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Effects of Drinking Cows' Milk at Breakfast in Promoting Sleep-Health in Japanese University Athletes

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Abstract

This study evaluates the effects of milk intake for 20 days at breakfast on diurnal type (chronotype), sleep habits and soccer performance in Japanese university male athletes attending a university soccer club. Seventy three athletes were asked to take 200 ml of cows' milk at breakfast for 21 consecutive days during November and December, 2014. Twenty athletes attending the same soccer club did not drink cows' milk for the same period of time and acted a control group. An integrated questionnaire was administered twice, before the intervention period and 1 month after it to all 93 participants. The questionnaire included questions on sleep habits and diurnal type. On the 10th day and 21st days of the intervention period, a questionnaire on performance/skill was administered to all participants. The group which drank cows' milk showed higher frequency of improvement of soccer performance than did the control group did (performance—where higher values indicate less skill: milk drinking group=29.92, control group=31.9 on day 10; milk drinking group=28.21, control group=31.9 on day 21), and also judged that their soccer performance had improved more after 21 days than 10 days of the intervention. Those participants who changed diurnal type to becoming more morning-typed were more likely to judge that their soccer performance had improved than did those who showed no change in diurnal type.

Keywords: Japanese athletes as university soccer club members, circadian typology, sleep habits, soccer performance

1. Introduction

Nongonierma and FitzGerald (2015) described how cows' milk proteins promoted human health in eight ways. The first way was "Improving satiety and weight-management", the second was "Reducing risk of heart disease"; the third was "Having an antimicrobial role", the fourth was "Having anti-inflammatory effects", the fifth was "Showing anticancer effects", the sixth was "Exerting antioxidant effects", the seventh was "Affecting insulin secretion and serum glucose regulation" and the eighth was "An action upon muscle protein synthesis". With regard to the relationship between sleep health and the intake of cows' milk, Laird and Drexel (1934) reported that a meal of cornflakes and milk strongly improved sleep quality (as judged from an uninterrupted night's sleep). Later, Brezinova and Oswald (1972) showed, using electroencephalography, that sleep was significantly improved (longer and un-interrupted night sleeps) in older people when they ate a combination of cows' milk and cereal before going to bed. However, there have been only a few studies on the effects of drinking cows' milk in the morning on any improvement in sleep health. For example, Takeuchi et al. (2014) reported the two results of a questionnaire study on Japanese infants aged 2-6 years old. The first result was that infants who ate carbohydrate (or carbohydrate and protein) plus milk at breakfast were more of a morning type and showed better sleep quality than those who ate only carbohydrate (or carbohydrate and protein). The second one was that infants who drank milk at breakfast were less frequently depressed than those who did not do so.

It has been suggested that protein intake at breakfast would raise blood tryptophan levels, and that this would, through the synthesis of serotonin (an anti-depressive agent), be effective in promoting mental health and morning-type habits and also, following synthesis into melatonin, which promotes sleep onset at night (Harada et al., 2007; Nakade et al., 2009, 2012; Wada et al., 2013). For Japanese children, drinking cows' milk at breakfast is an important source of tryptophan and could, on the basis of a questionnaire study (Takeuchi et al., 2014), be supposed to promote the mental and sleep health, although there have been no intervention studies to test this hypothesis. However, a group of adult athletes (Japanese university soccer team members) was given a leaflet titled: "Three benefits: Go to bed early! Get up early! Eat a nutritionally rich breakfast!" and then encouraged to act upon this advice for a period of one month. It was observed that their soccer performance, sleep health and mental health all improved after the intervention (Harada et al., 2016). It is possible that an increased intake of cows' milk at breakfast, as a source of tryptophan, could also improve soccer performance, sleep health and mental health. However, there have been no intervention studies to test this possibility, and so the purpose of this study is to test this hypothesis.

2. Participants and Methods

2.1 Participants

The study involved 93 participants, 60% of whom were from Shikoku Island, the others coming from all over Japan. Two authors of this manuscript are the general manager and the coach of the soccer club of Kochi University, and the subjects were recruited by them by personal contact and word of mouth. The participants were 19-25 years old.

