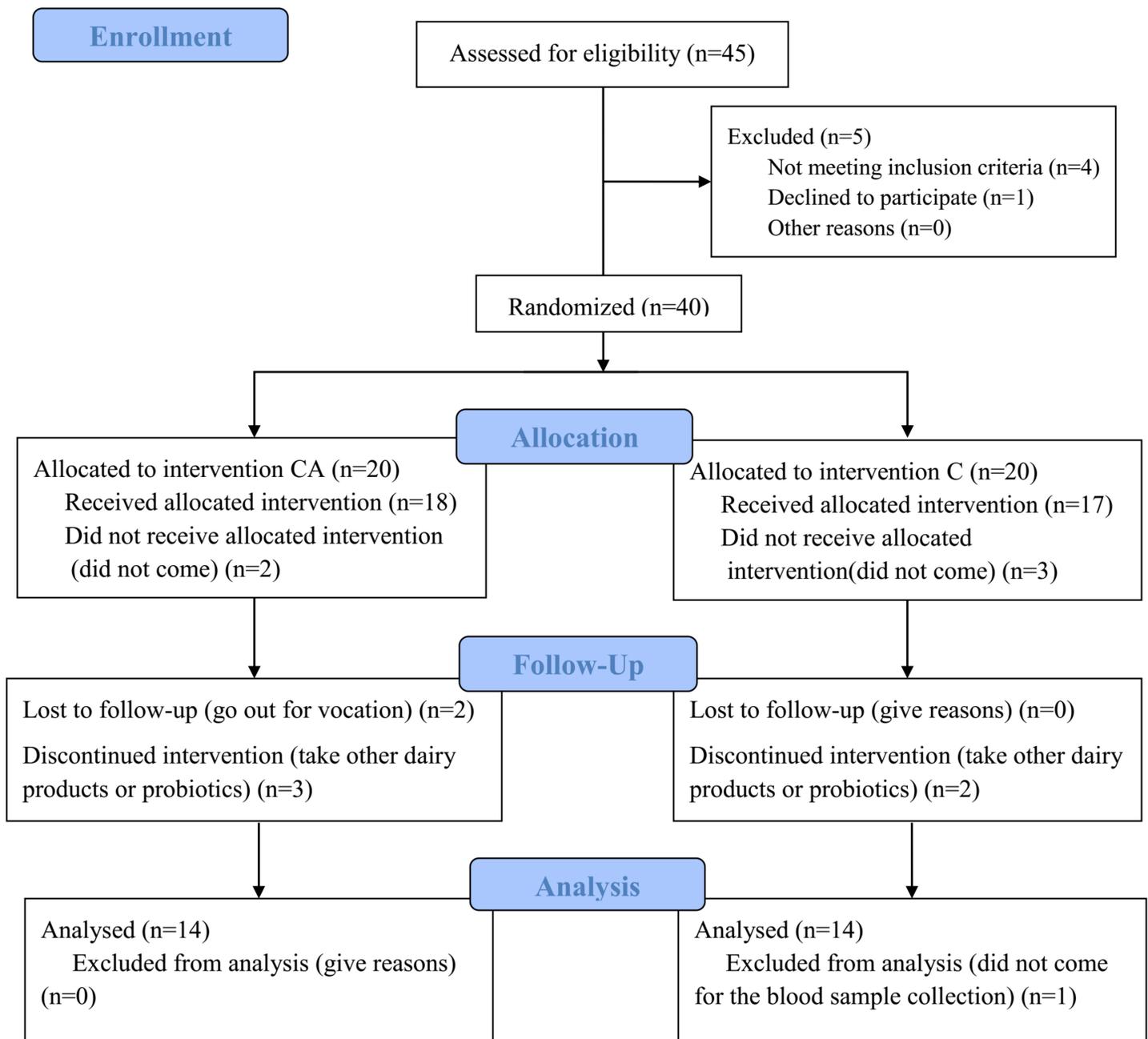


Figure 1

Flow diagram.

