

INTERVENTION STUDY TO PROMOTE SLEEP AND MENTAL HEALTH OF JAPANESE UNIVERSITY ATHLETES AND INFANTS ESPECIALLY FOCUSING ON INTAKE of COW MILK AT BREAKFAST

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Abstract: Cow milk is an important supplying of protein resources for human infants and sports athletes because it is easy to be taken and possible for vegetarians. There is one research example to show the relationship between cow milk contents and sleep. Guesdon et al. (2006) reported that the casein hydrolysate could promote the sleep of rats. In this study, rats were exposed to chronic stress in the form of environmental disturbances, while some of them took the aSI-casein hydrolysate (CH) as content of foods. Sleep duration in control rats was reduced during the first 2 days of the stress period. However, the stress-induced sleep disturbance was prevented in the CH-treated rats which showed longer slow wave sleep in NON-REM sleep and also slight longer REM sleep than the control rats.

Key words: Cow milk intake, breakfast, tryptophan, serotonin, melatonin, soccer performance achievement, Japanese University soccer athletes

1. Introduction

Some studies have been performed on the significance of human breast milk on human child health. For example, Cubbero et al. (2005) reported that tryptophan contents in the breast milk of mothers fluctuated with circadian rhythm with acrophase (peak time of day) at around 3:00. This rhythm was followed by another rhythm of the 6-sulfatoxymelatonin with the acrophase of 06:00 in the infants. This phase-delayed rhythm implies that tryptophan-serotonin-melatonin syntheses might be fundamental mechanism which would be related to the sleep quality of babies. As another report, infants who fed on exclusively breast milk showed significantly lower incidence of colic attacks, lower severity of irritability attacks and longer nocturnal sleep duration (Engler et al., 2012). They reported that breast milk (nocturnal) gave additional plasma melatonin, whereas artificial formula did not so. These results implied melatonin supplied to the infant via breast milk

might play a role in improving sleep and reducing colic in breast-fed infants compared to formula-fed ones.

On the other hand, cow's milk has long been thought as a relaxation agent with sleep-inducing substances (Guesdon et al., 2006). Laird & Drexel (1934) reported that adults who consume a meal of cornflakes and milk showed a stronger tendency of un-interrupted night sleep. Brezinova & Oswald (1972) performed electroencephalography and showed that older people who fed a milk and cereal meal at bedtime got an improved sleep quality.

How about the efficacy of protein consumption at breakfast on sleep health and circadian typology in human? Tryptophan intake at breakfast has been known to be effective on promoting better mental health and morning-typed life through serotonin and melatonin syntheses (Harada et al., 2007; Nakade et al., 2009, 2012; Wada et al., 2013). For Japanese children, cow milk seems to be important resource for taking tryptophan at

breakfast because of limited meal time in the morning. Takeuchi et al. (2014) recently reported the relationship between circadian typology and mental health, and cow milk consumption at breakfast as follows. Infants who took milk at breakfast showed 21.2 of DTSS on average which tended to be higher than 20.7 ($p = 0.085$) shown by those who did not take milk. Infants who took carbohydrate (or carbohydrate and protein resource) plus milk at breakfast were significantly morning-typed than those who took only carbohydrate (or carbohydrate and protein resource) ($p < 0.001$). Infants who took milk at breakfast tended to be less frequently depressed than those who did not ($p = 0.098$).

However, we cannot discuss the causal relationship of cow milk consumption at breakfast to human mental and physical issues including circadian typology and sleep health based on such questionnaire study. There have been no reports of intervention studies on the effect of cow milk intake at breakfast on circadian typology, sleep health and mental health of human. This study aims to access to the causal relationship from the view point of intervention study.

2 OBJECTIVE (HYPOTHESIS, RESEARCH QUESTIONS)

The hypothesis to be examined from the epidemiological view point is that intake of cow milk at breakfast can promote sleep health of Japanese infants and university athletes via the increased synthesis of serotonin and melatonin from tryptophan included in the cow milk.

3 METHODOLOGY (PROJECT CHARACTERISTICS, RESEARCH ORGANIZATION)

3-1. Pilot intervention study on whether cow milk consumption for two weeks enhance sleep quality of Japanese university soccer athletes (Takeuchi et al., unpublished)

An integrated questionnaire was administered to 90 team members attending Kochi University soccer club just before, just after and one month after the intervention period of 2 weeks. Participants were divided to three groups which were asked to consume the 200 ml package milk in the morning (~9:59), in the daytime (10:00~15:59) or in the evening (16:00~). The package which includes 200 ml of milk was distributed to 90 members everyday for the two weeks from 26th November to 11th December. Sleep diary was kept by the participants for the intervention 2 weeks.

