Correlation between Breakfast Tryptophan Content and Morningness–Eveningness in Japanese Infants and Students Aged 0–15 yrs

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Abstract Tryptophan can be metabolized via 5-hydroxytryptamine=serotonin to melatonin by a series of 4 enzymes in pineal body. Lack of serotonin in body fluid in the brain during daytime can lead to several psychiatric disorders, while shortage of plasma-melatonin at night can be related to sleep disorders. The Morningness–Eveningness (M–E) questionnaire and the original questionnaire including questions on sleep habits, mental symptoms, and contents of meals were administered to 1055 infants aged 0–6 yrs, 751 students attending an elementary school, and 473 students attending junior high school in Kochi City (33°N). The index of tryptophan taken at breakfast (Trp-Index) was calculated as tryptophan amount per one meal based on the tryptophan included in each 100 g of the foods and a standard amount of food per one meal. A significant positive-correlation between M–E scores and Trp-Index was not shown by relatively older students, aged 9–15 yrs (Pearson's test, $r=0.044$ – 0.123 , $p=0.071$ – 0.505), whereas a significant positive correlation was shown by infants and young elementary school students aged 0–8 yrs ($r=0.180$, 0.258 , $p<0.001$). The more frequently the infants had difficulty falling asleep at bedtime and waking up in the morning, the less the Trp-Indices taken at breakfast were (Kruskall–Wallis-test, $p=0.027$ for difficulty falling asleep; $p=0.008$ for difficulty waking up). The more frequently infants became angry even by a little trigger, or depressed, the lower (more evening-typed) the M–E scores were (Kruskal–Wallis test: $p\leq 0.001$). Tryptophan ingested at breakfast is very important for children to keep a morning-type diurnal rhythm, high quality of sleep, and indirectly good mental health, presumably, through the metabolism of tryptophan to serotonin in daytime and further to melatonin at night. *J Physiol Anthropol* 26(2): 201–207, 2007 <http://www.jstage.jst.go.jp/browse/jpa2>

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Introduction

Tryptophan is one of the essential amino acids which should be absorbed exclusively from meals. It is metabolized via 5-hydroxytryptamine=serotonin to melatonin by a series of 4 enzymes in pineal body (Moore et al., 2000; Zheng et al., 2004). Exposure to sunlight in the daytime seems to trigger the synthesis of serotonin in pineal (Rosenthal et al., 1997). Aggression, anxiety/aggression-driven depression, impulsive behavior, and suicidal attempts may be induced by lower serotonin (5-HA) or serotonin disturbance (Linnoila et al., 1993; Van Praag, 2001).

External melatonin treatment is effective for the phase advance of the sleep-wake cycle of DSPS (delayed sleep phase syndrome) patients (Turek and Czeisler, 1999). High plasma melatonin levels at night can be hypothesized to play an important role in sleep onset and sleep quality (Turek and Czeisler, 1999). In infants, the plasma melatonin level at night is several times as high as that in adolescents and adults (Waldhauser et al., 1988). Is the amount of tryptophan taken in at breakfast related to sleep habitats and the circadian typology shown by the Morningness–Eveningness score, especially in infants?

There have been several studies on the relationship between M–E preference and selected psychological variables. Levy (1985) found evening types more pessimistic than morning types. Moreover, there were negative correlation between the Horne and Östberg questionnaire score and the responses on the 3 depression scales of the BDI (Beck Depression Inventory), GDS-SF (Geriatric Depression Scale-Short Form, 15-item), and CESD (Center for Epidemiological Studies Depression Scale) (Chelminsky et al., 1999). Tankova et al. (1994), however, in their review of studies that examined the relationship between circadian typology and other individual difference variables, concluded that neuroticism and psychoticism are not associated with performance on “morningness–eveningness” scales. However, epidemiological studies by Harada (2004) showed that evening-type students had lower mood, higher irritation, and more easily triggered anger than morning-type ones. One causal factor for evening-

Table 1 A version for infants of the Morningness–Eveningness questionnaire Torsvall & Åkerstedt (1980) originally constructed.

