Original Article

http://www.jcpjournal.org
pISSN 2288-3649 · eISSN 2288-3657
https://doi.org/10.15430/JCP.24.007

JCP

The Effect of A2 Milk on Gastrointestinal Symptoms in Comparison to A1/A2 Milk: A Single-center, Randomized, Double-blind, Cross-over Study

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β-Casein, a major protein in cow's milk, is divided into the A1 and A2 type variants. Digestion of A1 β-casein yields the peptide β-casomorphin-7 which could cause gastrointestinal (GI) discomfort but A2 milk containing only A2 β-casein might be more beneficial than A1/A2 (regular) milk. The aim of this study was to evaluate the differences in GI discomfort after ingestion of A2 milk and A1/A2 milk. A randomized, double-blind, cross-over human trial was performed with 40 subjects who experienced GI discomfort following milk consumption. For each intervention period, either A2 milk first (A2→A1/A2) or A1/A2 milk was first consumed for 2 weeks (A1/A2→A2) following a 2-week washout period. GI symptom rating scale (GSRS) scores, questionnaire for digestive symptoms, and laboratory tests including fecal calprotectin were evaluated. For symptom analysis, generalized estimating equations gamma model was used. A2 milk increased bloating (P = 0.041) and loose stools (P = 0.026) compared to A1/A2 milk in GSRS. However, A2 milk caused less abdominal pain (P = 0.050), fecal urgency (P < 0.001) and borborygmus (P = 0.007) compared to A1/A2 milk in questionnaire for digestive symptoms. In addition, fecal calprotectin also decreased or less increased after consumption of A2 milk compared to A1/A2 milk (P = 0.030), and this change was more pronounced in males (P = 0.005) than in females. There were no significant adverse reactions during the trial. A2 milk alleviated digestive discomfort in Koreans following A2 milk consumption (ClinicalTrials.gov NCT06252636 and CRIS KCT0009301).

Key Words Caseins, Gastrointestinal tract, Milk, Opioid, Receptors

INTRODUCTION

Casein is one of the two major proteins in cow's milk, accounts for about 80% of milk proteins, and is known to be related to cancer [1] as well as a number of diseases including type 1 diabetes [2], cardiovascular diseases [3], autism and neurological disorders [4], sudden infant death syndrome [5], and allergy [6], and classified into αs1- (39%–46% of total casein), αs2- (8%–11%), β- (25%–35%), and κ-casein (8%–15%) [7]. Of these, β-casein exists in at least 13 different forms, while the two most common forms are A1 and A2 β-casein [8]. A1/A2 (regular) milk contains both A1 and A2 β-casein, but A2 (A2/A2) milk contains only A2 β-casein.

Recent studies suggest that A2 milk may be a safer choice than regular milk in terms of gastrointestinal (GI) discomfort after consumption. In animal experiments, casein delayed gastric emptying and GI transit time [9]. Rats fed A1 type β-casein exhibited delayed GI transit compared to those fed A2 type β-casein [10]. This is because that β-casomorphin-7 (BCM-7), an opioid peptide, is released during the digestion of A1 type β-casein [11,12]. The structure of BCM-7 is similar to that of an endogenous opioid peptide that can activate the μ-opioid receptors and affect the GI motility, mucus production, and hormone production [13]. Furthermore, BCM-7 may affect the production and activation of lactase, and unabsorbed lactose affects colonic inflammation through changes in gut microbiota, and the delayed GI transit may increase the opportunity for lactose fermentation [13].

Lactose intolerance appears at similar frequencies in both sexes, and is known to be common especially in American Indians and Asians [14,15]. In South Korea, the prevalence varies from 39.1% to 84.1% depending on the report, and

Received May 18, 2024, Revised June 4, 2024, Accepted June 11, 2024, Published on June 30, 2024
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actual prevalence has not been clearly identified, yet [16]. Typical symptoms of lactose intolerance include diarrhea, abdominal pain, borborygmus, and flatus that are caused by lactose not absorbed from the small intestine and reached the large intestine. However, GI discomfort after milk congestion can occur even in cases not diagnosed with lactose intolerance or does not meet the diagnostic criteria, so the actual proportion of people feel discomfort after milk consumption in South Korea is thought to be much higher. In fact, human trials in New Zealand, China, Australia, and the United States reported the correlation between A1 type β-casein and decreased GI motility similarly [13], and one of them suggested that the discomfort after milk consumption might be due to A1 type β-casein, not to lactose intolerance [17]. However, no research has been conducted in South Korea, yet. From this background, this study was conducted to evaluate the difference between A2 milk and A1/A2 milk in terms of discomfort after consuming milk.