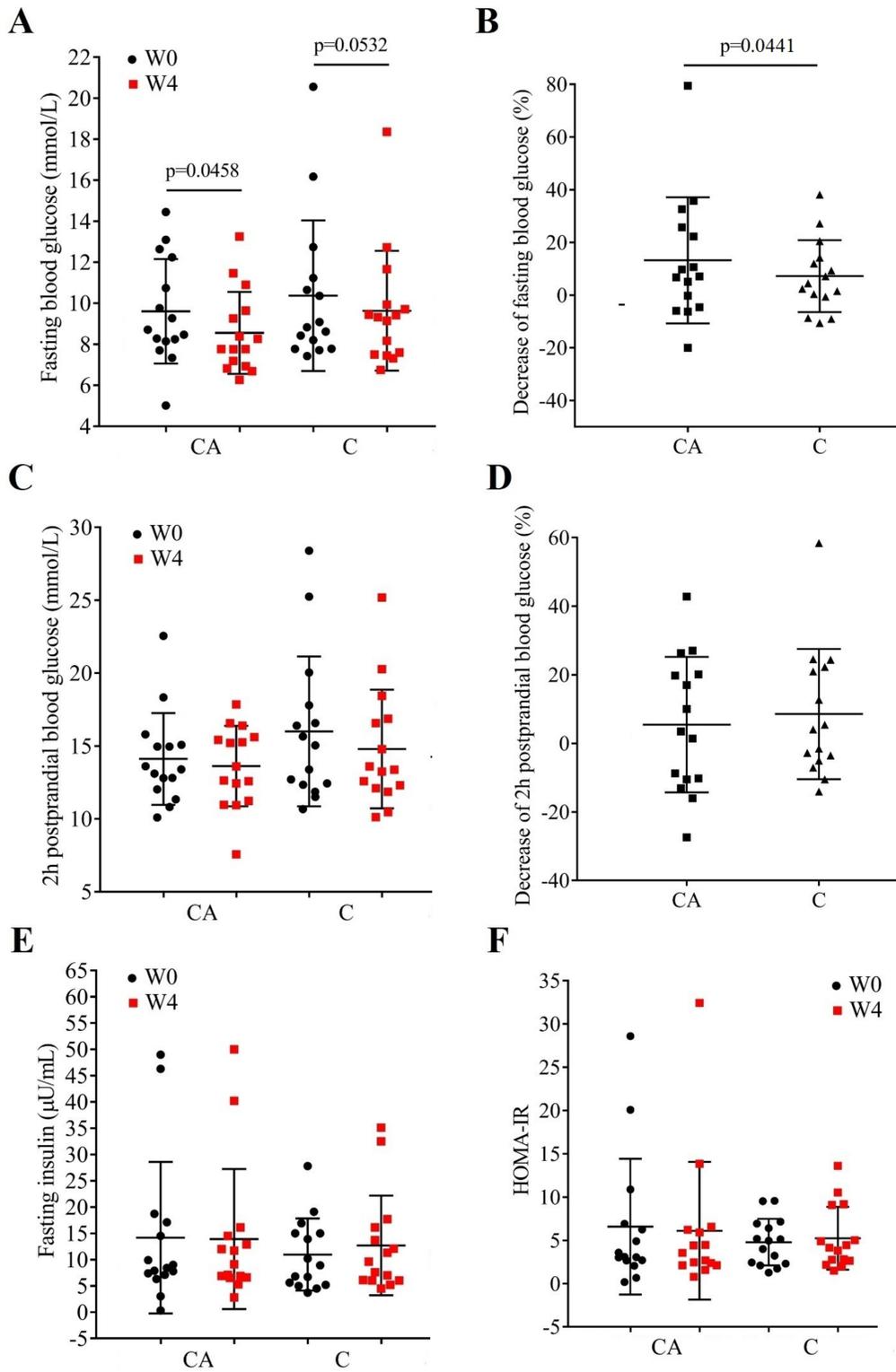


Figure 2

Fasting blood glucose, 2h postprandial blood glucose, fasting serum insulin and HOMA-IR in each group before (W0) and after (W4) the intervention. A, fasting blood glucose; B, decrease of fasting blood glucose; C, 2h postprandial blood glucose; D, decrease of 2h postprandial blood glucose; E, fasting serum insulin and F, HOMA-IR in patients intervened with cow milk (P, placebo), camel milk supplemented with BBA6 (CA), camel milk alone (C) and BBA6 alone (A)