The integrated questionnaire includes questions on habits of milk consumption and meals, sleep habit, The Diurnal Type Scale Score (Torsvall & Åkerstedt, 1980) and mental health.

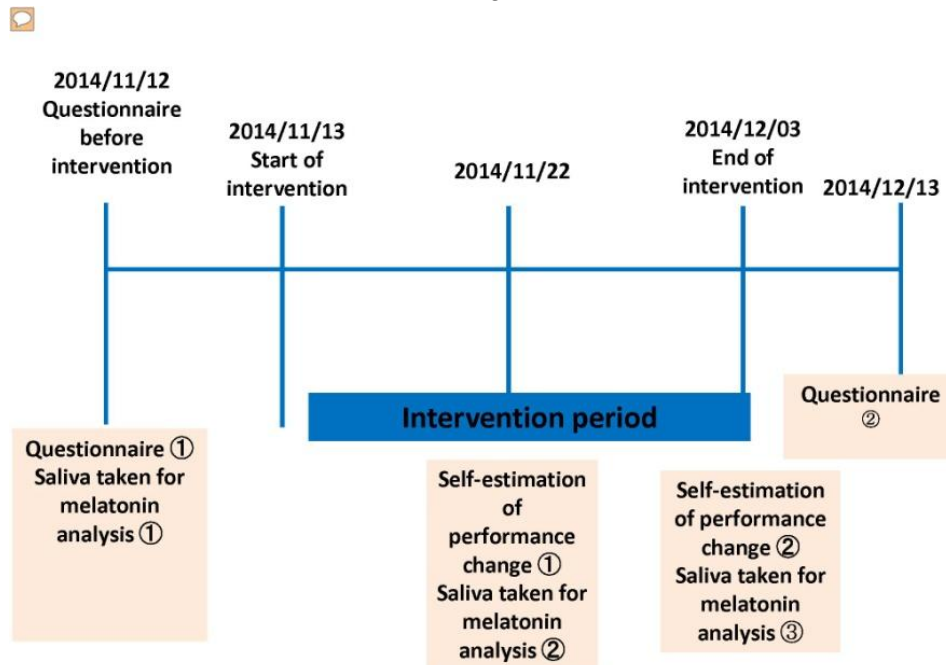
3-2. Intervention study on whether cow milk consumption for three weeks affects circadian typology, sleep health and mental health of Japanese university soccer athletes (Takeuchi et al., unpublished; Kawada et al., unpublished)

This intervention study was performed for 107 Japanese university students who attended Kochi university soccer team. An integrated questionnaire was administered just before (on 12th November 2014) and (on 10th January 2015) one month after the intervention three weeks from 13th November 2014 to 4th December 2014. A short questionnaire was also administered just after the intervention period. Ninety three participants (18-23 years old) answered the questionnaire before the intervention and occupied 88.6% of the 107 members. Twenty participants as control group members, actively, agreed with taking no milk through the intervention 3 weeks. The remained 73 participants were asked to take the 200 ml cow milk, in the morning before 10:00, with natural contents which had been distributed every day for the three weeks. All 93 participants were asked to keep sleep diary for the intervention 3 weeks. The 93 participants had been included in the three

teams which were in accordance with the soccer performance achievement (Team A: with highest achievement, B: moderate; C: lower). No other intervention out of milk

consumption at breakfast has been performed during the three weeks. The study schedule was shown in Fig. 1.

Fig.1



The integrated questionnaire which was administered before the intervention and also one month after that includes Food Frequency Questionnaire (FFQ), life habit questionnaire (bedtime, wake up time, difficulties for the sleep onset and offset, interrupted night sleep, sleep hours, The Diurnal Type Scale (Torsvall & Åkerstedt, 1980). Sleep diary which was kept for the 3 intervention period, consists of the questions on daily mood (judged as 0-100 scores), bedtime, sleep latency, sleep hours, interrupted times and duration for night sleep, satisfaction scores for fall-in-sleep (0-100 scores), feeling of the sleep deepness (0-100 scores), mood at fall-in-sleep (0-100 scores), lighting conditions at night, usage of PC, electric game and TV watching.

The change in the performance achievement from the beginning of the intervention was

estimated with the questionnaire shown by Table 1. Sum of the 11 items (1: Assessment of the present situation, 2: Visual field on playing, 3: Movement of foot, 4: Rudimentary mistake, 5: The first touch, 6: Irritation on playing, 7: Running out of stamina, 8: Injury on playing, 9: Body balance, 10: Precision of long kick, 11: Motivation for the practice) was defined as the soccer performance achievement index (SPAI: lower scores show higher achievement).