MATERIALS AND METHODS

This was a randomized, double-blind, cross-over human trial on subjects who experienced GI discomfort following milk consumption. Fifty subjects aged over 19 years were recruited between March and December 2023. Baseline screening tests were conducted two weeks before the first scheduled administration. Eligibility was based on the inclusion and exclusion criteria. The registered subjects were randomly assigned into two groups, and for each intervention period, either A2 milk or A1/A2 milk was consumed for 2 weeks following a 2-week washout period. Then A1/A2 milk or A2 was consumed for 2 weeks. The efficacy and safety of the intervention were assessed after each 2-week period (Fig. 1).

The random assignment of participants was performed using the permuted block randomized method, and the trial was designed as double-blinded to minimize bias. During the trial, use of medications that could affect the trial were prohibited, and the individual history of milk consumption was checked through questionnaires at each visit. To maintain double-blind nature of the trial, the details of the information on randomization for each group were sealed and kept undisclosed by the administrator. The study protocol was registered on ClinicalTrials.gov (NCT06252636) and Clinical Research Information Service (CRIS) (KCT0009301), and approved by the Seoul National University Bundang Hospital Institutional Review Board (IRB) (B-2302-808-001). Written informed consent was obtained from all the participants.

The results of a previous study that used the GI symptom rating scale (GSRS) as the primary efficacy variable were referred [18]. The average difference between the two groups was estimated to be 2.5, and the standard deviation was estimated to be 5.0 for calculating the required number of subjects. As a result, the number of subjects was decided to be 16 in each group, assuming Type I error of 5%, Type II error of 20%, and an elimination rate of 20%.

The primary efficacy measure in this study was based on the improvement in GSRS upper abdominal symptom scores after 12 weeks of administration compared to baseline. The GSRS is a symptom-specific instrument comprising 15 items combined into five symptom clusters depicting reflux, abdominal pain, indigestion, diarrhea and constipation, which has a five-point graded Likert-type scale [19]. The secondary efficacy measure included questionnaire for digestive symptoms designed for this trial asking about seven GI symptoms with a four-point graded Likert-type scale (Table S1), bowel habit changes, and laboratory tests including fecal calprotectin.

Laboratory tests including C-reactive protein, interleukin (IL)-4, immunoglobulin (Ig) G, IgE, and BCM-7 were measured. In addition, fecal calprotectin, which is a marker for organic GI disease and intestinal inflammation, was additionally measured [20]. Blood and stool samples were collected at before and after each period, and were analyzed by an outsourced laboratory, and discarded in accordance with laboratory regulations.

All treatment-emergent adverse events reported during the trial 157 were carefully documented and coded according to MedDRA (version 3.0). Any abnormal reactions observed after the ingestion of the trial food were charted and evaluated.

The data obtained from the participants in this trial were analyzed in three main forms: safety, full analysis (FA), and per protocol (PP). For evaluation variable, intragroup comparison of changes was analyzed using the paired *t*-test. The comparison between the two groups was analyzed using the two-sample *t*-test or Wilcoxon rank-sum test, depending on the normality of the data. In addition, generalized estimating equations (GEEs) gamma model was used for additional analyses of symptom scores. Statistical analyses were performed using SAS® version 9.4 (SAS Institute).

RESULTS

Study participation and demographics

Out of the 50 subjects, 40 were randomly assigned excluding 10 subjects who failed the screening process. Among the assigned subjects, finally 17 subjects in the AP group (A2→A1/A2) and 18 subjects in the PA group (A1/A2→ A2) were included in the PP set. Three subjects in the AP group and two subjects in the PA group were excluded from the PP set due to consent withdrawal or violation of the selection/exclusion criteria (Fig. 2). There were no significant differences in baseline clinicopathological characteristics, frequency and amount of milk consumption, and GI symptoms between the two groups (Table 1).

