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ORIGINAL ARTICLE

Effect of sleep efficiency on salivary metabolite profile and cognitive function during exercise in volleyball athletes

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Abstract

Purpose Sleep duration is known to affect physiological and circadian metabolites and human homeostasis. However, little is known about the relationship between sleep quality and metabolite and cognitive function during exercise. Therefore, the aim of the present study was to investigate the impact of sleep quality on metabolite level and cognitive function in female volleyball athletes.

Methods Twelve female volleyball athletes participated in this study. Sleep efficiency was measured for 1 week using NemuriSCAN (Paramount Bed Co. Ltd., Japan) as an index of sleep quality. The subjects were divided into better ($n=6$) and lesser ($n=6$) sleep quality groups by the median value of sleep efficiency. Saliva samples were collected using a Salimetric oral swab cotton and salivary metabolites were analysed using capillary electrophoresis and time-of-flight mass spectrometry. The subjects performed Stroop tasks (simple and difficult tasks) at rest and during aerobic exercise in recumbent cycle ergometer at light and heavy intensity.

Results Increased sleep efficiency was found in the better sleep quality group, whereas total sleep time was similar. There were differences in urea cycle and Krebs cycle metabolites between the two groups; their levels were correlated with sleep efficiency. The difficult-task response time during heavy exercise was faster in the better sleep quality group.

Conclusion We demonstrated that sleep efficiency was associated with urea cycle and Krebs cycle metabolite levels and response time during heavy exercise in volleyball athletes. These results suggested that sleep quality may affect amino acid and energy metabolism and cognitive function during heavy exercise.

Keywords Cognitive function · Sleep efficiency · Metabolome · Athlete · Exercise intensity

Abbreviations

CE-TOFMS	Capillary electrophoresis and time-of-flight mass spectrometry
HRR	Heart rate reserve
NO	Nitric oxide

Introduction

High performance in competitive sport is associated with the integration of perceptual, cognitive, and motor skills (Chiu et al. 2017). Expert athletes are thought to exhibit superior executive control and visual processing (Mann et al. 2007; Zaccagni et al. 2009). High-order cognitive function is important for fast ball sports such as volleyball, in which athletes need to consider the ball, their position, teammates, and the opposite team even during heavy exercise intensity during matches (Zaccagni et al. 2009). Therefore, the

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athletes should have a high level of cognitive function during exercise to ensure top athletic performance.

Sleep has a beneficial effect in athletic performance through the recovery of physiological and cognitive function (Fullagar et al. 2015). Taheri and Arabameri (2012) have demonstrated that partial sleep deprivation delayed choice response time in college-trained students. Jarraya et al. (2014) also reported that sleep deprivation affected selective attention in handball goalkeepers. In addition, it has been reported that many athletes tend to have not only shorter sleep duration, but also poorer sleep quality due to anxiety and nervousness prior to competition (Leeder et al. 2012). However, the effect of sleep quality on cognitive function, especially during exercise, is not fully understood. Furthermore, the connection of biological link mediators between sleep and cognitive function is unclear (Xie et al. 2013). Sleep has a vital role in modulating metabolic homeostasis, which in turn affects physiological functions. A previous study suggested that an abnormal metabolite profile is associated with decreased cognitive function (Lee et al. 2018). Sleep deprivation induces changes in neuronal metabolites such as serotonin, tryptophan, and taurine in sedentary healthy adults (Davies et al. 2014). However, the effect of the sleep quality on the metabolite profile in athletes and the relation between sleep-related metabolites and cognitive function during exercise is also unclear. The aim of this study was to investigate the impact of sleep quality on the metabolite profile and cognitive function in volleyball athletes. Therefore, we performed a comprehensive comparison of metabolite profile and cognitive function between athletes with lesser and better sleep quality.

Methods

Subjects

A total of 12 female volleyball players (20 ± 0.3 years, 169 ± 3 cm, 59.5 ± 2.3 kg) participated in this study. Subjects in this study were medication free and had no history of menstruation abnormalities. The volleyball players were members of a top level of Japanese college team and trained 6 days/week with each training session lasted 6 h. The present study was approved by the institutional review board at the University of Tsukuba. All subjects gave written informed consent prior to participate in the study.