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1. When would your child prefer to rise (provided your child has a full day's play-8h) if your child was totally free to arrange his (her) time ?
(4) before 06:30, (3) 06:30–07:29, (2) 07:30–08:29, (1) 08:30 or later
 2. When would your child prefer to go to bed (provided your child has a full day's play-8h) if your child was totally free to his (her) time ?
(4) before 21:00, (3) 21:00–21:59, (2) 22:00–22:59, (1) 23:00 or later
 3. If he (she) always had to go to bed at 21:00, what does your child think it would be like to fall asleep then ?
(4) easy—would fall asleep practically at once,
(3) rather easy—would like awake for a short while,
(2) rather difficult—would like awake for some time,
(1) very difficult—would like awake for a long time
 4. If he (she) always had to rise at 06:00, what does your child think it would be like to get up then ?
(4) easy—no problem at all
(3) a little unpleasant but not a great problem
(2) rather difficult and unpleasant
(1) very difficult and unpleasant
 5. When does your child usually begin to feel the first signs of tiredness and need for sleep ?
(4) before 21:00, (3) 21:00–21:59, (2) 22:00–22:59, (1) 23:00 or later
 6. How long a time does it usually take before your child “recovers his (her) faculties” in the morning after rising from a night's sleep ?
(4) 1–10 min., (3) 11–20 min, (2) 21–40 min, (1) more than 40 min
 7. Please, indicate to what extent your child is a morning or evening active individual.
(4) pronounced morning active (morning alert and evening tired)
(3) to some extent morning active
(2) to some extent evening active (morning tired and evening alert)
(1) pronounced evening active
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type students to have worse mental health is hypothesized to be inner de-synchronization of the main and slave clocks (Harada, 2004; Kripke et al., 1978). Another cause of the worse mental health shown by evening-type students can be supposed to be the lower level of serotonin in the brain in the daytime. The low level of serotonin may be due to the low amount of intake of tryptophan at breakfast by evening-typed students. The rapid tryptophan depletion technique (RTD), which is accomplished by consuming a tryptophan-free amino acid drink, was reported to induce lower levels of serotonin and melatonin followed by increased depression in several psychiatric patients (Moore et al., 2000). For example, RTD induced a depressive relapse in 14 of 21 inpatients hospitalized for major depressive disorder who had responded well to antidepressant therapy (Delgado et al., 1990). This study aims to answer the following questions from the epidemiological point of view.

1. Is the amount of tryptophan intake at breakfast related to sleep habits and circadian typology shown by the Morningness–Eveningness score, especially in infants?
2. Is the amount of tryptophan intake at breakfast related to the mental health of Japanese infants, pupils, and adolescents?

Materials and Methods

Morningness–Eveningness questionnaire and original questionnaire

A modified version for infants (Table 1) based on the Japanese version (Takeuchi et al., 2001a) for students of a Morningness–Eveningness Questionnaire (MEQ) was used in this study. The questionnaire was translated from the English

version and slightly modified for students (Takeuchi et al., 2001a) based on the MEQ that Torsvall and Åkerstedt (1980) originally constructed for general workers. Three of seven questions included in the MEQ pertain to sleep onset timing in the evening, three to sleep offset timing in the morning, and one to peak timing of activity during the daytime. Each question allows for choice, from evening- to morning-type (scored from 1 to 4). The M–E score is the summation of the seven answers. The minimal possible score (extreme evening-type) is 7, and the maximal possible score (extreme morning-type) is 28. The common MEQ questionnaire was used to compare the Morningness–Eveningness typology among ages 0–15 yrs. Infants within 6 months of birth were excluded from this study, because parents cannot answer the questions, due to specific sleep and feeding habits in this early stage (Korte et al., 2001). An original questionnaire was also used for the study, including the two questions on mental symptoms shown in Table 2, and original questions on sleep habits which Harada et al. (1998) originally constructed and were used in several papers (Takeuchi et al., 2001a; Takeuchi et al., 2001b; Harada et al., 2002; Takeuchi et al., 2003; Harada et al., 2004; Shinomiya et al., 2004). The MEQ and the original questionnaire were administered to 1055 infants aged 0–6 yrs, 751 students attending an elementary school, and 473 students attending a junior high school located in Kochi City (33°N). Parents, 93% of whom were mothers, answered, instead, the questionnaire for their children aged 0–8 yrs [740 infants and 195 students of the first and second grades]. 440 of the 480 elementary school students in the third to sixth grades and 445 of the 480 junior high students answered the questionnaire. All 740 infants were attending one of 9 kindergartens which were constructed by the government of Kochi City. The sex ratio

Table 2 Average tryptophan contents in foods and average gram of foods-intake per one meal to calculate the index of tryptophan.