Changes of GSRS scores

GSRS changes before and after milk consumption analyzed by GEE (gamma distribution) are described in Table 2. In case of A2 milk, in period 1, score for loose stools was sig-

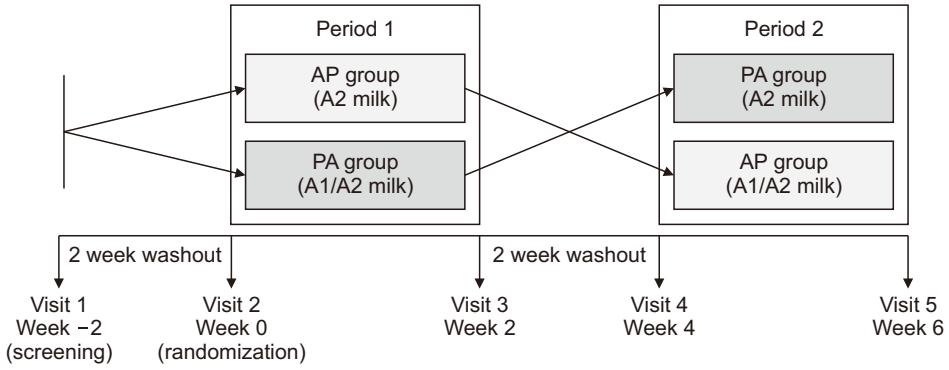

Figure 1. Study design. AP group, A2 milk consumption followed by A1/A2 milk consumption (A2→A1/A2); PA group, A1/A2 milk consumption followed by A2 milk consumption (A1/A2→A2).

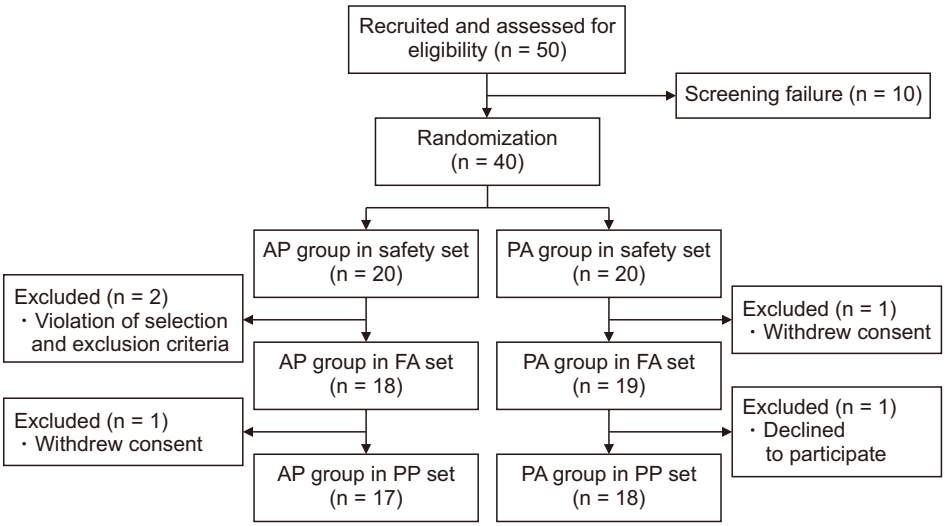

Figure 2. Study flow chart. AP group, A2 milk consumption followed by A1/A2 milk consumption (A2→A1/A2); PA group, A1/A2 milk consumption followed by A2 milk consumption (A1/A2→A2). FA, full analysis; PP, per-protocol.

nificantly increased (P = 0.031); scores for rumbling, bloating, constipation and loose stools were increased, and score for diarrhea was increased after A2 milk consumption. In period 2, score for loose stools was increased, and scores for rumbling, bloating, burping, flatus, constipation, and hard stools were decreased after A2 milk consumption. In case of A1/A2 milk, in period 1, score for rumbling was increased, and scores for acid reflux, hunger pain, nausea, bloating, burping, flatus, diarrhea, fecal urgency, and sensation of incomplete emptiness were decreased after A1/A2 milk consumption. In period 2, score for flatus was increased, and scores for loose stools and hard stools were increased after A1/A2 milk consumption. However, statistically significant difference in GSRS scores was not observed. A statistically significant difference was observed between A2 and A1/A2 milk in bloating (P = 0.041) and loose stools (P = 0.026).