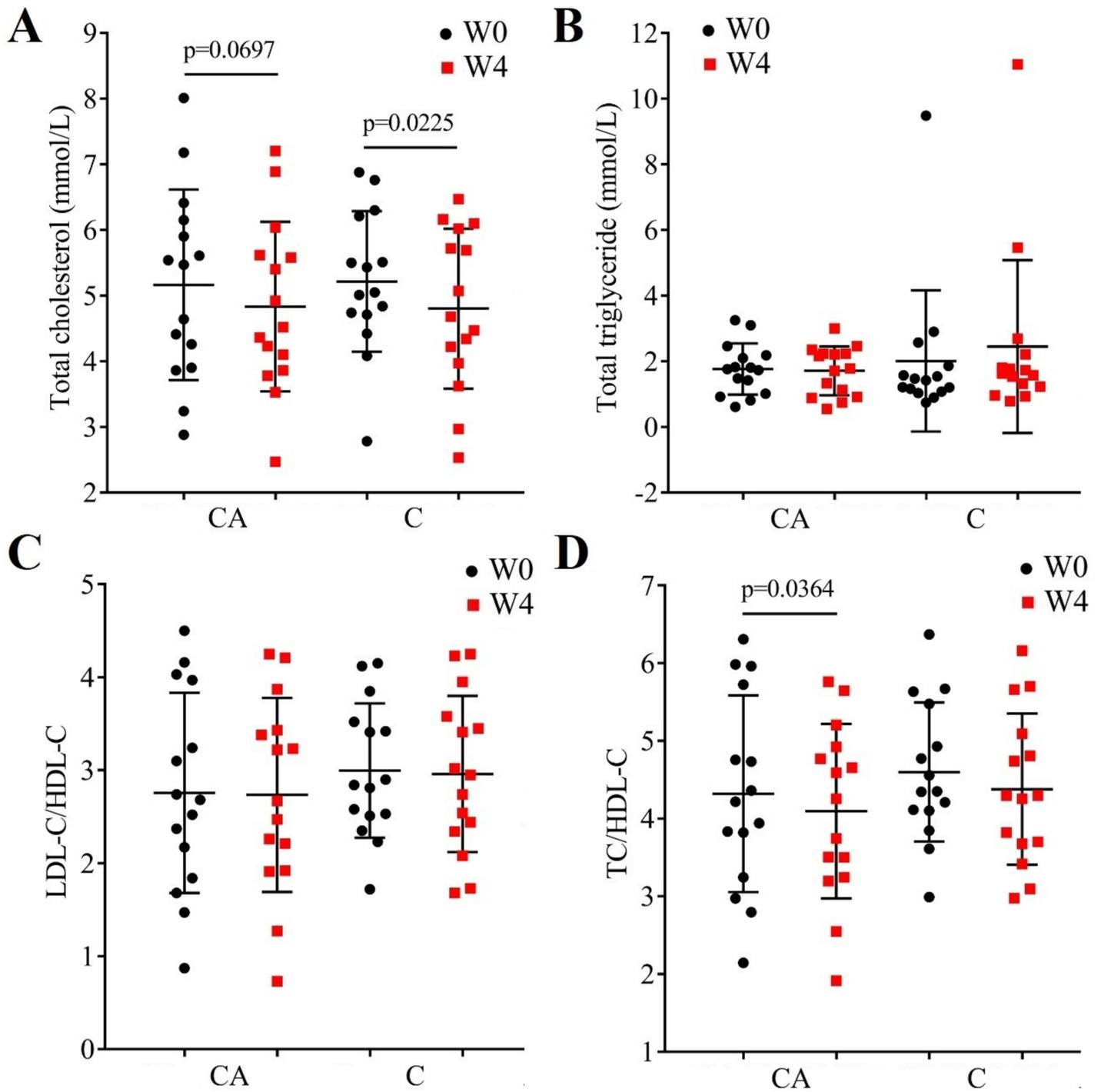


Figure 3

Lipid profile in each group before (W0) and after (W4) the intervention. A, total cholesterol; B, total triglyceride; C, the LDL cholesterol/HDL cholesterol ratio; D, total cholesterol/HDL-cholesterol ratio in patients intervened with cow milk (P, placebo), camel milk supplemented with BBA6 (CA), camel milk alone (C) and BBA6 alone (A).