Saliva from 12 participants in each of two groups (milk taken or no milk) were taken with the cotton cylinder (1cm diameter, 3cm long) which was set under the tongue for 3 min. Saliva was taken at 22:00 and 23:00 just before the intervention, 10 days and 20 days of the intervention at six times in total, and kept under -20 °C till the analysis of melatonin concentration. Data as melatonin concentrations in the saliva samples were measured using an

ELISA kit. Melatonin concentration of 48 samples from 8 participants in the experimental group and 42 samples from 7 participants of the

control group were correctly measured and statistically analyzed.

Table 1. Question: How was your current soccer performance which could be evaluated by 11 elements, changed in comparison with that just before the intervention period? (Kawada et al., unpublished)

| | ← Improved ----- Receded → | | | |
|---|----------------------------|--------------------------|--------------------------|-----------------|
| 1. Assessment of the present situation | ① Quicker | ② a little bit quicker | ③ a little bit delayed | ④ delayed |
| 2. Visual field on playing | ① Broader | ② a little bit broader | ③ a little bit narrower | ④ narrower |
| 3. Movement of foot | ① heavier | ② a little bit heavier | ③ a little bit lighter | ④ lighter |
| 4. Rudimentary mistake | ① more skillful | ② slightly more skillful | ③ slightly less skillful | ④ less skillful |
| 5. First touch | ① decreased | ② a little bit decreased | ③ a little bit increased | ④ increased |
| 6. Irritation on playing | ① decreased | ② a little bit decreased | ③ a little bit increased | ④ increased |
| 7. Running out of stamina | ① decreased | ② a little bit decreased | ③ a little bit increased | ④ increased |
| 8. Injury on playing | ① decreased | ② a little bit decreased | ③ a little bit increased | ④ increased |
| 9. Body balance | ① improved | ② a little bit improved | ③ a little bit receded | ④ receded |
| 10. Precision of long kick | ① higher | ② a little bit higher | ③ a little bit lower | ④ lower |
| 11. Motivation for the practice | ① higher | ② a little bit higher | ③ a little bit lower | ④ lower |

The short version of questionnaire was administered to all 93 participants just after the intervention. This questionnaire includes questions on life habit, sleep habit, breakfast frequency and contents, and the frequency of cow milk consumption.

SPSS statistical software (12.0 J for Windows, SPSS Inc, Chicago, IL, USA) was used for all statistical analyses in this study.

A full explanation with the code of the guideline for a study targeting humans (Portaluppi et al., 2010) was performed all participants before the beginning of the study. This explanation included that the results of the study are used only for the academic purposes and to promote the health of athletes and securely kept under the control. The

contents of the study have been examined before this study by the ethic committee of Laboratory of Environmental Physiology, Graduate School of Integrated Arts and Sciences, Kochi University and this committee judged this study was appropriate from ethic view point. All participants completely agreed with the participation in this study.

4 RESULTS AND DISCUSSION (PROCEDURE)

4-1-1. Pilot intervention study on whether cow milk consumption for two weeks enhance sleep quality of Japanese university soccer athletes (Takeuchi et al., unpublished)-Main results

Only in the group which occupied the evening-typed 25% of all participants, the sleep quality was significantly improved when the distributed milk was taken in the morning

(Wilcoxon-test: $z=-2.06$, $p=0.04$) (Fig. 2), whereas the sleep quality of the evening-typed participants were not improved when the milk was taken in the daytime and evening (Fig. 3).