2.2 Design-1: Experimental and Control Groups

The experimental group (n=73) drank 200 ml of cows' milk at breakfast each morning for a period of 21 days, from 13th November, 2014, to 4th December, 2014. The milk was provided for them. The control group (n=20) did not drink cows' milk for breakfast during this period. The control group consists of men who do not like to drink cows' milk. There were no significant differences in age, diurnal type scale and sleep habits (wake-up time, bedtime and sleep hours both in the weekdays and at the weekend) between the two groups. After the questionnaires and sleep diaries had been distributed, participants were allowed to answer them at home.

2.3 Design-2: Contents of an Integrated Questionnaire

An integrated questionnaire (Harada et al., 2016) was administered twice, just before the intervention and one month later, after the intervention. The integrated questionnaire included questions to determine diurnal type (7 questions, scored from 7 to 28, higher scores show morning-type: Torsvall and Åkerstedt (1980): in this occasion, half number of participants were defined as evening-type ones who scored 7 to 16 and another half morning type ones who scored 17 to 28), questions on sleep habits (sleep duration, bedtime, wakeup time, interruption of a night's sleep, difficulty in fall asleep and difficulty in waking up) and on mental health (inability to control their emotions, irritation, anger and depressive mood). The participants were always free to choose these. The questions on mental health had been validated in so far as the answers showed a significant positive correlation with those from the General Health Questionnaire (GHQ) (Harada et al., unpublished). During the intervention period, all participants kept a sleep diary. This included information on rising time, bedtime, time asleep, sleep latency, time of waking, and number of times when sleep was interrupted during the night.

2.4 Design-3: Self-Estimation of Change in Soccer Performance during Intervention

On the 10th and 21st day of the intervention, participants answered a questionnaire on subjective estimates of their change in soccer performance in comparison with the performance before the intervention (Harada et al., 2016). This questionnaire had been validated before use by two authors of this manuscript, the general manager of the soccer team at Kochi University and the head coach of the soccer team. Participants were asked not to play soccer on these two days. The eleven questions (Table 1) were divided into three categories which were, first, mental or psychological issue (Questions 1, 6 and 11), second, physiological function (Questions 2, 7 and 8) and physical techniques (Questions 3, 4, 5, 9 and 10).

Table 1. A questionnaire on the change in the performance between pre-intervention stage and (middle) point of one month intervention. In comparison with the performance before the intervention, how has each of the following 11 components of your soccer performance changed from beginning to the end of intervention month? Please put the circle (check) on the appropriated numeral. If you have the other kind of change in soccer performance and other changes in your life, please write down in the bottom space. (Harada et al., 2016)

Question 1: Assessment of the present situation, Question 2: Visual field on playing, Question 3: Movement of foot, Question 4: Rudimentary mistake, Question 5:First touch, Question 6: Irritation on playing, Question 7: Running out of Stamina, Question 8:Injury on playing, Question 9: Body balance, Question 10: Precision of long kick, Question 11: Motivation for the practice

	(Performance has been better) ◄			► (Performance has been worse)	
Question 1:	① faster	② faster a little	③ no change	④ slower a little	⑤ slower
Question 2:	①wider	② wider a little	③ no change	④ narrower a little	⑤ narrower
Question 3:	①lighter	② lighter a little	③ no change	④ heavier a little	⑤ heavier
Question 4:	①reduced	② lighter a little	③ no change	④ increased a few	⑤ increased
Question 5:	①better	② better a little	③ no change	④ worse a little	⑤ worse
Question 6:	①reduced	② lighter a little	③ no change	④ increased a few	⑤ increased
Question 7:	①reduced	② lighter a little	③ no change	④ increased a few	⑤ increased
Question 8:	①reduced	② lighter a little	③ no change	④ increased a few	⑤ increased
Question 9:	①better	② better a little	③ no change	④ worse a little	⑤ worse
Question 10:	①better	② better a little	③ no change	④ worse a little	⑤ worse
Question 11:	①better	② better a little	③ no change	④ worse a little	⑤ worse

Category 1: Mental or psychological issues (Question 1, 6 and 11).