Changes of GI symptom score

The changes of results of a questionnaire based on the frequency of GI symptoms analyzed by GEE (gamma distribution) are presented in Table 3. In case of A2 milk, in period 1, score for borborygmus increased, whereas scores for bloating, burping, postprandial distress, abdominal pain, rumbling,

Table 1. Baseline characteristics of the subjects

	AP group (n = 17)	PA group (n = 18)	*P*-value
Sex			
Female	5 (29.4)	5 (27.8)	>0.999[a]
Male	12 (70.6)	13 (72.2)	
Age (yr)	37.47 ± 13.30	37.11 ± 14.38	0.939[b]
Hight (cm)	162.35 ± 5.85	165.99 ± 9.19	0.174[b]
Weight (kg)	61.39 ± 10.90	60.87 ± 11.16	0.891[b]
Frequency of milk consumption (per week)			
1–2	17 (100)	17 (94.4)	>0.999[a]
3–4	0 (0)	1 (5.6)	
More than 5	0 (0)	0 (0)	
Amount of milk consumption (mL)			
<200	13 (76.5)	15 (83.3)	0.691[a]
200–400	4 (23.5)	3 (16.7)	
≥400	0 (0)	0 (0)	
GSRS total score	4.28 ± 2.67	5.28 ± 6.26	0.777[c]

Values were presented as number (%) or mean ± standard deviation. AP group, A2 millk consumption followed by A1/A2 milk consumption (A2→A1/A2); PA group, A1/A2 milk consumption followed by A2 milk consumption (A1/A2→A2). GSRS, gastrointestinal symptom rating scale. *P*-values were calculated by [a]Fisher's exact test, [b]two sample *t*-test or [c]Wilcoxon rank sum test.

Table 2. Gastrointestinal symptom rating scale changes before and after milk consumption