Fig.2

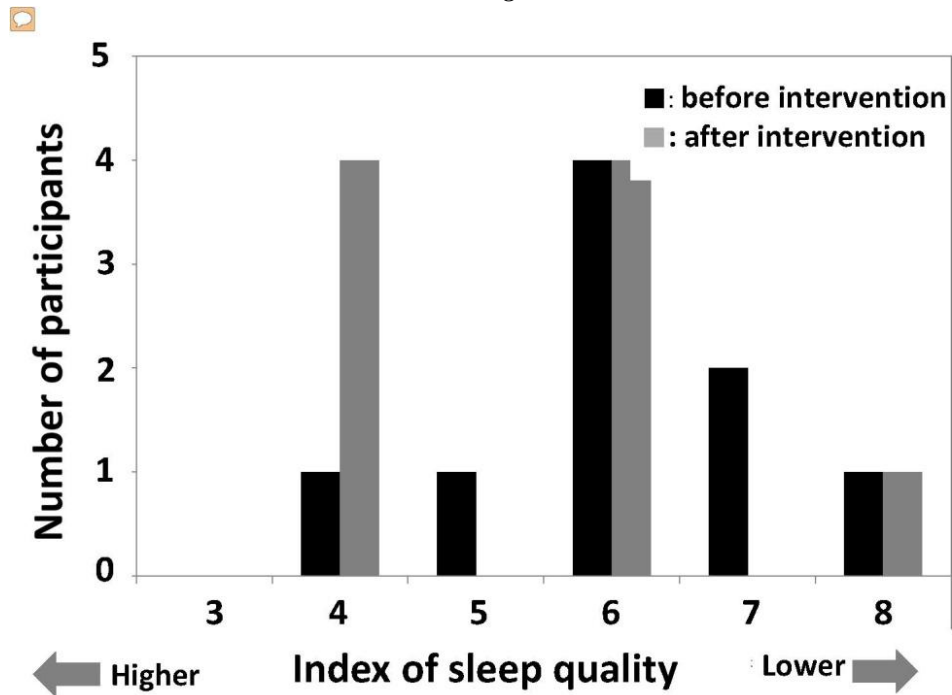
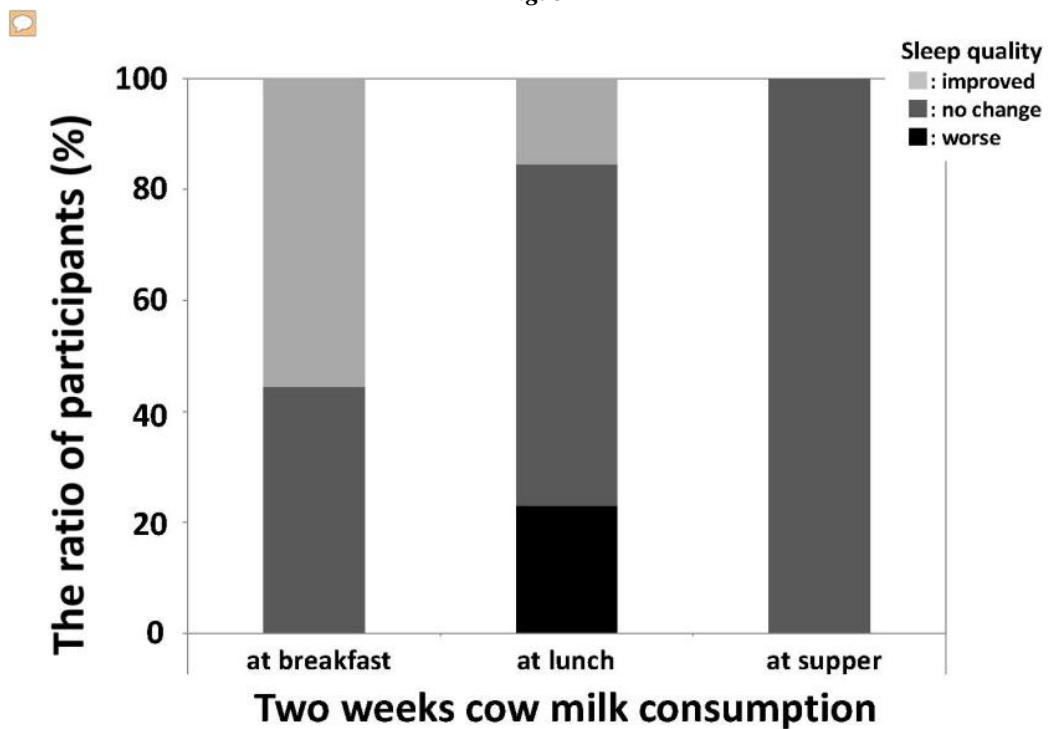


Fig. 3



4-1-2. Pilot intervention study on whether cow milk consumption for two weeks enhance sleep quality of Japanese university soccer athletes (Takeuchi et al., unpublished)-Discussion