Category 2: Physiological function (Questions 2, 7 and 8).

Category 3: Physical techniques (Questions 3. 4. 5. 9 and 10).

2.5 Ethic Treatment

Before administrating the intervention study, each participant was given a written explanation that detailed the concepts and purposes of the study and stated that their answers to the integrated questionnaire and the questionnaire on soccer performance (Table 1) would be used only for academic purposes. After the above explanation, all participants agreed completely with the proposal and gave written consent. They could withdraw at any time, and doing so would have no negative consequences for them, but there were no withdrawals. The study followed the guidelines established by the *Chronobiology International* journal for the conduct of research on human subjects (Portaluppi et al., 2010). The study was also permitted by the Kochi university soccer club committee and committee in Laboratory of Environmental Physiology, Graduate School of Integrated Arts and Sciences, Kochi University, which carried out ethical inspections regarding the contents of the questionnaires and the intervention program.

2.6 Statistic Analysis

The software used for statistical analysis was SPSS 12.0 J for Windows (SPSS Inc., Chicago, IL, USA. Mann-Whitney U-test was used for ranked variables. Friedman's test and Wilcoxon's test were used for paired variables. We were sometimes interested in changes during the intervention (Friedman) and sometimes only comparisons between the beginning and end of the intervention (Wilcoxon).

3. Results

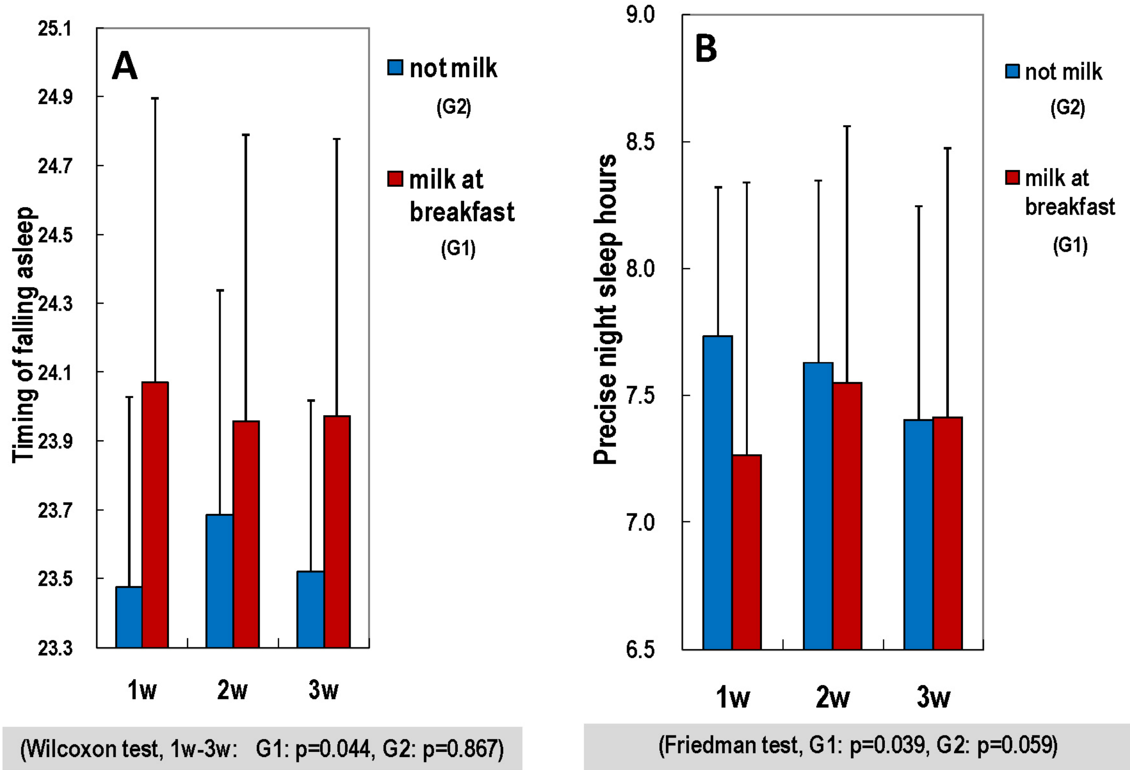

Figure 1. Change in sleep onset time (A) and precise night sleep hours (B) during the intervention period based on the sleep diary data in university soccer club members

