Japanese sake yeast supplementation improves the quality of sleep: a double-blind randomised controlled clinical trial

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SUMMARY

Activation of adenosine A_{2a} receptors in cerebral neurons induces sleep in various mammals. It was previously found that Japanese sake yeast enriched in adenosine analogues activates A_{2a} receptors *in vitro* and induces sleep in mice. Here it is reported that sake yeast activated A_{2a} receptors in a cultured human cell line and improved human sleep quality in a clinical trial. Sake yeast activated A_{2a} receptors in HEK cells in a dose-dependent manner with an EC_{50} of 40 $\mu g\ mL^{-1}$, and the activation was attenuated almost completely by the A_{2a} receptor antagonist ZM241385 with an IC_{50} of 73 nM. In a double-blind placebo-controlled crossover clinical study, 68 healthy participants ingested tablets containing either 500 mg of sake yeast powder or a placebo (cellulose) 1 h before sleep for 4 days. Electroencephalograms were recorded during sleep at home with a portable device for 4 week days. Electroencephalogram analyses revealed that sake yeast supplementation significantly ($P = 0.03$) increased delta power during the first cycle of slow-wave sleep by 110%, without changing other sleep parameters. Sake yeast supplementation also significantly increased growth hormone secretion in the urine on awakening by 137% from 3.17 ± 0.41 (placebo) to 4.33 ± 0.62 (sake yeast) pg mg^{-1} creatinine ($P = 0.03$). Subjective sleepiness ($P = 0.02$) and fatigue ($P = 0.06$) in the morning were improved by sake yeast. Given these benefits and the absence of adverse effects during the study period, it was concluded that sake yeast supplementation is an effective and safe way to support daily high-quality, deep sleep.

INTRODUCTION

Numerous studies have attempted to interpret sleep significance. Insufficient sleep, such as insomnia, is highly correlated with an increased risk for lifestyle diseases, such as obesity, hypertension and cerebral infarction (Beihai and Xiaomei, 2015; Guo *et al.*, 2013; Spiegel *et al.*, 2009). Thus, good sleep is indispensable for high quality of life. Despite the known importance of sleep, many people suffer from sleep-related problems (Ohayon, 2002).

Non-rapid eye movement (NREM) sleep with 0.5–4-Hz electroencephalographic (EEG) activity (delta power) is considered deep sleep, termed slow-wave sleep (SWS), and is essential for many physiological functions. Experimental SWS deprivation leads to daytime sleepiness and decreased daytime performance (Dijk, 2010). SWS is required for growth hormone (GH) secretion during sleep, memory consolidation and proper cognitive functioning (Born and Wilhelm, 2012; Ferrara *et al.*, 2000; Gronfier *et al.*, 1996; Van Cauter and Copinschi, 2000). SWS length

tends to decline with aging, along with a decrease in the subjective sleep quality (Dijk, 2010; Espiritu, 2008; Unruh *et al.*, 2008). One way to improve sleep quality was to increase the delta power activity and deepen SWS.

Genetic and pharmacological studies identified various signalling factors, such as γ-aminobutyric acid (GABA), adenosine, prostaglandin D_2, histamine and hypocretin/orexin (hcrt/orx), regulating wake/sleep behaviours. Inhibitory cerebral neurons secrete GABA to suppress neuronal activity maintaining a wakeful state (Nitz and Siegel, 1996). Histamine and hcrt/orx are known to activate neurons that positively control the awake state (Huang *et al.*, 2001; Mieda *et al.*, 2004). Hence, GABA receptor agonists and histamine or hcrt/orx antagonists act as hypnotics to reduce neural activity regulating the awake state to induce sleep. Although these hypnotics, including benzodiazepine, effectively induce sleep, patients should refrain from long-term daily use because of their adverse effects, such as headache, dizziness and abnormal dreams. (Equihua *et al.*, 2013; Lader, 2014).

Differing from GABA, histamine and hcrt/orx signalling, the adenosine and prostaglandin D_2 pathways are recently identified humoral sleep-regulation pathways and have high novelty in that these signallings positively regulate sleep-inducing neurons in the ventrolateral preoptic area or nucleus accumbens shell (Hayaishi and Urade, 2002; Huang *et al.*, 2011; Lazarus *et al.*, 2011; Urade and Hayaishi, 2011). Local extracellular adenosine level increases in the brain proportionally with waking time, and the accumulated adenosine stimulates adenosine A_1 and A_{2a} receptors (Gallopin *et al.*, 2005). Although the contribution proportions of the A_1 and A_{2a} receptors to sleep remain controversial, studies suggested that the A_{2a} receptor is the main sleep induction mediator by adenosine; for example, cerebroventricular infusion of a selective agonist of A_{2a} receptors, CGS-21680, promotes markedly NREM sleep in mice to the peak level of physiological sleep, but a selective A_1 agonist, cyclopentyl adenosine, did not affect the vigilance state at all (Urade *et al.*, 2003). Moreover, the arousal effect of caffeine is completely attenuated in A_{2a} receptor knockout (KO) mice but unchanged in A_1 receptor KO mice at all, indicating that A_{2a} receptors are critical for caffeine-induced wakefulness (Huang *et al.*, 2005). Hence, A_{2a} receptor stimulation is a possible target for inducing deep sleep. However, there are currently no hypnotics or supplements for improving sleep by stimulating A_{2a} receptors. Therefore, the aim was to develop functional and safe supplements to improve sleep quality by stimulating A_{2a} receptors in the brain.