Foods	Tryptophan (mg) /100 g	Average food intake (g)	Try.-Index (mg)
Eggs	180	50	90
Meats (beef, pork, chicken)	205	100	205
Milk	45	100	45
Bread or rice	105	100	105
Vegetable	20	100	20
Fruit juice	2	100	2
“Natto”	245	40	98
Seaweed	150	10	15
Non-dry fish	215	100	215
Dry fish	530	10	53
Coffee or tea	30	100	30
“Miso”	125	20	25

(DATAbase on functional foods factors Tokyo Agriculture University Lab of Public Nutrition Health Home Page, 2004)

was almost half and half in all grades of participants aged 0–15 yrs. Answers to the questionnaire were collected in June–November, 2004.

Statistical analysis

Data were statistically analyzed using Pearson’s correlation test, the Wilcoxon test, the Mann–Whitney U-test, the Kruskal–Wallis test and ANOVA, using SPSS statistical analyzing software (version 11.5) for personal computer. M–E scores were expressed as means plus or minus the standard deviation (Mean±SD).

Procedure

The study followed the guidelines established by the journal Chronobiology International for the conduct of research on human subjects (Touitou et al., 2004). We carefully explained the concepts and purposes of this study in writing and orally: “Questionnaires are completely unregistered and answers to them will be used only for academic purposes,” to kindergarten nurses and the parents of the infants. This study was permitted by the kindergarten nurses’ committees of nine kindergartens and the elementary and junior high schools which carried out an ethical inspection of the contents of the questionnaire. After the explanation, all the parents of the participants completely agreed with our proposal for epidemiological work. The survey was administered at home for younger children, aged 0–8 yrs (infants and students in the first and second grades), and in the classrooms of the schools for older students (students in the third to sixth grades of elementary school and junior high students).

Question on the contents of breakfast

“Please mark all the foods taken by your child at a usual breakfast from the following”:

Fig. 1 Relationship between amount of tryptophan intake in breakfast and M–E score in Japanese infants.

(1) eggs	(9) seaweed or laver
(2) meats [including ham and bacon]	(10) non-dried fish
(3) milk	(11) dried fish
(4) vegetables	(12) coffee
(5) boiled rice	(13) tea
(6) bread	(14) green tea
(7) fruit juice	(15) soybean soup
(8) fermented soybeans (“Natto”)	

The index of tryptophan (Trp-Index) taken from breakfast was calculated as the tryptophan amount per one meal based on the tryptophan included in each 100 g of the foods and a standard amount of food per one meal (Table 2) (Gomyo and Hasegawa, 1993).

Results

M–E scores and breakfast tryptophan content by infants and students aged 0–15 yrs

The average and SD of the M–E scores were 20.04 ± 3.58 (657 infants aged 0–6 yrs), 19.79 ± 3.48 (211 students in the first and second grades of elementary school, 6–8 yrs), 17.74 ± 3.83 (214 students in the third and fourth grades, 8–10 yrs), 16.42 ± 3.98 (226 students in the fifth and sixth grades, 10–12 yrs), 15.33 ± 3.79 (419 junior high students, 12–15 yrs). A significant positive-correlation between M–E scores and the Trp-Index was not shown by relatively older students, aged 9–15 yrs, but was shown by infants and young elementary school students aged 0–8 yrs (Pearson’s test: $r=0.180$, $p<0.001$ for infants; $r=0.258$, $p<0.001$ for students in first and second grades; $r=0.123$, $p=0.071$ for students in the third and fourth grades; $r=0.044$, $p=0.505$ for the fifth and sixth grades; $r=0.081$, $p=0.098$ for junior high students) (Figs. 1, 2).

Breakfast tryptophan content and sleep quality by infants aged 0–6 yrs

The Trp-Index of the infants who had a very short sleep-

Fig. 2 Correlative analysis between amount of tryptophan intake in breakfast and M-E score in Japanese students in first and second grades (A), third and fourth grades (B), and fifth and sixth grades (C) of elementary school, and junior high school students (D).

Fig. 3 Relationship between tryptophan intake in breakfast and subjective sleep latency in Japanese infants aged 0–6 yrs.

latency of less than 5 min was 440 mg and significantly higher than that (302 mg) of those who showed a sleep-latency of more than 60 min (Kruskal–Wallis test ; $\chi^2=14.719$, $df=5$, $p=0.011$) (Fig. 3). The more frequently the infants had difficulty falling asleep at bedtime and difficulty waking up in the morning, the less their Trp-Indices at breakfast were (Kruskal–Wallis-test: $\chi^2=10.922$, $df=4$, $p=0.027$, falling asleep; $\chi^2=13.740$, $df=4$, $p=0.008$, waking up) (Figs. 4, 5).