Study protocol

The subjects performed a cognitive task at rest and during exercise after evaluation of sleep quality for one week. They performed the cognitive task test in the early morning after a 12-h overnight fast after the collection of a saliva sample to establish base metabolite levels. Subjects abstained from alcohol and caffeine for at least 12 h prior to performance tests. The subjects sat on the cycle ergometer (Angio, LODE, The Netherlands) for measurement at rest and during exercise in a semi- recumbent position, which cause a lower increase in heart rate and higher increase in stroke volume. They underwent two 8-min bouts of cycling aerobic exercise at light (30% heart rate reserve, HRR) and heavy (70% HRR) intensity (Lucas et al. 2012). An investigator monitored each subject's heart rate using a 3-lead electrocardiogram and recorded the rating of perceived exertion using Borg's 6–20 scale (Borg 1970).

Measurements

Sleep quality

Sleep quality was measured using a non-wearable actigraphy device (NemuriSCAN; Paramount Bed Co. Ltd., Tokyo, Japan), which is placed under a mattress (Kogure et al. 2011). NemuriSCAN device has a highly sensitive pressure sensor and detects human movement through the mattress. The activity score, which reflects intensity and frequency of body movement (except for breathing and heartbeats), automatically determines the "sleep/awake" status every minute using an original algorithm. We analysed several other sleep variables, such as sleep latency time (awake status time from going to bed until sleep onset), arousal time (awake status time after sleep onset), total sleep time (sleep status time from going to bed), and sleep efficiency [total sleep time divided by the time in bed (%)]. These sleep variables have been shown to have a strong correlation with polysomnography results in both sleep and awake time and in each sleep stage (Kogure et al. 2011).

Salivary metabolite

Saliva samples were collected using an oral swab cotton swab and storage tube (Salimetrics oral swab; Salimetrics, USA) before the cognitive task test. Participants were asked to abstain from any drink and food before the saliva collection. They sat, rinsed their mouth with distilled water three times, and then rested for at least 5 min. Saliva production was stimulated by chewing on cotton for 1 min at a rate of 1 chew/s (Ra et al. 2014). The obtained saliva samples were separated from the cotton by centrifugation (1500 g), and the samples were frozen at − 80 °C until analysis. Metabolite levels were determined using capillary electrophoresis and time-of-flight mass spectrometry (CE-TOFMS) analysis (Soga et al. 2002). A saliva sample of 25 µL and Mili-Q water of 25 µL were combined with 400 µmol/L of commercial standard solution (H3304-1002; Human Metabolome Technologies, Japan) and passed through a 5 kDa cut-off

filter to remove proteins and macromolecules. The filtrate was analysed using CE-TOFMS performed using an Agilent capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) as described previously (Ra et al. 2014). Briefly, cationic metabolites were analysed through a fused silica capillary (50 µm internal diameter, 80 cm length) with a commercial buffer as the electrolyte. The sample was injected at a pressure of 5 mbar for 10 s. The applied voltage was set at 28 kV. Electrospray ionization–mass spectrometry was conducted in the positive ion mode. The spectrometer was scanned with mass-to-charge ratio (m/z) ranging from 50 to 1000. Anionic metabolites were analysed through a fused silica capillary (50 µm internal diameter and 80 cm length) with a commercial buffer as the electrolyte. The sample was injected at a pressure of 50 mbar for 25 s. The applied voltage was set at 30 kV. Electrospray ionization–mass spectrometry was conducted in the negative ion mode. The spectrometer was scanned using m/z ratios ranging from 50 to 1000. The obtained data were analysed using proprietary autonomic integration software (MasterHabds; Human Metabolome Technologies, Tsuruoka, Japan). Each metabolite was identified and quantified by the peak information including m/z ratio, migration time, and peak area.

Cognitive function

Participants performed the Stoop task to evaluate their cognitive function. In this study, the Stroop task involved two experimental conditions: non-executive simple task and executive difficult-task conditions. In the simple task condition, a colour name (e.g., red, blue, yellow, and green) was presented in black colour ink, and the participants were asked to identify the corresponding word in black colour. In the difficult-task condition, they received colour words displayed in incongruent colour (e.g., the word BLUE was presented in red) and they were asked to identify the corresponding ink colour name displayed in incongruent colour word (Akazawa et al. 2018). Participants had three previous practice sessions (1–2 weeks pre-test, 1 h before the test, and immediately before the test). The participants conducted the Stroop task at rest and during low and heavy exercise 5 min after the start of each exercise intensity.