To find a component that activated A_{2a} receptors, 80 food materials were investigated and it was found that sake yeast markedly activated the A_{2a} receptor. Sake yeast, but not beer yeast and baker's yeast, is interestingly enriched in adenosine analogues. Sake yeast is used to produce Japanese rice wine (sake) and also used for traditional Japanese foods, Kasujiru (a soup containing sake yeast) and Kasuzuke (pickles containing sake yeast). It was predicted that sake yeast induced sleep, and it was found that oral administration of sake yeast induced sleep in mice.

In this study, it was shown that sake yeast directly activates A_{2a} receptors on cultured human cells and improves sleep quality in healthy humans.

MATERIALS AND METHODS

Materials

Sake yeast powder (GSP6) was purchased from Mitsubishi Gas Chemical Company (Tokyo, Japan). Sake yeast tablets for ingestion were prepared by pressing 125-mg sake yeast powder, containing approximately 75-mg dry sake yeast, into 300-mg tablets. The inactive ingredients in the tablets were crystalline cellulose, cyclodextrin, calcium carboxymethylcellulose, hydroxypropyl methylcellulose, silicon dioxide, calcium stearate and glycerol. Placebo tablets flavoured to taste like sake yeast with Sanfixbutter No. 24644 (Saneigen, Osaka, Japan) and tartrazine (Saneigen) were prepared according to the same basic composition but without the sake yeast (crystalline cellulose was added instead).

Adenosine A_{2a} receptor activity assay *in vitro*

Adenosine A_{2a} receptor activity was quantified by cyclic AMP (cAMP) accumulation in HEK cells expressing human adenosine A_{2a} receptor (PerkinElmer, Waltham, MA, USA). The manipulation was performed according to the manufacturer's instructions (LANCE Ultra cAMP Kit, PerkinElmer). HEK cells were suspended in Hanks' balanced salt solution containing 5 mM HEPES (pH 7.4), 0.1% protease-free bovine serum albumin and 0.5 mM methylisobutylxanthine in 384-well microplates. Dried sake yeast powder or adenosine receptor agonist, 5′-*N*-ethylcarboxamidoadenosine (NECA, 1.0 μM; Tocris Bioscience, MN, USA), was added to the cell suspension, adjusted to contain 1000 cells per well, in the absence or presence of an adenosine A_{2a} receptor antagonist (0.1 and 1.0 μM ZM241385; Sigma-Aldrich, MO, USA), and incubated for 30 min at room temperature. The detection mixture containing Eu-cAMP tracer and ULight-anti-cAMP was added and incubated for 1 h at room temperature. Fluorescence resonance energy transfer signals were measured with a microplate reader (Tecan Infinite 200 PRO, Männedorf, Switzerland; excitation: 320 nm; emission: 665 nm). The standard cAMP curve included with the kit was used to determine cAMP production.

Study design of clinical test

The study design was approved by the Institutional Review Board of Lion Corporation. All the study procedures were conducted according to the Helsinki Declaration and ethical guidelines for epidemiology research authorised by the

Japanese government. Written informed consent was obtained from all participants.

This double-blind placebo-controlled crossover trial was conducted over two 4-day periods (Tuesday–Friday), separated by a 3-day washout period. Participants were randomly allocated to either group I or II (Fig. 1). They ingested four sake yeast tablets (125 mg sake yeast powder per tablet) or four placebo (cellulose) tablets per day for 4 days, 1 h before bedtime. Supplementation effects were evaluated by measuring EEG activity and GH amount secreted, and by having participants fill in a sleep questionnaire.

Participants

One-hundred and eight Japanese volunteers were recruited. They were all weekday full-time employees, and were assessed for eligibility with the Pittsburgh Sleep Quality Index (PSQI) questionnaire (Buysse *et al.*, 1989). Before starting the clinical trial, 35 volunteers who already had good sleep quality (PSQI < 5; Buysse *et al.*, 1989) and five volunteers who declined to participate were excluded. Thus, 68 participants proceeded to the clinical trial (average age ± SE = 38.2 ± 1.1 years; 35 female and 33 male; PSQI average ± SE = 6.8 ± 0.2).