Diurnal-type scores increased during the intervention month in the athletes who were originally designated as "evening-type" whereas, in those originally designated as "morning-type", these scores decreased (Table 2). There were no differences in the diurnal type scores of control group between before and after the intervention (Wilcoxon-test: z=-0.06, p=0.95). Sleep latency was decreased from 29 min on average to 23 min in the evening-type group, whereas it remained unchanged, at 15-16 min, in the morning-types (Table 2).

Table 2. The diurnal-type scale scores and sleep latency before and after the intervention, in the two halves of the sample designated as "evening-type" and "morning-type" before the intervention

	The Diurnal Type Scale Scores		Subjective Sleep Latency (min)	
	Before	After	Before	After
Evening typed group	14.08±1.81	14.63±2.57	29.34±23.86	23.44±12.21
Wilcoxon test:	*Z=-2.068, p=0.039*		*Z=-1.972, p=0.049*	
Morning typed group	19.13±2.05	18.17±2.98	16.05±9.62	15.41±11.08
Wilcoxon test	*Z=-2.696, p=0.007*		*Z=-1.009, p=0.272*	

Sleep diary data showed that sleep onset time tended to become earlier during the intervention three weeks (Figure 1A) and other sleep parameters of sleep offset, wake up time, bedtime and sleep latency were not changed in the milk-intake group (Table 3). All the five parameters were not change in the control group (Table 3). During the intervention period of 3 weeks, the hours from sleep onset to offset and the precise sleep hours excluding interrupted times during night sleep, increased in the milk-intake group (Figure 1B), whereas those did not changed in the control group (Table 4). However, the hours in bed and mid-point of sleep hours did not change during the intervention period in both groups (Table 4). This means that it took shorter minutes for the fall-in-sleep in the milk intake group, although there were no significant differences in sleep latency data between the first and third weeks of intervention (Table 3).

The total scores of 11 questions on soccer performance was decreased (it means "in-advance" of soccer performance) in the milk-intake group, whereas that did not change in the control group (Table 5). Of the 11 questions, soccer performance was improved during the 3 weeks at No 1, 2, 6, 8, 9 and 11: (1) Assessment of the present situation became faster; (2) Visual field on playing became wider; (6) Irritation on playing was reduced; (8) Injury on playing was reduced; (9) Body balance became better; and (11) Motivation for the practice became better (Table 1 and Table 6).

Table 3. Mean values for sleep habits during the 3-week intervention, based on the sleep diary data (Mean±SD)

Cow milk at breakfast	Bedtime	Sleep onset	Sleep offset	Wake up	Sleep latency
The first week	23.70±0.78	24.07±0.82	7.48±0.66	7.63±0.62	0.34±0.19
The second week	23.63±0.77	23.96±0.83	7.56±0.76	7.71±0.74	0.31±0.20
The third week	23.64 ±0.68	23.97±0.80	7.41±0.75	7.58±0.71	0.33±0.21
Freadman's test (1st 2nd and 3rd weeks)					
X^2-value	1.50	2.66	2.76	1.21	4.17
df	2	2	2	2	2
p-value	0.472	0.265	0.252	0.546	0.124
Wilcoxon test (the first and third weeks)					
z	-1.11	-1.77	-0.99	-0.70	-0.99
p	0.266	0.077	0.322	0.482	0.320
No cow milk	Bedtime	Sleep onset	Sleep offset	Wake up	Sleep latency
The first week	23.18±0.51	23.48±0.55	7.29±0.69	7.39±0.66	0.30±0.12
The second week	23.35±0.59	23.69±0.65	7.32±0.62	7.47±0.64	0.34±0.14
The third week	23.25±0.40	23.52±0.50	7.15±0.61	7.34±0.64	0.27±0.14
Freadman's test (1st 2nd and 3rd weeks)					
X^2-value	5.51	5.66	1.83	0.50	1.64
df	2	2	2	2	2
p-value	0.064	0.059	0.401	0.779	0.441
Wilcoxon test (the first and third weeks)					
z	-1.42	-0.47	-1.02	-0.12	-0.12
p	0.155	0.638	0.308	0.906	0.906