		A2 milk			A1/A2 milk			*P*-value[b]
		Before	After	*P*-value[a]	Before	After	*P*-value[a]	
Period 1	Abdominal pain	-	-	-	-	-	-	-
	Heartburn	-	-	-	-	-	-	-
	Acid reflux	1.00 ± 0.00	-	-	1.20 ± 0.18	1.00 ± 0.00	0.500	-
	Hunger pain	-	1.00 ± 0.00	-	1.20 ± 0.18	1.00 ± 0.00	0.125	-
	Nausea	-	-	-	1.33 ± 0.27	1.00 ± 0.00	0.500	-
	Rumbling	0.96 ± 0.03	1.17 ± 0.11	0.289	1.07 ± 0.18	1.27 ± 0.16	0.453	0.905
	Bloating	0.91 ± 0.11	1.37 ± 0.22	>0.999	1.38 ± 0.25	1.15 ± 0.10	0.531	0.041
	Burping	1.00 ± 0.00	1.00 ± 0.00	0.125	1.22 ± 0.14	1.05 ± 0.15	0.063	0.318
	Flatus	1.18 ± 0.12	1.18 ± 0.12	0.789	1.38 ± 0.38	1.15 ± 0.23	>0.999	0.661
	Constipation	1.00 ± 0.00	1.50 ± 0.43	>0.999	1.00 ± 0.00	1.00 ± 0.00	>0.999	0.160
	Diarrhea	1.61 ± 0.39	1.31 ± 0.24	>0.999	1.39 ± 0.27	1.12 ± 0.07	>0.999	0.984
	Loose stools	0.96 ± 0.04	1.11 ± 0.10	0.031	1.28 ± 0.23	1.28 ± 0.23	>0.999	0.623
	Hard stools	1.17 ± 0.15	1.17 ± 0.15	0.375	1.13 ± 0.12	1.13 ± 0.12	0.109	0.266
	Fecal urgency	1.00 ± 0.00	1.00 ± 0.00	>0.999	1.25 ± 0.22	1.00 ± 0.00	>0.999	0.198
	Sensation of incomplete emptiness	1.00 ± 0.00	1.00 ± 0.00	>0.999	1.17 ± 0.15	1.00 ± 0.00	0.625	0.237
	Total	4.44 ± 0.61	4.86 ± 0.63	0.770	5.43 ± 1.46	4.40 ± 0.81	0.174	0.255
Period 2	Abdominal pain	-	-	0.688	-	-	0.500	-
	Heartburn	-	-	-	-	-	-	-
	Acid reflux	-	-	>0.999	-	-	-	-
	Hunger pain	-	-	>0.999	-	-	>0.999	-
	Nausea	-	-	>0.999	-	-	-	-
	Rumbling	1.13 ± 0.12	1.00 ± 0.00	0.109	1.00 ± 0.00	1.00 ± 0.00	>0.999	0.257
	Bloating	1.29 ± 0.17	1.00 ± 0.00	>0.999	1.00 ± 0.00	1.00 ± 0.00	>0.999	0.058
	Burping	1.13 ± 0.12	1.00 ± 0.00	0.188	1.00 ± 0.00	1.00 ± 0.00	>0.999	0.257
	Flatus	1.33 ± 0.27	1.00 ± 0.00	>0.999	1.00 ± 0.00	1.20 ± 0.18	>0.999	0.063
	Constipation	1.25 ± 0.22	1.00 ± 0.00	0.500	1.00 ± 0.00	1.00 ± 0.00	>0.999	0.198
	Diarrhea	-	-	>0.999	-	-	>0.999	-
	Loose stools	1.00 ± 0.00	1.50 ± 0.35	0.531	1.33 ± 0.27	1.00 ± 0.00	0.453	0.026
	Hard stools	1.50 ± 0.35	1.00 ± 0.00	>0.999	1.25 ± 0.22	1.00 ± 0.00	0.250	0.533
	Fecal urgency	-	-	>0.999	-	-	>0.999	-
	Sensation of incomplete emptiness	-	-	>0.999	-	-	0.625	-
	Total	5.42 ± 0.84	3.53 ± 0.67	0.398	4.23 ± 0.47	3.00 ± 0.53	0.847	0.801

Values are presented as least squares mean ± standard error. Period 1, the consumption period for the first intervention milk; Period 2, the consumption period for the second intervention milk. *P*-values were calculated using [a]Wilcoxon signed rank test or [b]generalized estimating equation (gamma distribution).

and fecal urgency decreased after A2 milk consumption. In period 2, scores for bloating, burping, postprandial distress, rumbling, borborygmus, and fecal urgency increased after A2 milk consumption. In case of regular milk, in period 1, scores for rumbling, and fecal urgency were increased, and scores for bloating, burping, and postprandial distress decreased after A1/A2 milk consumption. In period 2, scores for bloating, burping, postprandial distress, rumbling, and borborygmus increased, and score for fecal urgency decreased after A1/A2 milk consumption. Compared to A1/A2 milk, A2 milk showed beneficial changes (more decrease or less increase of symptom scores after consumption) for abdominal pain ($P = 0.050$) and fecal urgency ($P < 0.001$) in period 1, and for borborygmus ($P = 0.007$) in period 2.

Changes of fecal calprotectin

The changes of fecal calprotectin are presented in Table 4. In case of A2 milk, fecal calprotectin level was increased in period 1 and decreased in period 2 after A2 milk consumption. In case of regular milk, a fecal calprotectin level was increased in period 1 and decreased in period 2 after A1/A2 milk consumption. A statistically significant difference was observed in comparison between A2 and A1/A2 milk in period 1 ($P = 0.030$). In addition, subgroup analysis according to sex revealed that this change was more pronounced in males ($P = 0.005$) than in females.

Other laboratory tests including C-reactive protein, IL-4, IgG, IgE, and BCM-7 were measured and analyzed (Table S2). However, no significant statistical differences or trends were identified.