The participants were instructed to maintain their usual lifestyle, and to not ingest excessive alcohol or caffeine amount. They were asked to not take substances, such as medicinal drugs and supplements, which could affect sleep quality. During the trial, eight participants dropped out (three caught influenza, three could not maintain their usual lifestyle, one took an antihistamine drug, and one consumed an excessive alcohol amount). Data analysis was conducted for 60 participants (average age ± SE = 37.7 ± 1.1 years; 29 female and 31 male; PSQI average ± SE = 6.8 ± 0.2; Fig. 1).

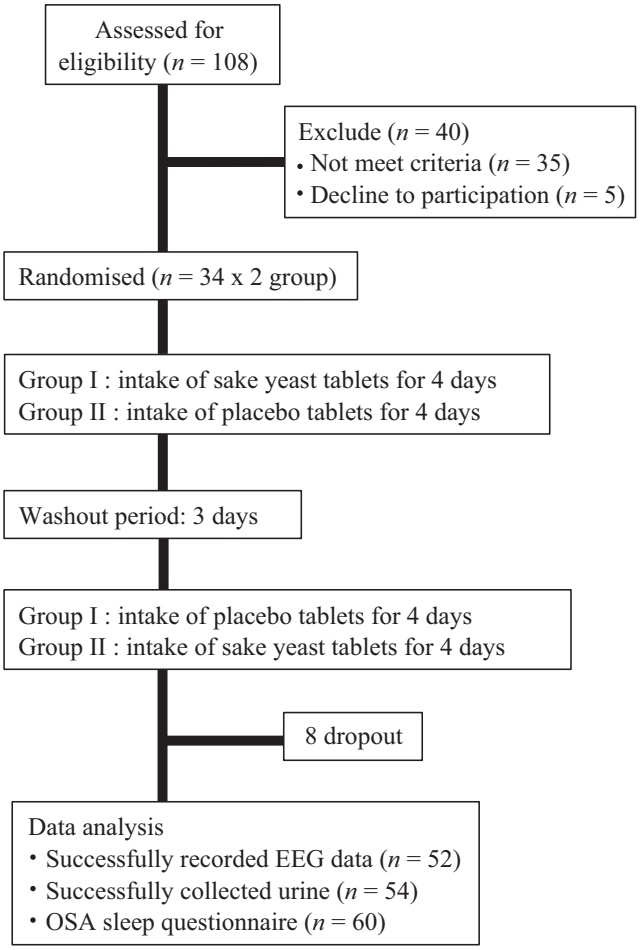

Figure 1. Flow chart of participation and study design. The flow chart shows the study design of the clinical trial. We divided 68 participants who met the criteria randomly into two groups, groups I and II. Participants in each group ingested four tablets of sake yeast or placebo, respectively, daily for 4 days. During the study period, 8 participants dropped out.

EEG measurement and sleep-stage evaluation

Electroencephalographic data were collected at a sampling rate of 128 Hz to analyse the participants' daily sleep using a single-channel portable EEG device (Sleepscope, Sleepwell, Osaka, Japan) with disposable Ag/AgCl surface electrodes placed on their foreheads and behind their ears at home (Takahashi *et al.*, 2013). Sleep stage during each 30-s epoch was evaluated by EEG data visual analysis. The participants were instructed to record EEG in their home for four nights per test condition. EEG data were classified into four different sleep stages: awake; REM sleep; light (stage 1 and 2) NREM sleep; and deep (stage 3 and 4) NREM sleep. Delta power during the first SWS cycle was calculated to evaluate sleep depth during SWS, and this was used as the primary clinical endpoint. Additionally, sleep latency, sleep efficiency, total sleep time, light NREM sleep time, deep NREM sleep time, REM sleep time and awaking time were calculated. Time courses of EEG power density at different frequencies (0.5–2.0, 4.0–8.0, 8.0–12 and 0.5–35 Hz) for 120 min after sleep onset were calculated. For individual records, average and standard deviation (SD) of each frequency epoch were calculated. Any epoch data that exceeded the average +3 × SD were excluded from the analysis. The appropriate epoch data in each frequency were calculated as relative values, based on the mean of the 0.5–35 Hz record for 120 min after sleep onset in the placebo condition (n = 52). Of the 60 participants, EEG data for eight were not recorded correctly because of disconnected electrodes and were excluded from the statistical analysis.

Urinary GH concentration

Urine samples were collected every morning on awakening. Urinary GH and creatinine concentrations were measured at Macrophi (Kagawa, Japan). Briefly, GH amount was determined with an enzyme-linked immunosorbent assay kit

(DGH00, R&D systems, MN, USA), according to the manufacturer's instructions. Creatinine concentration in the urine samples was determined with a creatinine (urinary) colorimetric assay kit (Cayman Chemical, MI, USA), according to the manufacturer's instructions. GH concentration was corrected for urinary creatinine (pg mg^{-1} creatinine). Because six participants forgot to collect urine, these participants' data were excluded from the statistical analysis.