At the 10th day from the start of intervention, about 70% of the soccer club members of milk intake group who showed shorter sleep latency than that at the beginning of intervention, improved their soccer performance, whereas only about 45% of those who showed same or longer sleep latency did so (Figure 2A). At the 21st day from the start of intervention, about 60% of the soccer club members of milk intake group who showed shorter sleep latency than that at the beginning of intervention, improved their soccer performance, whereas only about 30% of those who showed same or longer sleep latency did so (Figure 2B).

Participants who drank cows' milk, which had been distributed, on all 21 days showed higher increase in the diurnal type scores (change to more morning-typed) (p=0.056) (Figure 3) and judged their soccer performance to be improved with higher frequency (p=0.009) than those who took cows' milk on only less than 21 days.

Table 4. Change in sleep habit during the intervention 3 weeks based on the sleep diary data

Cow milk at breakfast	Hours in bed	Onset to offset	Precise hours	Mid point of sleep
The first week	7.91±0.93	7.39±0.97	7.27±1.07	27.77±0.56
The second week	8.08±0.93	7.63±0.96	7.55±1.01	27.77±0.64
The third week	7.92±0.95	7.45±1.06	7.42±1.06	27.71±0.57
Freadman's test (1st 2nd and 3rd weeks)				
X^2-value	3.16	10.19	6.47	0.64
df	2	2	2	2
p-value	0.206	0.006	0.039	0.727
*Wilcoxon test (the first and **third** weeks)*				
z	-0.16	-0.54	-1.19	-1.28
p	0.873	0.586	0.233	0.199
No cow milk	Hours in bed	Onset to offset	Precise hours	Mid point of sleep
The first week	8.17±0.64	7.82±0.6	7.73±0.59	27.39±0.56
The second week	8.13±0.69	7.68±0.65	7.63±0.72	27.53±0.56
The third week	8.07±0.7	7.58±0.71	7.41±0.84	27.31±0.43
Freadman's test (1st 2nd and 3rd weeks)				
X^2-value	2.17	2.17	4.67	1.50
df	2	2	2	2
p-value	0.338	0.338	0.097	0.472
*Wilcoxon test (the first and **third** weeks)*				
z	-1.02	-1.33	-2.04	-1.10
p	0.308	0.182	0.041	0.272

Table 5. Index (total scores) of the change in soccer performance 10 days and 20 days later in comparison with the beginning of intervention

	10 days later*		20 days later*		*Wilcoxon test*	
	Mean	SD	Mean	SD	*z*	*p*
Cow milk at breakfast	29.920	3.291	28.211	4.699	*-3.961*	*<0.001*
	10 days later		20 days later		*Wilcoxon test*	
	Mean	SD	Mean	Mean	*z*	*p*
No cow milk at breakfast	31.938	2.886	31.867	2.386	*-3.961*	*<0.001*
	z	*p*	*z*	*p*		
Mann-Whitney U-test	*-2.698*	*0.007*	*-3.058*	*0.002*		

* These scores are the values as "the comparison values" between 0 day and 10 day (or 20 days). So there is no value at "0" day.