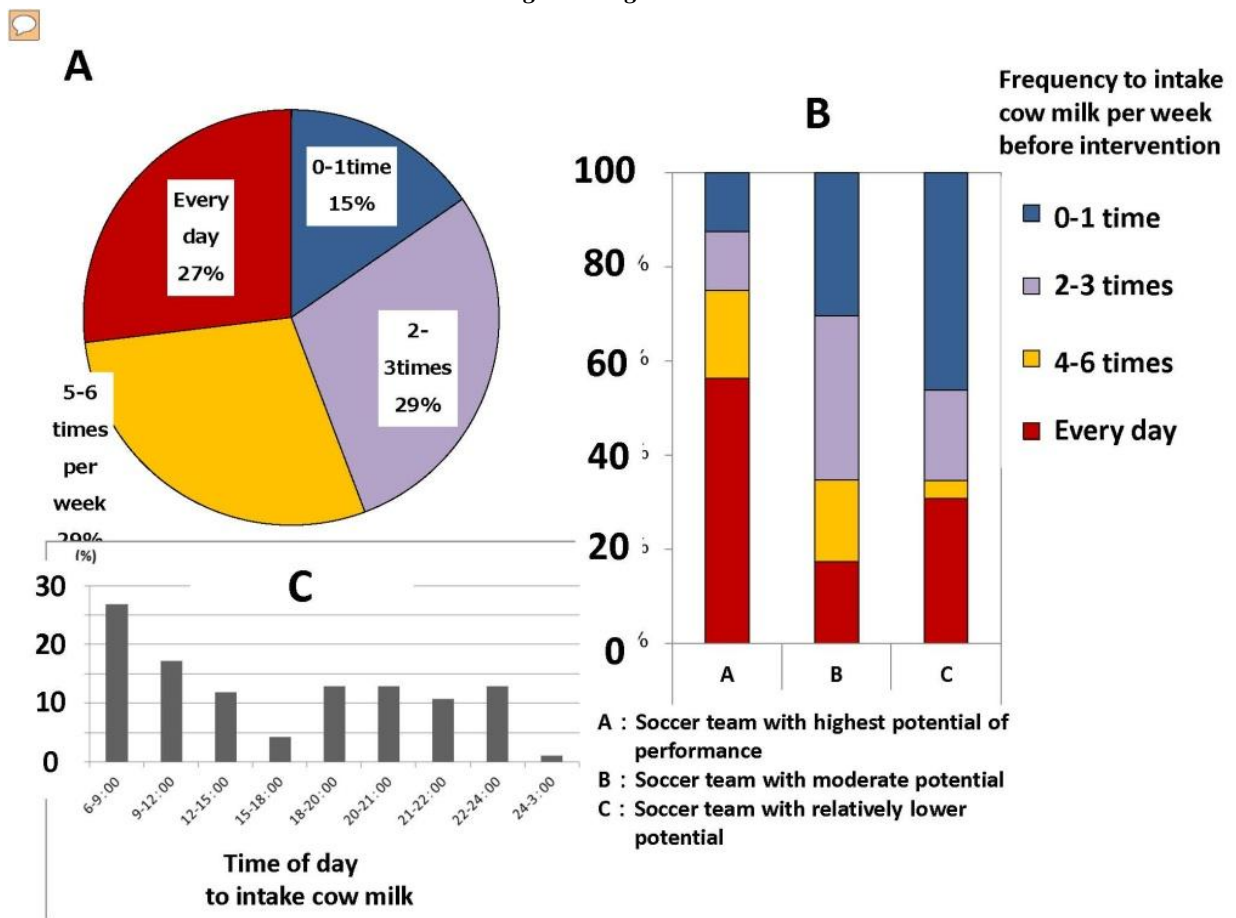
Tryptophan included in the milk taken at the breakfast could be transferred to serotonin in the daytime in the pineal and melatonin could be synthesized at night from the serotonin in the brain. This melatonin might be possible to enhance the sleep quality of the evening-typed soccer team members which had shown originally lower quality of sleep.

4-2-1. Intervention study on whether cow milk consumption for three weeks affects circadian typology, sleep health and mental health of Japanese university soccer athletes

(Takeuchi et al., unpublished; Kawada et al., unpublished)-Main results

Just before the intervention, the ration of milk consumer was 64%, and the participants to take milk every day occupied 27% and those to do it with the frequency of 0-1 time per week was 15% (Fig. 4A). Twenty four % of milk consumers took milk in 6:00-9:00, whereas in 9:00-12:00 15% of the consumers did it (Fig. 4B). There was no difference in the ratio of milk consumers among the three teams made due to the soccer performance achievement (χ^2 -test: χ^2 -value = 3.602, df = 2, p = 0.165), and no difference in the frequency to take cow milk was shown among the three teams (χ^2 -test: χ^2 value = 10.553, df = 9, p = 0.308). Sixty-three % of the participants took breakfast and 69% of the breakfast consumers took it at regular time.