Sleep questionnaire

The participants filled out the Ogri–Shirakawa–Azumi sleep questionnaire (OSA; Yamamoto *et al*., 1999) every morning within 30 min of rising to assess the subjective effects of sake yeast on sleep quality. The OSA sleep questionnaire has 16 questions rated on a scale of 1–4, and consolidates results into five factors: 'sleepiness on rising' (calculated from questions 2, 4, 8 and 14); 'initiation and maintenance of sleep' (from questions 3, 7, 10, 13 and 16); 'frequency of dreams' (from questions 9 and 12); 'refreshed on rising' (from questions 1, 5 and 11); and 'sleep duration' (from questions 6 and 15). Sixty OSA responses for the statistical analysis were evaluated.

Statistical analysis

All statistical analysis was performed using Statlight version 2 software (Yukms, Kanagawa, Japan). For biochemical analysis of adenosine A_{2a} receptor activity, cAMP production was averaged across five trials and evaluated by Tukey–Kramer test. A value of $P < 0.05$ was considered significant. In the clinical trial, the first day under each sake yeast and placebo condition was considered an adaptation period, and data from those days were excluded from statistical analysis. For each participant, EEG parameters, OSA scores and GH urinary concentrations from the remaining test period were averaged, and the differences between the sake yeast and placebo conditions were analysed by paired *t*-tests. A two-tailed *P*-value of <0.05 was considered statistically significant.

RESULTS

Sake yeast stimulated human adenosine A_{2a} receptors *in vitro*

Because adenosine A_{2a} receptor activation results in an increase in the intracellular concentration of cAMP, the receptor activating activity was quantified by measuring the intracellular concentration of cAMP in HEK cells expressing human A_{2a} receptors after incubation with 80 different foodstuffs. From this primary screening, it was found that sake yeast powder significantly increased cAMP production (Fig. 2a). The EC_{50} concentration, which is equal to 50% of activation with an excess concentration (1.0 μM) of NECA, was 40 μg mL^{-1}. The sake yeast-induced increase in the intracellular cAMP level was completely inhibited by a selective antagonist of adenosine A_{2a} receptors, ZM241385, in a dose-dependent manner with an IC_{50} value of 73 nM (Fig. 2b), indicating that the sake yeast treatment activated human adenosine A_{2a} receptors.

Sake yeast ingestion increased EEG delta power in the first cycle of SWS and enhanced GH secretion during sleep in the clinical trial

Then, a double-blind, randomised, placebo-controlled clinical trial was conducted on 68 healthy volunteers to test whether sake yeast affects human sleep. EEG parameters are summarised in Table 1. Ingestion of sake yeast tablets significantly ($P = 0.03$) increased the EEG delta power in the

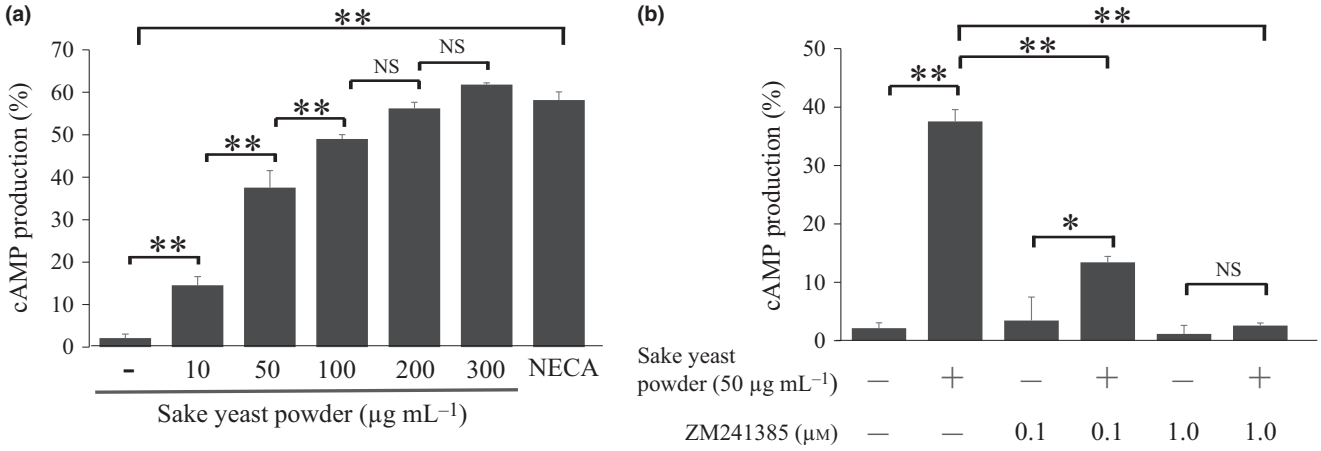

Figure 2. (a) Effects of various amounts of sake yeast powder or 1.0 μM NECA on intracellular cAMP concentration in A_{2a} receptor-expressing HEK cells. (b) Suppression of sake yeast (50 μg mL^{-1})-induced increase in cAMP production by the adenosine A_{2a} receptor antagonist ZM241385 (0.1 and 1.0 μM). *y* axis shows cAMP level normalized to the signal from 1.0 μM of cAMP as 100%. Error bar shows SE. n= 5; **P < 0.01; *P < 0.05; NS, not significant.