Statistical analyses

We divided the subjects into lesser ($n=6$) and better ($n=6$) sleep quality groups by their median value of sleep efficiency. Normality of the distribution for outcome measures was tested using the Shapiro–Wilk test. Normally distributed data were analysed using unpaired Student's t test to compare differences between the groups. The correlation between sleep efficiency, metabolite levels, and response time in the Stroop tests was analysed using Pearson's correlation coefficient. Data that were not normally distributed were analysed using nonparametric Mann–Whitney test for group differences and Spearman correlation coefficient for relationships. Data are expressed as the means ± SD. Statistical significance was set a priori at $P<0.05$ for all comparisons. Statistical analyses were performed using SPSS ver. 24 software (Chicago, IL, USA).

Results

Table 1 depicts the sleep characteristics of both groups. There were no differences in time in bed and total sleep time between groups. In the better sleep quality group, sleep efficiency was greater and sleep latency and arousal time was shorter compared to the lesser sleep quality group.

Figure 1 shows the metabolites with significant differences between the lesser and better sleep quality groups. Lysine, proline, tyrosine, ornithine, citrulline, 2-oxoglutaric acid, and caffeine levels were greater in the better sleep quality group. Arginine levels tended to be greater in the better sleep quality group. Urea and myo-inositol 3-phosphate levels were lower in the better sleep quality group. There were no differences in other metabolites between the two groups.

In the simple task, response time decreased during both light and heavy exercise intensity compared to rest in both groups (Fig. 2). There were no differences in response time in the simple task at rest and during exercise between the lesser and better sleep quality groups. In the difficult task, there was an interaction effect between exercise intensity and sleep quality in response time. The response time in the lesser sleep quality group did not change during exercise, while in the better sleep quality group, it decreased during heavy exercise intensity ($P<0.05$). Furthermore, the response time was significantly faster in the better sleep quality group during heavy exercise.

Figure 3 shows the relationship between sleep efficiency and response time at rest and during exercise. Sleep efficiency was significantly correlated with response time only

Table 1 Sleep characteristics in lesser and better sleep quality groups

	Lesser sleep quality group	Better sleep quality group
Time in bed (min)	420 ± 22	419 ± 45
Total sleep time (min)	379 ± 21	399 ± 45
Sleep quality (%)	90 ± 3	95 ± 1*
Sleep latency (min)	13 ± 8	8 ± 4*
Arousal (min)	35 ± 25	18 ± 15*