Fig.4A a Fig.4.B

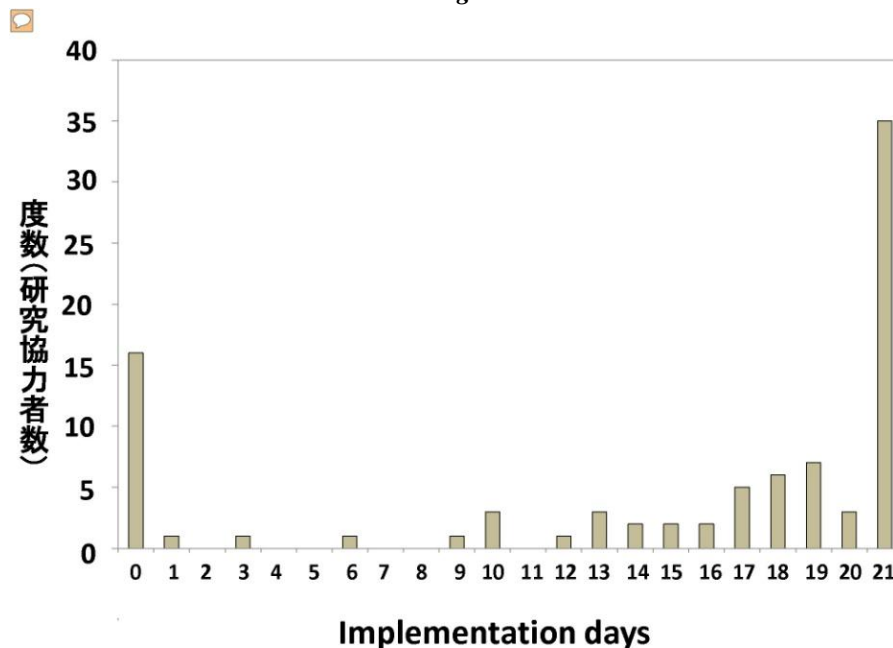


The participants who took breakfast with stable food (carbohydrates), main dish (protein) and side dish (vitamins and minerals) occupied only 44% of all participants. There were no significant differences in the ratio of participants who took breakfast among the three groups made due to the soccer performance achievement (χ^2 -test: χ^2 value = 3.410, df = 6, p = 0.756). The ratio of students who took breakfast with nutritionally balanced contents (carbohydrates, protein and vitamins & minerals) was higher in the team with highest performance achievement than the other groups (χ^2 -test: χ^2 value= 17.543, df = 6, p = 0.007) (Fig. 4B). There were no differences

in the regularity of breakfast time among the three groups (χ^2 -test: χ^2 value = 5.863, df = 4, p = 0.210).

The implementation of cow milk consumption was shown in Fig. 5, and 35 participants took cow milk distributed every day for the 3 weeks intervention period. The days when participants in the milk-intake group actually took cow milk distributed was 17.5 days (\pm 3.3 days of SD) on average. The students who actually took the cow milk distributed on all 21 days as intervention period and more than 15 days occupied 39.8% and 80.0%, respectively, of all participants of the milk-intake group.

Fig.5



In the control group, there are no significant change during the intervention 3 weeks in the diurnal type scale scores (DTSS), GHQ scores and sleep latency in the milk-intake group (Wilcoxon-test, DTSS: z = -0.06, p = 0.952; GHQ: z = -0.702, p = 0.483 ; Sleep latency: z = -0.67, p = 0.50), whereas the sleep quality was worse after the intervention than that before it (z = -1.71, p = 0.09) (Table 2).

On the other hand in the milk-intake group, there were no significant change during the intervention 3 weeks in DTSS, GHQ and sleep quality (DTSS: z = -0.406, p = 0.685; GHQ: z = -1.223, p = 0.221; Sleep quality: z = -4.43, p = 0.66), whereas the sleep latency after the intervention was significantly shorter than that before it (z = -2.80 ; p = 0.01) (Table 2).

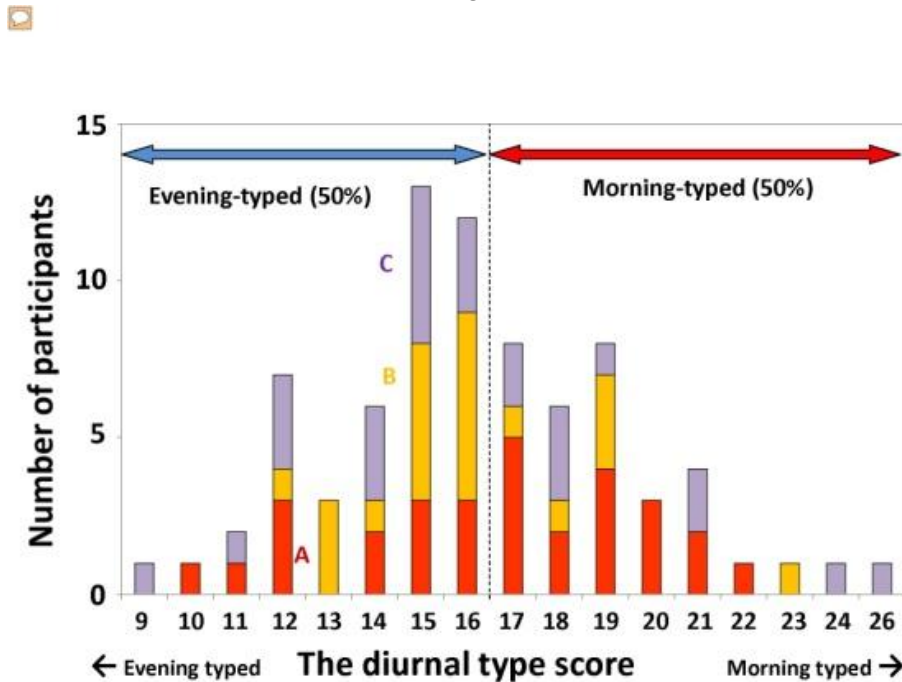
Table 2: Diurnal Type Scale scores, General Health Questionnaire scores, sleep quality and subjective sleep latency after the intervention, compared with those before that in Japanese university soccer team members (Takeuchi et al., unpublished; Kawada et al., unpublished).