Table 1 Objective sleep parameters analysed by EEG and urine analysis on sake yeast and placebo conditions

Evaluated factor	*Sake yeast*	*Placebo*	N	P-*value*
EEG				
Delta power during first NREM ($\times 10^5$ μV^2)	2.90 ± 0.31	2.64 ± 0.27	52	0.03*
Sleep latency (min)	11 ± 1	10 ± 2	52	0.48
Sleep efficiency (%)	92 ± 0	93 ± 0	50	0.64
Total sleep time (min)	340 ± 7	334 ± 7	50	0.43
Light NREM sleep (min)	212 ± 6	208 ± 7	50	0.49
Deep NREM sleep (min)	36 ± 5	33 ± 5	50	0.23
REM sleep (min)	92 ± 3	93 ± 3	50	0.87
Awaking (min)	16 ± 1	17 ± 1	50	0.36
Urine analysis				
GH in urine (pg mg^{-1} creatinine)	4.33 ± 0.62	3.17 ± 0.41	54	0.03*
Creatinine in urine (mg mL^{-1})	1.60 ± 0.07	1.55 ± 0.08	54	0.64

The numbers in the table represent means ± SE.
EEG, electroencephalogram; NREM, non-rapid eye movement; REM, rapid eye movement.
*$P < 0.05$.

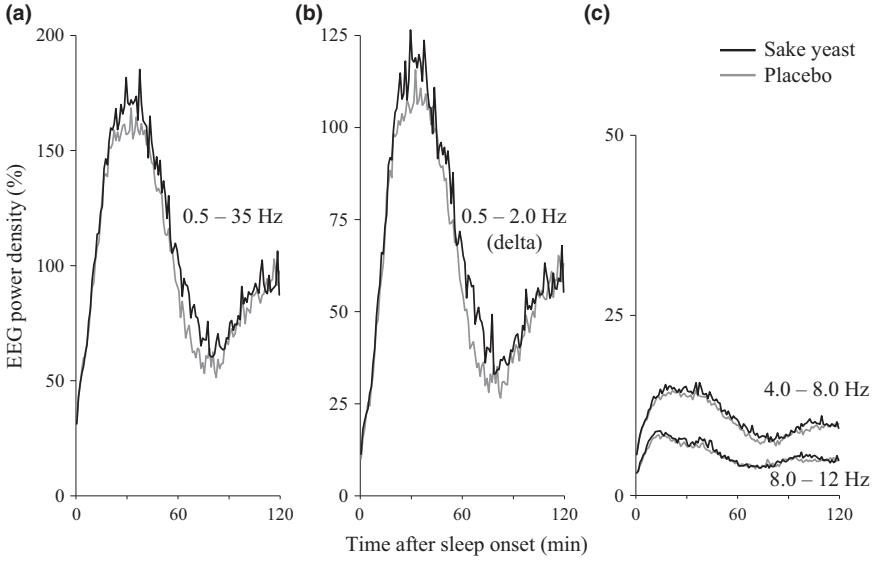

Figure 3. Time courses of EEG power density at different frequencies [0.5–35 Hz (a), 0.5–2.0 Hz(b), 4.0–8.0 and 8.0–12 Hz (c)] for 120 min after sleep onset. *y* axis represents the relative values of each frequency, based on the mean of the 0.5–35 Hz record for 120 min after sleep onset in placebo condition. The curves were smoothed by two epoch average. $n = 52$.

first SWS cycle by 110% compared with ingestion of the placebo. The time courses data of EEG power density in Fig. 3 showed that the major effect of sake yeast intake was the increasing of EEG delta power density among other frequency bands. No significant differences were observed in sleep latency, sleep efficiency and total amounts of sleep, light NREM sleep, deep NREM sleep, REM sleep or waking, indicating that sake yeast intake did not affect sleep architecture defined by the length of REM and NREM sleep and of wakefulness.

As GH secretion during sleep is known to be well-correlated with sleep depth, also the urinary concentration of GH on rising of participants was measured. Sake yeast ingestion significantly ($P = 0.03$) increased GH secretion during sleep by 137%, from 3.17 ± 0.41 (pg mg^{-1} creatinine) in the placebo group to 4.33 ± 0.62 in the sake yeast group (Table 1). In contrast, the creatinine concentration was unchanged between the placebo and sake yeast groups ($P = 0.64$).