Table 3. Gastrointestinal symptom score changes before and after milk consumption

		A2 milk			A1/A2 milk			*P*-value[b]
		Before	After	*P*-value[a]	Before	After	*P*-value[a]	
Period 1	Bloating	1.41 ± 0.35	1.20 ± 0.22	>0.999	1.63 ± 0.25	1.31 ± 0.22	0.031	0.886
	Burping	1.76 ± 0.35	1.33 ± 0.28	0.484	1.60 ± 0.24	1.24 ± 0.14	0.699	0.959
	Postprandial distress	1.74 ± 0.41	1.26 ± 0.21	>0.999	2.02 ± 0.29	1.31 ± 0.28	0.250	0.776
	Abdominal pain	2.00 ± 0.71	1.00 ± 0.00	>0.999	1.00 ± 0.00	1.00 ± 0.00	>0.999	0.050
	Rumbling	2.19 ± 0.45	1.35 ± 0.37	0.750	1.69 ± 0.50	1.70 ± 0.21	0.906	0.351
	Borborygmus	1.50 ± 0.35	1.67 ± 0.27	0.500	1.00 ± 0.00	1.50 ± 0.35	>0.999	0.419
	Fecal urgency	2.50 ± 0.35	1.00 ± 0.00	0.813	1.00 ± 0.00	1.50 ± 0.31	0.375	<0.001
Period 2	Bloating	1.00 ± 0.16	1.50 ± 0.23	0.063	1.00 ± 0.00	1.17 ± 0.15	0.109	0.219
	Burping	1.00 ± 0.00	1.57 ± 0.19	0.055	1.33 ± 0.30	1.67 ± 0.30	0.789	0.468
	Postprandial distress	1.13 ± 0.17	1.36 ± 0.20	>0.999	1.09 ± 0.08	1.17 ± 0.15	0.063	0.516
	Abdominal pain	-	-	-	-	-	-	-
	Rumbling	1.00 ± 0.00	1.40 ± 0.22	0.125	1.00 ± 0.00	1.50 ± 0.25	0.375	0.763
	Borborygmus	1.00 ± 0.00	1.25 ± 0.22	0.500	1.00 ± 0.00	2.00 ± 0.00	0.750	0.007
	Fecal urgency	1.14 ± 0.17	1.29 ± 0.20	>0.999	1.37 ± 0.29	1.32 ± 0.20	0.656	0.538

Values are presented as least squares mean ± standard error. Period 1, the consumption period for the first intervention milk; Period 2, the consumption period for the second intervention milk. *P*-values were calculated using [a]Wilcoxon signed rank test or [b]generalized estimating equation (gamma distribution).

Table 4. Fecal calprotectin levels before and after milk consumption

		A2 milk			A1/A2 milk			*P*-value[c]
		Before	After	*P*-value	Before	After	*P*-value	
Period 1	Total	41.55 ± 20.35	56.07 ± 24.61	0.762[a]	20.56 ± 3.66	330.82 ± 299.77	0.464[a]	0.030
	Male	83.90 ± 62.97	11.70 ± 2.27	0.313[a]	21.88 ± 4.73	25.77 ± 9.21	0.829[b]	0.005
	Female	23.90 ± 7.39	76.15 ± 34.10	0.232[a]	20.05 ± 4.72	424.20 ± 388.33	0.414[a]	0.080
Period 2	Total	29.63 ± 10.76	22.04 ± 6.20	0.787[a]	109.14 ± 77.94	31.39 ± 11.09	0.225[a]	0.218
	Male	43.14 ± 21.74	14.54 ± 3.83	0.373[b]	46.00 ± 20.23	21.54 ± 8.21	0.189[b]	0.623
	Female	23.91 ± 11.80	24.12 ± 8.09	0.978[b]	135.44 ± 109.20	35.50 ± 15.18	0.519[a]	0.118

Values are presented as least squares mean ± standard error (mg/kg). Per protocol analysis. Period 1, the consumption period for the first intervention milk; Period 2, the consumption period for the second intervention milk. *P*-values were calculated using [a]Wilcoxon signed rank test, [b]paired *t*-test or [c]generalized estimating equation (gamma distribution).