| | | | Diurnal type scale score | GHQ score | Sleep quality | Subjective sleep latency |
|--------------|---------------------|------|--------------------------|-----------|---------------|--------------------------|
| Evening type | Before intervention | Mean | 14.08 | 4.72 | 5.55 | 29.34 |
| | | SD | 1.81 | 2.40 | 1.66 | 23.86 |
| | After intervention | Mean | 14.63 | 4.23 | 5.24 | 23.44 |
| | | SD | 2.57 | 2.15 | 1.25 | 12.21 |
| | Wilcoxon test | Z | -2.068 | -0.843 | -1.427 | -1.972 |
| | | p | 0.039* | 0.399 | 0.154 | 0.049* |
| Morning type | Before intervention | Mean | 19.13 | 4.16 | 4.56 | 16.05 |
| | | SD | 2.05 | 2.63 | 1.48 | 9.62 |
| | After intervention | Mean | 18.17 | 3.92 | 4.51 | 15.41 |
| | | SD | 2.98 | 2.29 | 1.57 | 11.08 |
| | Wilcoxon test | Z | -2.696 | -0.909 | -0.187 | -1.099 |
| | | p | 0.007** | 0.363 | 0.852 | 0.272 |

There were no significant difference in the distribution of the diurnal type scale scores among the three teams made due to soccer performance achievement before the intervention period (Mean \pm SD = 16.32 \pm 3.16 in all three teams) (Kruskal-Wallis test,

χ^2 -value = 1.554, p = 0.460) (Fig. 6). Participants were divided to two groups due to the diurnal type scale scores as the morning-typed half and evening-typed half for the further analysis.

Fig.6

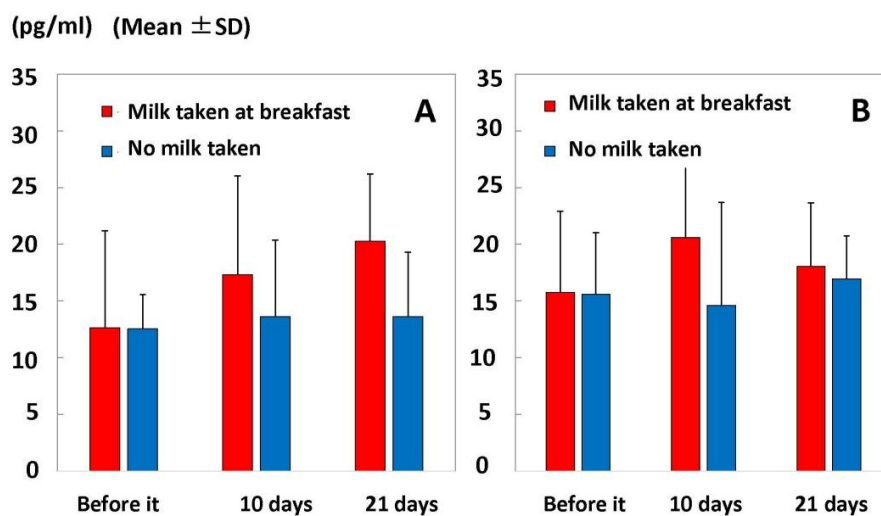


In the morning-typed group, there were no differences in GHQ scores (mental health index), sleep quality and sleep latency during the intervention 3 weeks (Wilcoxon-test, GHQ: $z = -0.909$, $p = 0.363$; sleep quality: $z = -0.852$, $p = 0.394$; sleep latency: $z = -1.099$, $p = 0.272$). In the evening-typed group, there were no differences in GHQ and sleep quality, whereas DTSS was increased and sleep latency was shorter than those before the intervention ($z = -2.068$, $p = 0.039$; $z = -1.972$, $p = 0.049$) (Table 2).

The milk intake group felt the advance in performance both at 10 days and 20 days of intervention than the control group (Mann-Whitney U-test: the 10 days: $z = -2.698$, $p = 0.007$; 10 days: $z = -3.058$, $p = 0.002$). There was no difference in the amount of performance advance between 0-10 days and 0-20 days in the control group, whereas the milk-intake group felt performance advance with higher degree just after the 20 days intervention than just after the 10 days intervention (Wilcoxon-test: the milk intake group: $z = -3.96$, $p < 0.001$; the control group: $z = 0.00$, $p = 1.00$).