Sake yeast supplementation improved subjective sleep quality

Table 2 shows the OSA scores from the sake yeast and placebo groups. Compared with the placebo condition, there was a significant improvement in 'sleepiness on rising'

Table 2 Subjective effects of sake yeast on sleep quality

Evaluated factor	*Sake yeast*	*Placebo*	*P-value*
Sleepiness on rising	16.7 ± 0.6	15.5 ± 0.6	0.02*
Initiation and maintenance of sleep	19.5 ± 0.6	19.1 ± 0.5	0.44
Frequent dreaming	23.3 ± 0.8	23.3 ± 0.8	0.95
Refreshed on rising	16.6 ± 0.6	15.5 ± 0.5	0.06
Sleep length	15.8 ± 0.8	15.2 ± 0.6	0.42

According to the OSA questionnaire method, each score from 16 questions was standardised and categorised into five factors as indicated. Higher scores indicate better subjective condition.
The numbers in the table represent means ± SE.
$n = 60$; $^*P < 0.05$.

($P = 0.02$) on sake yeast supplementation (higher scores denote improvement). A tendency toward improved 'refreshed on rising' scores ($P = 0.06$) was also observed. No significant differences or tendencies were observed for 'initiation and maintenance of sleep', 'frequency of dreams' or 'sleep duration'. Sake yeast supplementation produced no adverse events during the study period.

DISCUSSION

The present results showed that 500 mg sake yeast powder supplementation improved both objective and subjective measures of sleep quality in healthy participants. Sake yeast has a long history of use in food in Japan, giving assurance that sake yeast supplementation is safe. Sake yeast supplementation produced no adverse events during the study period. The current findings indicated that the daily sake yeast intake had ample potential for improving sleep quality. The biochemical data revealed that the incubation with sake yeast increased the intracellular cAMP level in human A_{2a} receptor-expressing cells, and that this effect was completely attenuated by the A_{2a} receptor antagonist ZM241385. These results indicated that sake yeast directly activated human A_{2a} receptors, and suggested that sake yeast affected sleep *in vivo* by this mechanism. Practically, in mice sake yeast-induced NREM sleep was abolished by an intraperitoneal pretreatment with an A_{2a} receptor-selective antagonist ZM241385 (unpublished data).

The EEG analyses indicated that sake yeast ingestion increased the delta power in the first cycle of SWS without changing the sleep architecture composed of awake state, REM state and SWS duration. It differed from benzodiazepines, widely used to treat insomnia, which alter sleep architecture by reducing SWS sleep (Borbély *et al.*, 1983; Parrino and Terzano, 1996). Because sake yeast intake induced natural and deep sleep in healthy participants without disturbing the balance of sleep architecture, it might offer the advantage of inducing physiological deep sleep, differing from medication with sleeping pills.

The EEG result of an increase in the delta power in the first cycle of SWS was in good agreement with the observed increase in urinary GH levels. GH is secreted mostly during the first SWS cycle, and the amount of GH secretion depends on the quality of the first SWS cycle (Gronfier *et al.*, 1996; Van Cauter and Copinschi, 2000). For instance, pharmacological enhancement of the first SWS cycle leads to increased GH secretion (Van Cauter *et al.*, 1997). Accordingly, it was inferred that sake yeast intake contributed to the increase in GH secretion by deepening sleep during the first cycle of SWS (Table 1). The role of GH in metabolic homeostasis is well studied, and GH deficiency is associated with disorders of lipid metabolism, decrease in lean body mass and cognitive impairment (Daniel *et al.*, 1990; Maruff and Falleti, 2005; Salomon *et al.*, 1989). Although GH is indispensable for homeostasis of these various functions, the amount of GH secretion decreases with aging (Feinberg, 2000; Van Cauter *et al.*, 2000). The depth of SWS also tends to decline with aging (Landolt *et al.*, 1996; Landolt and Borbély, 2001). Thus, it was expected that sake yeast intake will have beneficial effects on maintaining metabolic, vascular and brain functions, especially in older people, by improving sleep quality in the first SWS cycle and thereby enhancing GH secretion. Further case studies will be conducted to test this hypothesis.

Given that sleep quality is usually subjectively assessed, it was of great interest to know whether participants experienced a change in their quality of sleep after ingesting sake yeast. In this regard, the result of the subjective OSA questionnaire was remarkable. The result revealed that the sake yeast supplementation improved the subjective sense of 'sleepiness on rising'. The subjective improvement presumably reflected good sleep resulting from improved deep SWS after taking the sake yeast tablets.