Table 5. Bowel habit changes before and after milk consumption

		Before	After			*P*-value[a]	*P*-value[b]
			Constipation	Normal	Diarrhea		
A2 milk	Constipation	2 (5.7)	2 (5.7)	0 (0)	0 (0)	0.543	0.207
	Normal	21 (60.0)	1 (2.9)	16 (45.7)	4 (11.4)		
	Diarrhea	12 (34.3)	1 (2.9)	3 (8.6)	8 (22.9)		
A1/A2 milk	Constipation	5 (14.3)	3 (8.6)	1 (2.9)	1 (2.9)	0.456	
	Normal	20 (57.1)	2 (5.7)	10 (28.6)	8 (22.9)		
	Diarrhea	10 (28.6)	1 (2.9)	3 (8.6)	6 (17.1)		

Values are presented as number (%). Per protocol analysis. *P*-values were calculated by [a]McNemar test or [b]generalized estimating equation (multi-nominal).

Changes of bowel habit

No significant differences were found in bowel frequency and stool consistency between A2 milk and A1/A2 milk consumption. However, there was a tendency that more subjects with normal bowel movements had developed diarrhea after A1/A2 milk consumption than consumption of the other, although the difference was not significant (Table 5).

Safety

There were no reported adverse reactions during the study

period, regardless of severity and causality.

DISCUSSION

In this randomized, cross-over study, we compared A2 milk with regular milk in Koreans with discomfort symptoms following milk consumption. We observed the beneficial effect of A2 milk, such as the improvement or lesser increase of GI discomfort symptom scores and fecal calprotectin level, a marker for colon inflammation, although not all the results were consistently significant. These results suggest that A2 milk may alleviate GI discomfort in people who appeal milk-related discomfort symptoms.

As described previously, regular milk contains both A1 and A2 type β-casein, and the digestion of A1 type β-casein produce BCM-7 [11,12], which can activate the μ-opioid receptors and affect the GI motility, mucus production, hormone production, and the production and activation of lactase [13]. A number of animal studies have demonstrated that casein or their derivatives can delay GI motility via the μ-opioid receptor pathway. Experiments in young Wistar rats showed that casein delayed gastric emptying and GI transit time, and the effect was offset by naloxone, the opioid antagonist [9]. Another study in dogs also reported that casein significantly reduced the amplitude and frequency of small intestine contraction, and naloxone inhibited these effect by casein [21]. According to the subtype of casein, rats fed A1 type β-casein exhibited delayed GI transit time, and administration of naloxone offset the effect, but such changes were not observed in rats fed A2 type β-casein [10]. These results demonstrate that A1 type β-casein affects GI function via the opioid-dependent pathway.

There are also human trials demonstrating that A2 milk reduces discomfort symptoms compared with regular milk in people with GI discomforts after milk consumption [17,22-26]. In particular, Jianqin et al. [17] reported that A1/A2 milk consumption significantly prolonged the colonic and overall GI transit time compared to A2 milk, and GI symptoms were associated with A1 type β-casein rather than lactose itself. Furthermore, Pal et al. [13] suggested that the inflammatory properties of BCM-7 may affect the production and activation of lactase. Consequently, unabsorbed lactose affected colonic inflammation through changes in the intestinal microbiota, and delayed GI migration might increase the chances of lactose fermentation [13]. Taken together, it seems that A2 milk is superior to regular milk in terms of discomfort after consumption.

As mentioned above, there are studies demonstrating that A1 β-casein or BCM-7 increases the risk of certain diseases [27], including type 1 diabetes [2], cardiovascular diseases [3], autism and neurological disorders [4], sudden infant death syndrome [5], allergy [6], and even cancers [1]. However, it is not known whether these results mean medical causality or a simple correlation. In contrast, A2 milk seems to be out of this controversy. In terms of diseases of GI tract, A1 β-casein was reported to be associated with chronic constipation, altered GI transit, inflammation, and lactose intolerance symptoms, while the advantages of A2 β-casein with superiority in terms of easier digestion and absorption, and fewer symptoms of lactose intolerance than A1 β-casein were rather well supported [27].

In this study, elevation of the fecal calprotectin level occurred in some subjects, especially after A1/A2 milk consumption. There have been few previous studies on the correlation between casein protein and intestinal inflammation. In addition, a pilot study showed abnormally high fecal calprotectin values after 14 days of A1 β-casein exposure but not of A2 β-casein [22]. This result suggests the pro-inflammatory effect of A1 β-casein, in connection with findings from previous studies [10,28]. However, the number of cases was too small to verify statistical significance and draw a conclusion. In the future, a large-scale study about the inflammatory effects of A1 β-casein including symptoms, fecal calprotectin levels, and endoscopic findings are needed.