Table 6. Comparison of the change in soccer performance (based on that at the beginning of intervention) between 0 day and 10 days later (or 20 days later) in the three categories (Category 1: Mental or psychological issue, Category 2: Physiological function and Category 3: Physical techniques) in soccer performances. (Analysis with Wilcoxon's test)

	Category 1		Category 2		Category 3	
	z	*p*	*z*	*p*	*z*	*p*
Cow milk at breakfast	-4.21	<0.001	-3.01	0.003	-2.65	0.008
No cow milk	-1.61	0.106	-1.41	0.157	-1.84	0.066

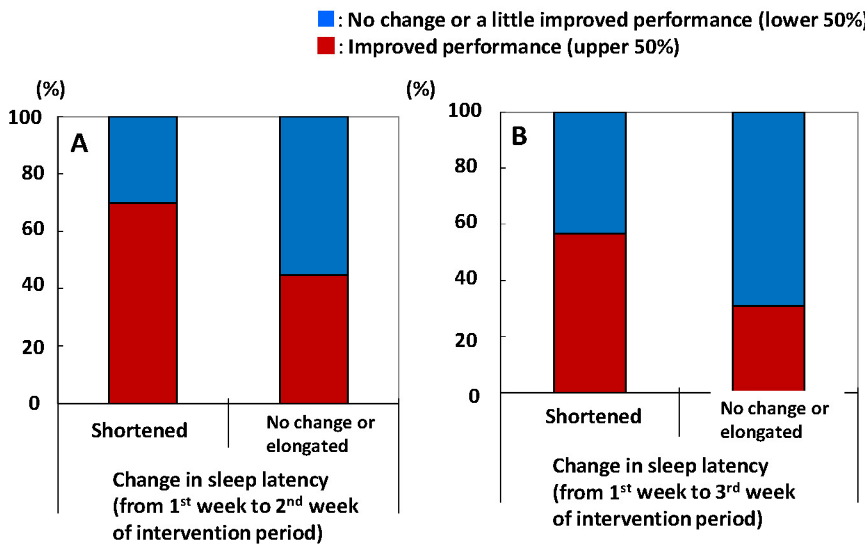

Figure 2. Relationship between the change in sleep latency from the 1st week to 2nd or 3rd week of the intervention period and the change in performance (red: become longer; blue: same or shorter) from the start to 10th day (A) (Fisher's exact probability test: p=0.067) or the end of intervention period (21st day); (B) (Fishers' exact probability test: p=0.067) in university soccer club members