Data are means ± SD

*$P<0.05$ vs. lower sleep quality group

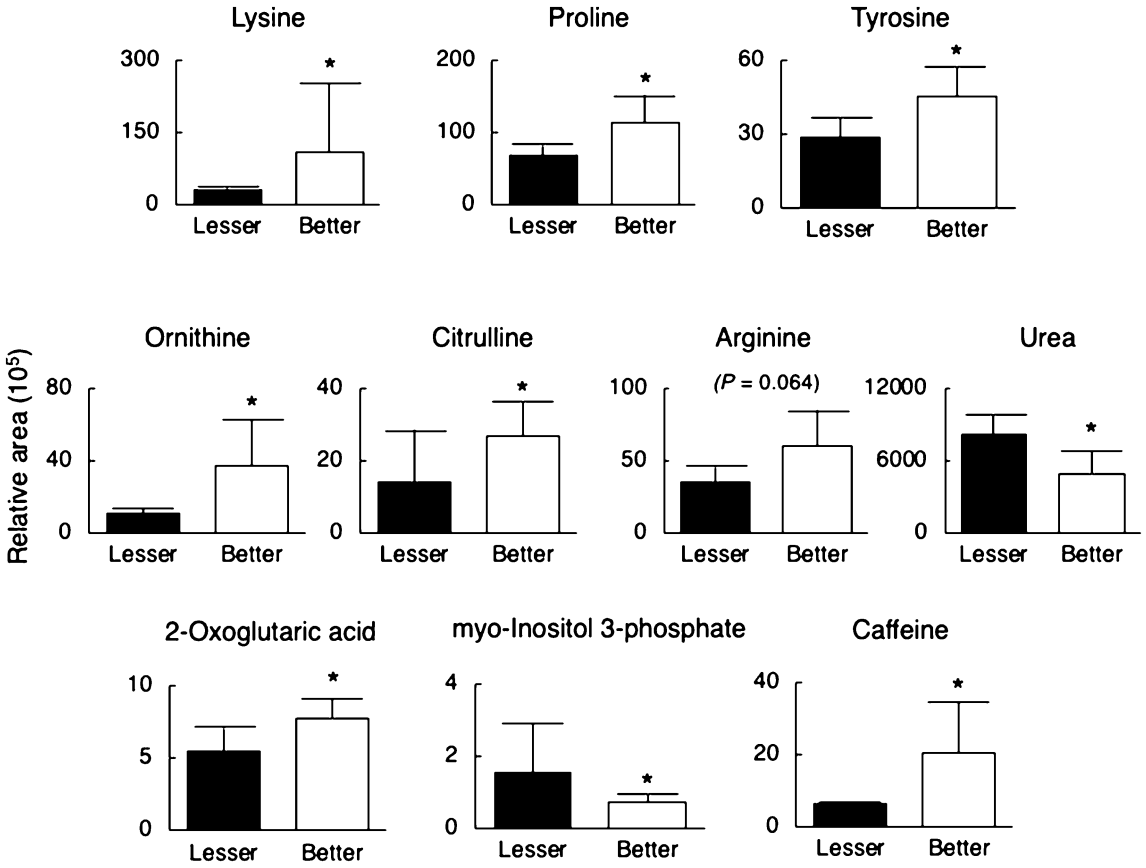

Fig. 1 Salivary metabolites in which differences were detected between the lesser and better sleep quality groups. $*P < 0.05$ vs. the lesser sleep quality group

during heavy exercise, with no significant correlations at rest and during light exercise. Table 2 depicts the matrix of correlation between selected metabolites and sleep quality and cognitive function. Lysine, tyrosine, ornithine, citrulline, arginine, 2-oxoglutaric acid, myo-inositol 3-phosphate, and caffeine were significantly correlated with sleep efficiency. Furthermore, citrulline was significantly correlated with response time during heavy exercise intensity.

Discussion

In this study, we investigated the effects of sleep quality on salivary metabolites and cognitive function during exercise in female volleyball players. The main findings of this study were as follows: first, there were significant differences in lysine, proline, tyrosine, ornithine, citrulline, and urea between the better and lesser sleep efficiency groups. Second, although there were no significant differences in response time in Stroop tasks at rest and during light intensity exercise, response time was faster in the better sleep efficiency group than in the lesser sleep efficiency group during heavy intensity exercise. Third, lysine, tyrosine, citrulline and arginine levels were significantly correlated with sleep efficiency; furthermore, citrulline levels were found to be correlated with Stroop response time during heavy exercise. These results suggested that sleep quality may be associated with glucose and ammonia metabolism and cognitive performance during heavy exercise in female volleyball athletes.

Emerging evidences demonstrate that sleep duration is associated with athletic performance (Thun et al. 2015). Mah et al. (2011) reported that increased sleep duration enhanced sprint time and shoot accuracy in male basketball players. Reilly and Piercy (1994) demonstrated that sleep deprivation decreased weight-lifting performance. In addition, Taheri and Arabameri (2012) found that sleep deprivation reduced selective response time in athletes. In this study, we first investigated the effect of sleep efficiency on cognitive performance during exercise in volleyball players. The results indicated that the response time in a Stroop task was faster in the better sleep quality group during heavy exercise. Therefore, sleep quality also seems to be relevant to cognitive performance during exercise.