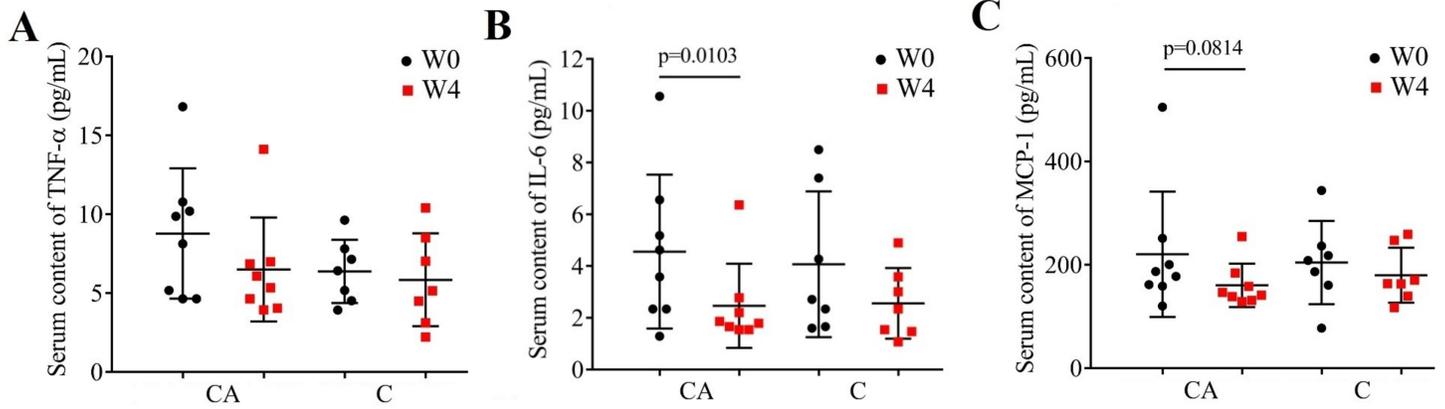


Figure 4

Serum contents of inflammatory cytokines in each group before (W0) and after (W4) the intervention. A, TNF- α ; B, IL-6; C, MCP-1 in patients intervened with cow milk (P, placebo), camel milk supplemented with BBA6 (CA), camel milk alone (C) and BBA6 alone (A).