It has been reported that casein protein modulates gut microbiota in studies using animal models [29-33]. For instance, A2 β-casein-fed 20-month-old mice showed higher content of beneficial fecal short chain fatty acids (SCFAs), in particular, isobutyrate, and higher proportion of intestinal CD4+ and CD19+ lymphocytes in the intraepithelial compartment as well as improved villi tropism [29]. In comparison, the A1/A2 group showed higher percentages of intestinal TCRγδ+ lymphocytes, and Ruminococcaceae became the most discriminant family [29]. Taken together, these findings indicate that A2 β-casein seems to have a positive effect on gut immunology and morphology in an aged mice model [29]. There have been human studies on the association of milk protein allergy or lactose intolerance with the gut microbiota [34,35].

The relation of casein protein to cancer is not well understood, but there are some reports on this. The beneficial action of casein protein is generally thought to be based on immune enhancement. Casein peptides are reported to act as enhancers of the immune system and induce death of malignant cells [1], and could be involved in the development of the mucosal immune system [36] and erythropoiesis [37] in mice. Casein also inhibited the azoxymethane-induced colon carcinogenesis in rats [38]. In addition, there are reports on the role of α-casein protein in suppressing cancer [39,40]. High α-casein expression was associated with increased recurrence-free survival in triple negative breast cancer patients via regulating the STAT/HIF-1α signaling pathway [39]. In particular, a difference in fecal calprotectin levels between groups according to A2 milk consumption was observed in this study, which theoretically raises the possibility that A2 β-casein may reduce inflammation and thus prevent the colon cancer carcinogenesis.

Notably, preliminary data from the follow-up study showed that the proportion of *Bifidobacterium longum* was significant-

ly increased after A2 milk consumption. *B. longum* is known to show beneficial effects by producing butyrate, and the decrease of *B. longum* was observed in patients with colonic adenoma and cancer compared to healthy controls [41]. In addition, the administration of *B. longum* reduced stress-induced gut dysbiosis in a water-avoidance stress rat model [42]. However, there are no studies on the role of casein in carcinogenesis according to the subtype of β-casein so far, and more studies focusing on cancer prevention are required.

This study has several limitations. First, not all indicators and items were statistically significant. Basically, it is thought to be because most of the subjects who participated in this trial had only mild symptoms after drinking milk, so the difference between before and after milk consumption may be not dramatically significant. If they had moderate or severe symptoms, they would have refused to participate in this crossover study. In addition, diets other than milk tested cannot be restricted in human experiments unlike animal experiments, which may have acted as a limitation in the analysis. However, we educated the participants not to eat dairy products.

Second, the results of the analyses of GSRS total score and questionnaire for digestive symptoms were not completely matched. This is also likely due to the small difference in symptoms between before and after milk consumption. There is still another reason; in the case of GSRS, it asks study subjects to respond from 0 to 4 depending on the severity of symptoms, but in the questionnaire used in our study, the scale was 0 to 3, based on the frequency, not the severity of symptoms because a questionnaire based on the frequency of symptoms may be more accurate in the cases of trials targeting subjects with mild symptoms.

Nevertheless, this study is the first trial to compare A2 milk and A1/A2 milk in Koreans as far as we know. We observed some beneficial effects of A2 milk on discomforts after milk consumption and fecal calprotectin changes, and suggested the possibility of differences between males and females. In addition, gut microbial analyses are undergoing to illustrate the underlying mechanisms, since modulatory effects of milk proteins on gut microbiota is emerging [29-33].

In conclusion, A2 milk is thought to be an effective and safe alternative for people with GI discomfort after milk ingestion. Further human trials with an increased sample size are needed to obtain clearer results.

FUNDING

This work was supported by the Seoul National University Bundang Hospital Research fund (grant no. 06-2023-0088). In addition, the clinical study was supported by Seoulmilk.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.15430/JCP.24.007.

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