The active ingredients in sake yeast and the mechanism of action on sleep are of great interest. Sake yeast is classified as the same yeast, *Saccharomyces cerevisiae*, as baker's yeast and beer yeast. Unlike sake yeast, however, baker's yeast and beer yeast do not activate adenosine A_{2a} receptors. Interestingly, sake yeast, and not other yeasts, contains a large amount of S-adenosyl-L-methionine (SAM) and methylthioadenosine (MTA), a deaminated metabolite of SAM (Shiozaki *et al.*, 1984), probably owing to the unique condition in the production process for sake. The identical chemical structure of SAM and MTA is thioadenosine; therefore, these ingredients were termed as thioadenosine analogues. Thioadenosine is a derivative of adenosine, the ligand of endogenous A_{2a} receptors and, in fact, activates human A_{2a} receptors *in vitro* (EC_{50} in HEK cells expressing the receptors: SAM, 155 μM; MTA, 21.7 μM; preliminary data).

Given that A_{2a} receptors are crucially involved in sleep regulation (Hayaishi and Urade, 2002; Huang *et al.*, 2005, 2011; Urade *et al.*, 2003), it was speculated that thioadenosine analogues in the ingested sake yeast stimulated human A_{2a} receptors in the brain and enhanced SWS, resulting in deep sleep. To test this hypothesis, it has been investigated

whether thioadenosines induce sleep in A_{2a} receptor KO mice, and whether A_{2a} receptor antagonists attenuate the sleep-inducing effect of thioadenosines in wild-type mice. Furthermore, it is planned to investigate whether or not A_1 receptors mediate the sake yeast-induced sleep-promoting effect using A_1-receptor KO mice and A_1 receptor antagonists.

Pharmacokinetic analysis is important to understand how long sake yeast affects sleep after the intake. As thioadenosine analogues disappeared in blood within 120 min after oral administration of sake yeast in mice, the effect may be limited in the early sleep period (first cycle of SWS in the case of humans).

In conclusion, it has been shown in this study that sake yeast activated human A_{2a} receptors and that its supplementation improved human sleep quality in a clinical trial. Sake yeast supplementation increased the delta power during the first cycle of SWS without changing other sleep parameters and also upregulated GH secretion during sleep. Because of its efficacy and safety, sake yeast ingestion is a promising way to support daily high-quality, deep sleep.

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CONFLICT OF INTEREST

All parts of this study were funded by Lion Corporation. The authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

AUTHOR CONTRIBUTIONS

N. M., A. M., Y. Nagamori and T. S. performed and analysed the clinical experiment. E. K., Y. Nakamura, K. O., T. S. and T. M. performed and analysed the *in vitro* experiment. M. M., A. U., K. S., H. N. and Y. U. prepared the manuscript.

REFERENCES

Beihai, G. and Xiaomei, G. Short and long sleep durations are both associated with increased risk of stroke: a meta-analysis of observational studies. *Int. J. Stroke*, 2015, 10: 177–184.

Borbély, A. A., Mattmann, P., Loepfe, M. *et al.* A single dose of benzodiazepine hypnotics alters the sleep EEG in the subsequent drug-free night. *Eur. J. Pharmacol.*, 1983, 89: 157–161.

Born, J. and Wilhelm, I. System consolidation of memory during sleep. *Psychol. Res.*, 2012, 76: 192–203.

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R. and Kupfer, D. J.. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res.*, 1989, 28: 193–213.

Daniel, R., Axel, G. F., Hoskote, S. *et al.* Effects of human growth hormone in men over 60 years old. *N. Engl. J. Med.*, 1990, 323: 1–6.

Dijk, D. J. Slow-wave sleep deficiency and enhancement: implications for insomnia and its management. *World J. Biol. Psychiatry*, 2010, 11: 22–28.

Equihua, A. C., De La Herrán-Arita, A. K. and Drucker-Colin, R. Orexin receptor antagonists as therapeutic agents for insomnia. *Front Pharmacol.*, 2013, 4: 163.

Espiritu, J. R. Aging-related sleep changes. *Clin. Geriatr. Med.*, 2008, 24: 1–14.

Feinberg, I. Slow wave sleep and release of growth hormone. *JAMA*, 2000, 284: 2717–2718.

Ferrara, M., De Gennaro, L., Casagrande, M. and Bertini, M. Selective slow-wave sleep deprivation and time-of-night effects on cognitive performance upon awakening. *Psychophysiology*, 2000, 37: 440–446.

Gallopin, T., Luppi, P. H., Cauli, B. *et al.* The endogenous somnogen adenosine excites a subset of sleep-promoting neurons via A_{2A} receptors in the ventrolateral preoptic nucleus. *Neuroscience*, 2005, 134: 1377–1390.

Gronfier, C., Luthringer, R., Follenius, M. *et al.* A quantitative evaluation of the relationships between growth hormone secretion and delta wave electroencephalographic activity during normal sleep and after enrichment in delta waves. *Sleep*, 1996, 19: 817–824.