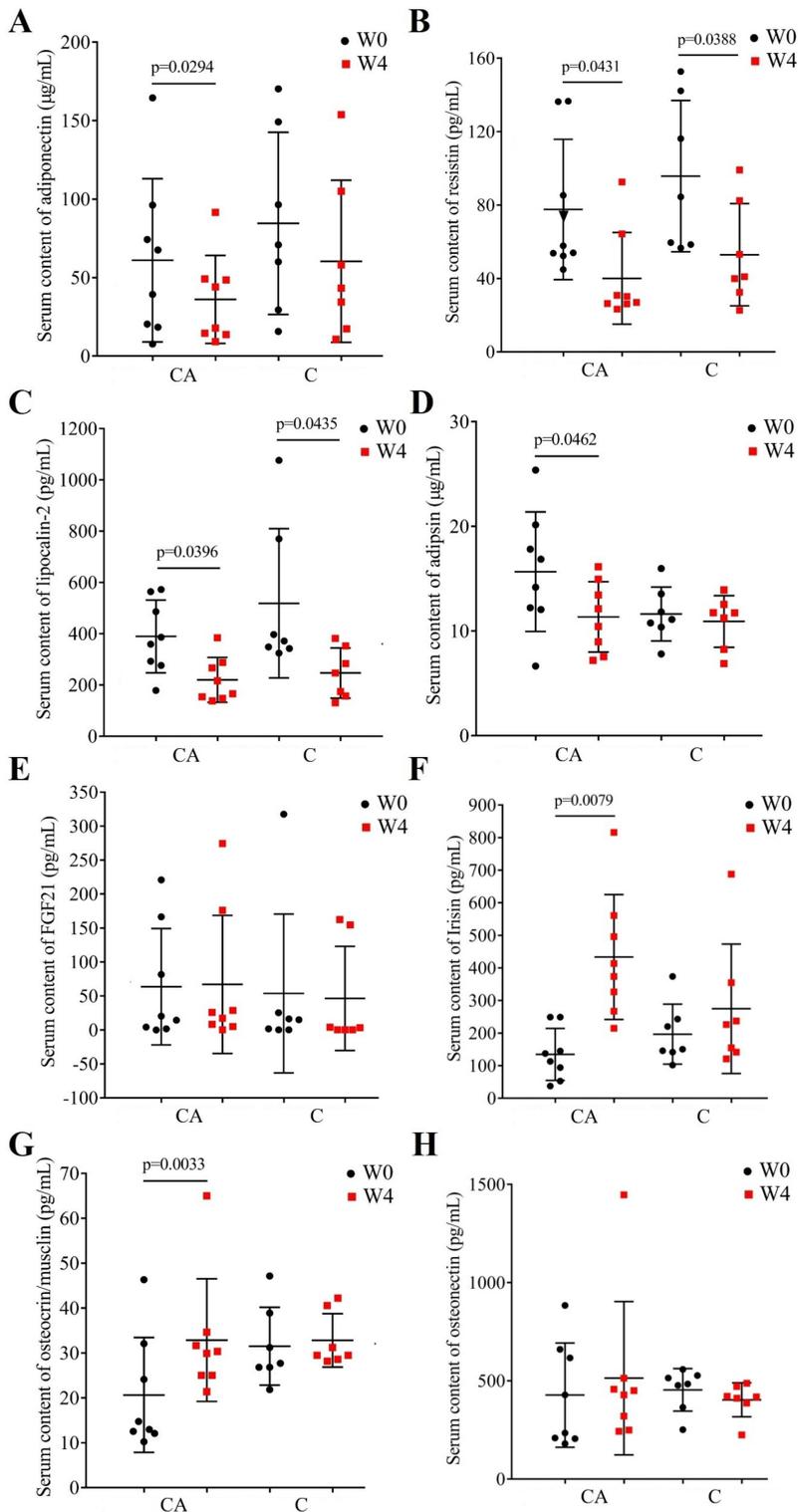


Figure 5

Serum contents of adipokines and myokines in each group before (W0) and after (W4) the intervention. A, adiponectin; B, resistin; C, lipocalin-2; D, adipsin; E, FGF-21; F, irisin; G, osteocrin; H, osteonectin in patients intervened with cow milk (P, placebo), camel milk supplemented with BBA6 (CA), camel milk alone (C) and BBA6 alone (A).

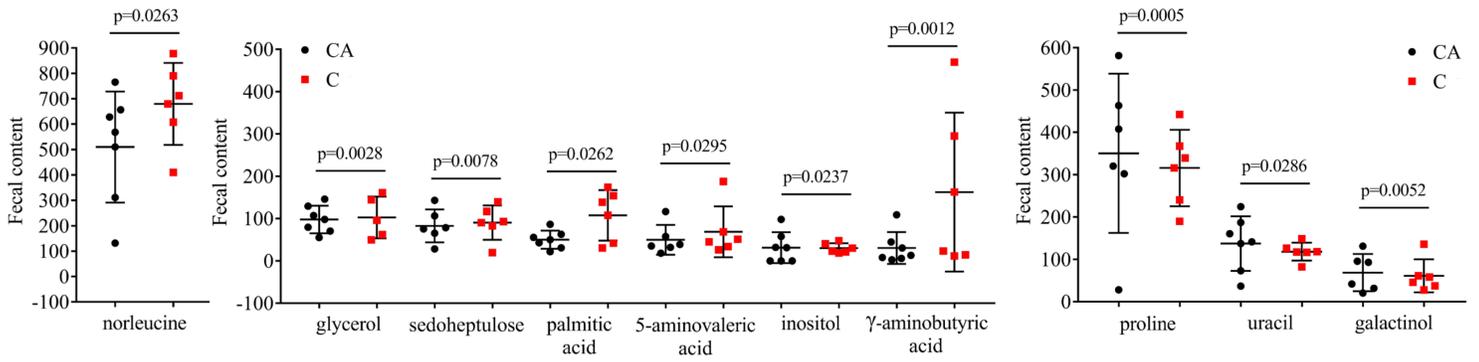


Figure 6

Different gut bacteria at the genus level in patients intervened with cow milk (P, placebo) and camel milk supplemented with BBA6 (CA) before (W0) and after (W4) the intervention analyzed by two-tailed Student's t-test. C and D, relative proportion of *Bifidobacterium animalis* in patients intervened with camel milk supplemented with BBA6 (CA) and BBA6 alone (A).