Salivary melatonin concentration at 22:00 significantly increased during the intervention in the milk-intake group (Friedman test, χ^2 -value=6.250, $df=2$, $p=0.044$), whereas there were no significant differences in the melatonin concentration during the intervention period in the control group (Friedman test, χ^2 -value=0.286, $df=2$, $p=0.867$) (Fig. 7A). However, there were no significant differences in the salivary melatonin concentration during the intervention 3 weeks both in the two groups (Friedman test, the milk-intake group: χ^2 -value=1.750, $df=2$, $p=0.417$; the control group, χ^2 -value=0.286, $df=2$, $p=0.867$) (Fig. 7B). Wilcoxon-test was performed salivary melatonin concentration at the beginning of the intervention and that at the end of that and showed significant increase of it ($z = -2.521$, $p = 0.012$). The difference in the melatonin conc. From the beginning to the end of the intervention 3 weeks in each participant salivary melatonin was calculated and the differences in the melatonin concentration of the milk-intake group were significantly larger than those in the control group Mann-Whitney U-test, $z = -1.680$, $p = 0.093$).

Fig.7A a Fig. 7B



4-2-2. **Intervention study on whether cow milk consumption for three weeks affects circadian typology, sleep health and mental health of Japanese university soccer athletes (Takeuchi et al., unpublished; Kawada et al., unpublished)-Discussion**

The milk consumption at breakfast could make the phase advance of the start of melatonin synthesis in advance in this study. The beginning timing of the serotonin synthesis in the daytime and following melatonin synthesis at night might be in advance because of the cow milk consumption at the fixed time in the morning. Such advances in phase shift can make the phase of circadian clocks in human in advance which leads to more morning-typed life in the soccer team members. Why can the cow milk intake in the morning make the soccer performance achievement increased during the 3 weeks intervention period?

The three answers would be possible as follows.

Memory consolidation during REM sleep of newly learned soccer-techniques (1)

When the soccer team athletes get morning typed life, amount of REM sleep could increase and the temporal new memories taken at training or practice in the last day can be kept temporally in hippocampus (Huijgen and Samson, 2015). Such temporal memory can be fixed in the brain in the course of memory consolidation during REM sleep (Karni et al., 1994; Hornung et al., 2007; Landmann et al., 2015). Because whole memories might be reconsolidated in order, the correct and appropriate judgments on playing would be possible in some scenes of games, for example.

Mental health improved due to the well inner synchronization of main clock driving autonomic nervous system and slave clock driving sleep-wake cycle (2)

There are 2 separate internal biological clocks in our body. The de-synchronization shows that the two clocks become out of the phase-coupling which could lead to lower motivation (Golombek et al., 2013) and also lower mental health with a possibility of

depression (Abreu & Bragança, 2015). Based on the epidemiological studies by Harada et al.(2012), the morning typed persons work the entrainment well to 24 hours fluctuation in environmental factors like as light-dark cycle, and so his/hers coupling of two biological clocks would be good and also his/hers mental health would be better.

Morning-typed life leads to intake of rich-protein breakfast which promotes serotonin synthesis enhancing concentration during game and practice (3)

The third mechanism would be via a higher amount of serotonin synthesis in the morning due to a rich-protein breakfast (Harada et al., 2007; Nakade et al., 2009; Nakade et al., 2012). The high concentration of serotonin in the body blood of the brain could improve the concentration on playing. The serotonin turns into melatonin at night, and it can promote sleep onset at night for the athletes. The melatonin orders our brain to go to bed earlier (Harada, 2004; Wada et al., 2013; Higuchi et al., 2014).

5 CONCLUSIONS (AND EVENTUALLY ACKNOWLEDGMENT)

Cow milk intake at breakfast seems to be powerful for improvement of sleep and mental health for athletes through the metabolisms of serotonin and melatonin synthesis based on tryptophan intake at breakfast.

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7 SUMMARY

The intervention study was done in 2013-2014 for promoting sleep and mental health of Japanese university athletes. This study consisted of three sessions. The first session was basic questionnaire study on the infants. The latter two sessions are intervention studies including milk intake at breakfast for the athletes and the infants.

The intervention to Kochi university soccer team was performed for 21 days in November 2014. Seventy three participants continued to intake 200 ml of cow milk which was distributed to all 73 soccer team members every morning for 21 days (experimental group), whereas milk intake was prohibited to 20 members for the 21 days (control group). Using a subjective evaluation method as performance questionnaire, soccer performance on playing (Assessment of the situation, field of vision, hardness of irritation, body balance, motivation to exercise) was improved after the intervention, whereas no change in the control group. Saliva melatonin level at 22 o'clock was higher after the intervention in the experimental group than that in the control group, whereas no difference in saliva melatonin at 23 o'clock between the two groups. This result would imply that milk intake at breakfast might lead to phase advance of circadian clock.

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