Guo, X., Zheng, X., Wang, J., Zhang, X., Li, J. and Sun, Y. Epidemiological evidence for the link between sleep duration and high blood pressure: a systematic review and meta-analysis. *Sleep Med.*, 2013, 14: 324–332.

Hayaishi, O. and Urade, Y. Prostaglandin D2 in sleep-wake regulation: recent progress and perspectives. *Neuroscientist*, 2002, 8: 12–15.

Huang, Z. L., Qu, W. M., Li, W. D. *et al.* Arousal effect of orexin A depends on activation of the histaminergic system. *Proc. Natl Acad. Sci. USA*, 2001, 98: 9965–9970.

Huang, Z. L., Qu, W. M., Eguchi, N. *et al.* Adenosine A_{2A}, but not A_1, receptors mediate the arousal effect of caffeine. *Nat. Neurosci.*, 2005, 8: 858–859.

Huang, Z. L., Urade, Y. and Hayaishi, O. The role of adenosine in the regulation of sleep. *Curr. Top. Med. Chem.*, 2011, 11: 1047–1057.

Lader, M. Benzodiazepine harm: how can it be reduced? *Br. J. Clin. Pharmacol.*, 2014, 77: 295–301.

Landolt, H. P. and Borbély, A. A. Age-dependent changes in sleep EEG topography. *Clin. Neurophysiol.*, 2001, 112: 369–377.

Landolt, H. P., Dijk, D. J., Achermann, P. and Borbély, A. A. Effect of age on the sleep EEG: slow-wave activity and spindle frequency activity in young and middle-aged men. *Brain Res.*, 1996, 738: 205–212.

Lazarus, M., Shen, H. Y., Cherasse, Y. *et al.* Arousal effect of caffeine depends on adenosine A_{2A} receptors in the shell of the nucleus accumbens. *J. Neurosci.*, 2011, 31: 10.

Maruff, P. and Falleti, M. Cognitive function in growth hormone deficiency and growth hormone replacement. *Horm. Res.*, 2005, 64: 100–108.

Mieda, M., Willie, J. T., Hara, J., Sinton, C. M., Sakurai, T. and Yanagisawa, M. Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. *Proc. Natl Acad. Sci. USA*, 2004, 101: 4649–4654.

Nitz, D. and Siegel, J. M. GABA release in posterior hypothalamus across sleep-wake cycle. *Am. J. Physiol.*, 1996, 271: 1707–1712.

Ohayon, M. M. Epidemiology of insomnia: what we know and what we still need to learn. *Sleep Med. Rev.*, 2002, 6: 97–111.

Parrino, L. and Terzano, M. G. Polysomnographic effects of hypnotic drugs. A Review. *Psychopharmacology*, 1996, 126: 1–16.

Salomon, F., Cuneo, R. C., Hesp, R. and Sönksen, P. H. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N. Engl. J. Med.*, 1989, 321: 1797–1803.

Shiozaki, S., Shimizu, S. and Yamada, H. Unusual intracellular accumulation of S-adenosyl-L-methionine by microorganisms. *Agric. Biol. Chem.*, 1984, 48: 2293–2300.

Spiegel, K., Tasali, E., Leproult, R. and Van Cauter, E. Effects of poor and short sleep on glucose metabolism and obesity risk. *Nat. Rev. Endocrinol*, 2009, 5: 253–261.

Takahashi, H., Masaki, C., Makino, M. *et al.* Management of sleep-time masticatory muscle activity using stabilisation splints affects psychological stress. *J. Oral Rehabil.*, 2013, 40: 892–899.

Unruh, M. L., Redline, S., An, M. W. *et al.* Subjective and objective sleep quality and aging in the sleep heart health study. *J. Am. Geriatr. Soc.*, 2008, 56: 1218–1227.

Urade, Y. and Hayaishi, O. Prostaglandin D_2 and sleep/wake regulation. *Sleep Med. Rev.*, 2011, 15: 411–418.

Urade, Y., Eguchi, N., Qu, W. M. *et al.* Sleep regulation in adenosine A_{2A} receptor-deficient mice. *Neurology*, 2003, 9: S94–S96.

Van Cauter, E. and Copinschi, G. Interrelationships between growth hormone and sleep. *Growth Horm. IGF Res.*, 2000, 10: S57–S62.

Van Cauter, E., Plat, L., Scharf, M. B. *et al.* Simultaneous stimulation of slow-wave sleep and growth hormone secretion by gamma-hydroxybutyrate in normal young men. *J. Clin. Invest.*, 1997, 100: 745–753.

Van Cauter, E., Leproult, R. and Plat, L. Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. *JAMA*, 2000, 284: 861–868.

Yamamoto, Y., Tanaka, H., Takase, M. and Shirakawa, S. Standardization of revised version of OSA sleep inventory for middle age and aged. *Brain Sci. Mental Disorders*, 1999, 10: 401–409.