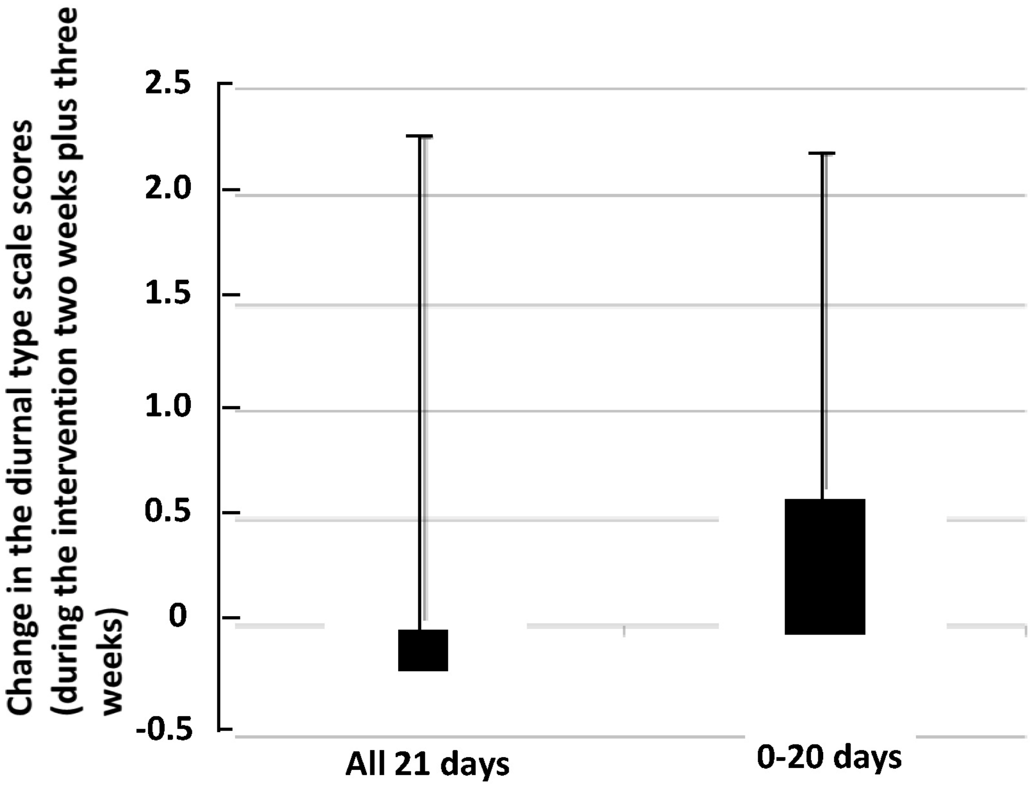

Figure 3. Comparison of the change in the diurnal type scale scores during the intervention 3 weeks and following three weeks, five weeks in total between the participants who took, every day, cow milk distributed for 21 days and those who took it for only 0-20 days

4. Discussion

The intake of cow milk at the breakfast would be possible to take the evening-typed members into more morning-typed ones, whereas the diurnal type of the morning-typed members could shift to more evening-typed. Morning typed members would be speculated to changed to more-evening typed ones, because the shortage of morning sun lights occurred due to later sun rise in this season of late November and early December. Tryptophan included in the cows' milk could be metabolized to serotonin in the morning and serotonin again would be synthesized to melatonin at night (Harada et al., 2013; Nakade et al., 2009, 2012; Wada et al., 2013; Harada et al., 2016) in the pineal of brains of the participants. Therefore, the sleep latency seems to become shorter during the intervention due to the melatonin synthesis which has been known as the natural sleep inducer (Bruni et al., 2015) based on the cow milk intake at the breakfast. This shorter sleep latency could lead to the longer sleep hours for the university soccer club members. In this experiment, saliva melatonin was measured at 22:00 and 23:00 (Takeuchi et al., unpublished), and only melatonin level at 22:00 was increased after the intervention, whereas it was similar to the value before the intervention at 23:00 (Takeuchi et al., unpublished). This result would imply that the phase of onset of melatonin synthesis was in advance by 1 hour due to drinking cows' milk for the three weeks.

How can we explain the improvement of soccer performance due to the intake of cow milk at the breakfast? The serotonin synthesis promotion would get the concentration improved during play, and also serotonin could be natural anti-depression agent (St-Pierre et al., 2016). Therefore, Category 1 as mental or psychological issue was most effectively improved among the three categories (Category 2: Physiological function and Category 3: Physical techniques).

Due to drinking cows' milk, the sleep onset seemed to be earlier due to earlier melatonin synthesis. Earlier sleep onset might promote growth hormone synthesis (Cauter & Plat, 1996) and could lead to "reduction of injury on playing" (Question No. 8) (Harada et al., 2016). Accompanying with the longer sleep hours, the amount of REM sleep may be increased after the intervention. Because REM sleep improves the memory-consolidation especially including integrated memory system which consists of sensory input, information processing and motor output (Wieser et al., 2015; Goerke et al., 2013), a new soccer technique which was learned during a training in the previous day could be fixed due to a sufficient REM sleep in the following night.

An advance of the circadian phase which would be caused by the intake of cow milk at breakfast might lead to the improvement of soccer performance. Recently, a mutant mouse created by a reduction of the BMAL1 gene that encodes proteins regulating circadian rhythm was reported to show about a 30% of reduction in muscle force and 40% reduction in mitochondrial volume (Andrews et al., 2010). Skeletal muscle amounts and mitochondria volume via BMAL1 gene are speculated to be related to the circadian phase of humans, although no studies have been done on the relationship in humans which remains to be studied in the future. The longer intervention more than 3 months might be effective for the increase of body muscles and mitochondria volume for athletes.

As a limitation of this study, the self report nature of the soccer performance scoring was adopted and also the participants know that they might be in the intervention group and so expect an effect. In near future, a coach or similar assess of performance instead will be adopted and also a placebo drink (without proteins) will be given for the control group in another study as a sham experiment. which could delete the side and "psychological" effects.

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