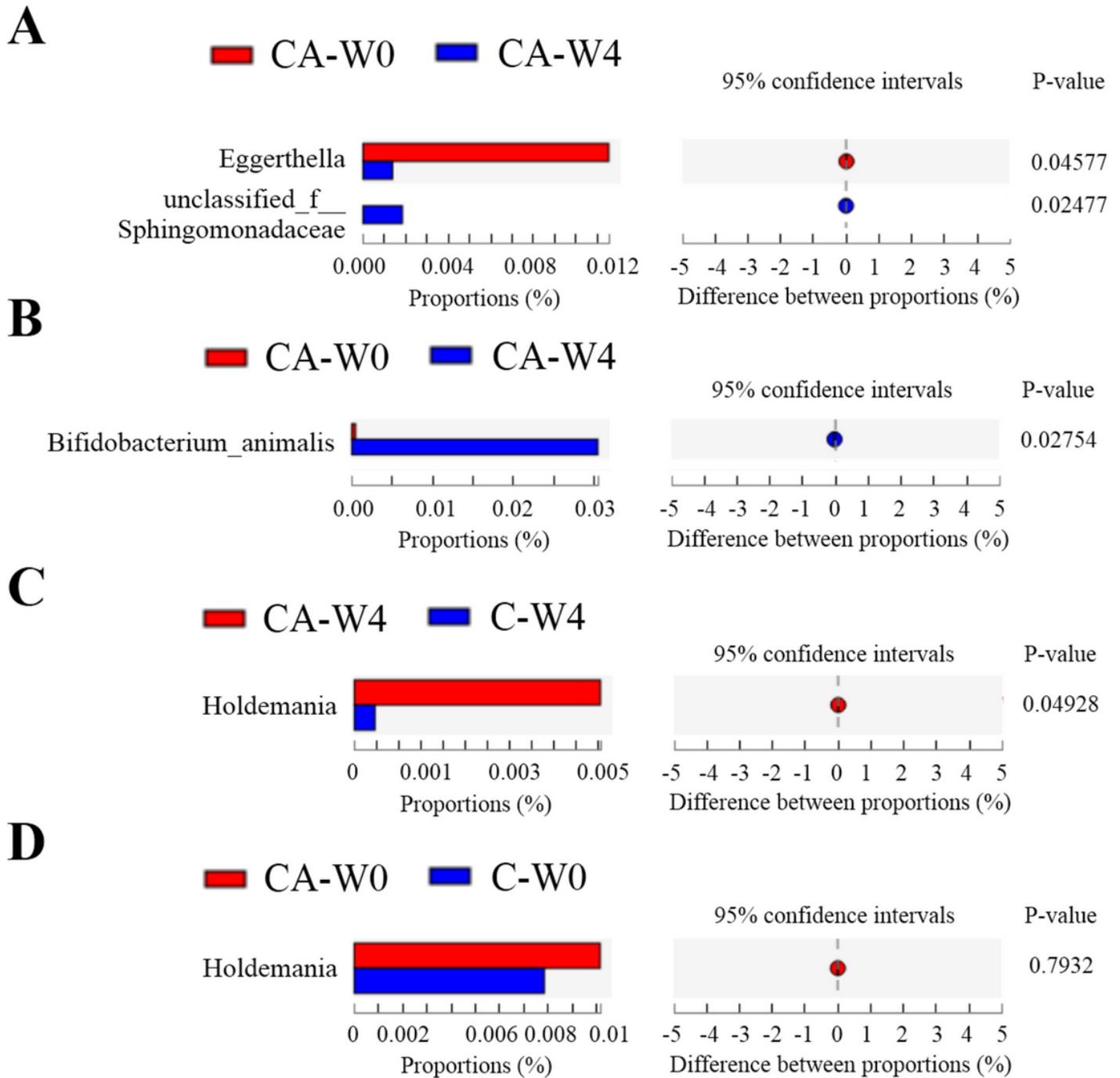


Figure 7

Different gut bacteria at the genus level in each group before (W0) and after (W4) the intervention analyzed by two-tailed Student's t-test. Patients intervened with A, cow milk (group P); B, camel milk supplemented with BBA6 (group CA); C, camel milk alone (group C) and D, BBA6 alone (group A).

Figure 8 was not provided with this version.

Figure 8

Amino acids (A) and amino acids metabolites (B) in fecal with significant changes between patients in group CA and P after the 4-week intervention.

Supplementary Files

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