Fig. 4 Relationship between tryptophan intake in breakfast and frequency to feel difficulty to fall asleep in Japanese infants aged 0–6 yrs.

Relationship between mental symptoms and Morningness–Eveningness preference or breakfast tryptophan content of infants aged 0–6 yrs

No significant differences in Trp-index were shown among infants feeling anger or depression with different frequencies (Kruskal–Wallis test: χ^2 -value=4.38, $df=3$, $p=0.224$ on anger, χ^2 -value=3.80, $df=3$, $p=0.284$ on depression). However, the more frequently infants became angry even by a little trigger,

Fig. 5 Relationship between tryptophan intake in breakfast and frequency to feel difficulty to be awakened in Japanese infants.

or depressed, the lower (more evening-typed) the Morningness–Eveningness scores were (Kruskal–Wallis test: χ^2 -value=18.43, $df=3$, $p<0.001$ on anger, χ^2 -value=15.72, $df=3$, $p=0.001$ on depression) (Figs. 6, 7).

Discussion

Morning tryptophan intake affecting sleep habit and circadian typology

Among 20 kinds of amino acids, only tryptophan can be metabolized finally to melatonin, which is the key substance to trigger sleep. The amount of tryptophan digested at breakfast may affect the synthesized amount of serotonin in the daytime, especially in the morning. The amount of serotonin can decide, in turn, the amount of synthesized melatonin at night. This serotonin synthesis is hypothesized to occur mainly in the morning hours, because the amount of tryptophan at supper has neither significant effects on M–E scores (Pearson's correlation test: $r=0.191$, $n=591$, $p<0.001$ in breakfast; $r=0.100$, $n=600$, $p=0.067$ in supper) nor an effect on sleep habit in another study on Japanese infants performed in 2005 (Harada et al., unpublished).

Tryptophan intake at breakfast is effective for the onset and offset of sleep in infants (Figs. 3, 4, 5). A high amount of melatonin synthesized from tryptophan taken in the morning may produce good sleep habits. Tryptophan taken in at supper may be used not for serotonin synthesis the next morning but for the general synthesis of several proteins. A high amount of serotonin in the morning and the subsequent high amount of melatonin in the evening can be efficient zeitgebers to circadian oscillators, making the infants more morning-typed. The amount of plasma melatonin at night derived from tryptophan may affect sleep quality directly and/or through the phase advance of the so-called “slave-oscillator” driving sleep-wake cycle (Aschoff et al., 1967). The amount of daytime serotonin may be related to SCN circadian phase control by daytime light in humans (Pickart and Rea, 1997).

Age-specific aspects are possible in the intensity of the

Fig. 6 Relationship of frequency to be angry even by a little trigger to Morningness–Eveningness preference and amount of tryptophan to be taken in breakfast of Japanese infants aged 0–6 yrs.

Fig. 7 Relationship of frequency to be depressed to Morningness–Eveningness preference and amount of tryptophan to be taken in breakfast of Japanese infants aged 0–6 yrs.

morning-type-driven effects of tryptophan taken in at breakfast. A significant positive correlation between M–E scores and the amount of tryptophan taken in at breakfast was not shown for elementary school children in the third to sixth grades and adolescents aged 8–15 yrs, but it was shown for infants aged 0–6 yrs and elementary school children aged 6–8 yrs (Fig. 2). Plasma melatonin concentration at night is very high in the infants and young children aged 1–7 yrs, the value being 5 to 8 times that in adults (Waldhauser et al., 1988). A high blood concentration of serotonin in the brain in the morning and, in turn, high concentration of melatonin in the evening is necessary for infants and young children.

Therefore, a low intake of tryptophan taken at breakfast may easily induce disorders in sleep quality and the circadian typology of infants and young children aged 1–8 yrs.

Breakfast tryptophan intake and mental symptoms

Based on the results of this study, tryptophan intake at breakfast has no direct effect on mental symptoms such as depression and anger in infants. However, there is some potential for indirect linkage of a permanent lack of tryptophan intake to the induction of low mental symptoms via the shift to the evening typology.

A shortage of catecholamines, adrenaline, noradrenaline, and dopamine is known as neuroamines to induce affective disorders (Ikuta, 2005). Phenylalanine and tyrosine taken at

mealtimes as a source of catecholamines may promote the improvement of mental health of children. The analysis of this remains to be done in the future.

In conclusion, tryptophan absorbed at breakfast is very important for children to keep to a morning-type diurnal rhythm and a high quality of sleep, presumably through the metabolism of tryptophan to serotonin in the daytime and further to melatonin at night.

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