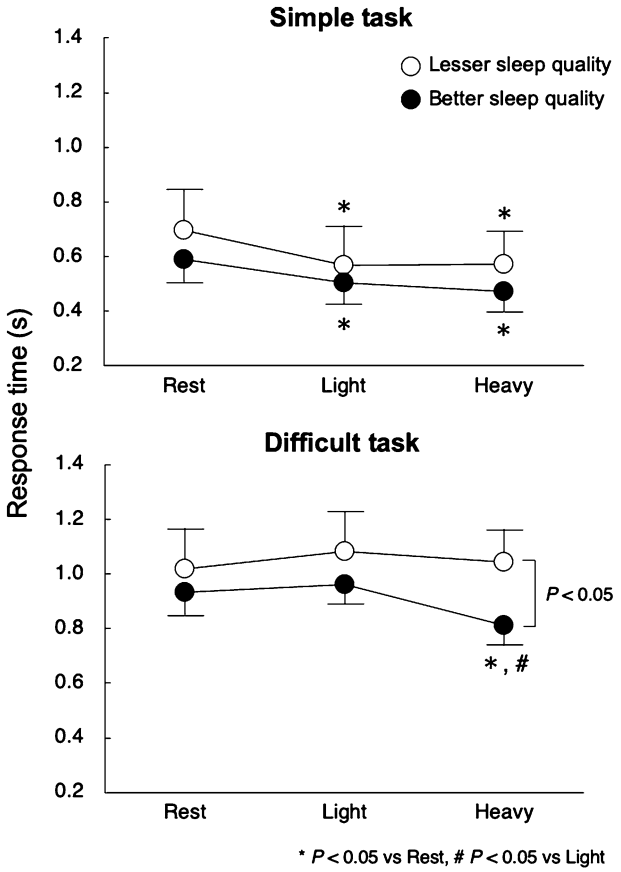

Fig. 2 Response time in Stroop simple and difficult tasks at rest and during light and heavy exercise. *$P<0.05$ vs. rest, #$P<0.05$ vs. light exercise

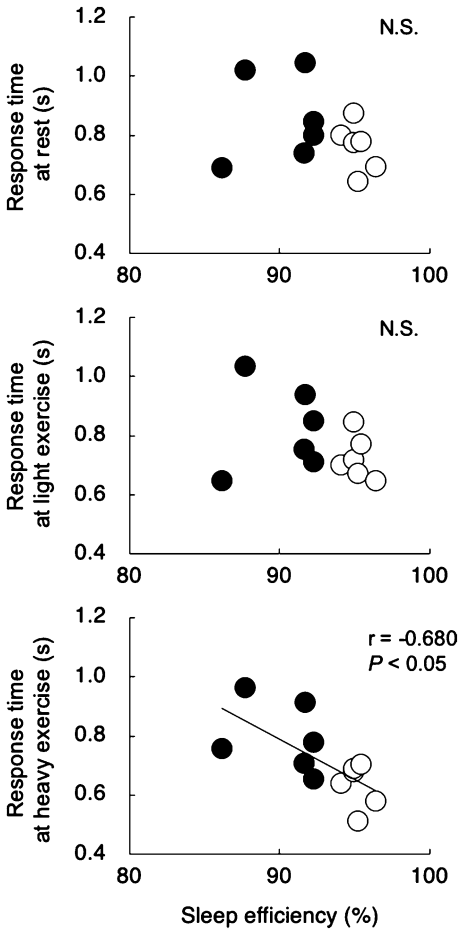

Fig. 3 Relationships between sleep and response time at rest and during light and heavy exercise. Response time is presented as the average of simple and difficult tasks. Open circles represent the better sleep quality group and filled circles represent the lesser sleep quality group

It has been demonstrated that cognitive performance can be enhanced during exercise (Chang et al. 2012). The response time in the Stroop task was shorter during light and heavy cycling exercise compared to that during rest (Lucas et al. 2012). Consistently with the previous studies, the results of the present study demonstrated that response time decreased during exercise. In the simple task condition, response time was equally decreased during light and heavy exercise in both the better and lesser sleep quality group, while the better sleep quality group showed a lower response time during heavy exercise. Furthermore, sleep efficiency was significantly correlated with response time during heavy exercise intensity. Thus, sleep quality may affect cognitive performance at relatively higher exercise intensities.

We identified some metabolites that were altered by sleep efficiency. Recently, it was reported that fatigue symptoms after intense exercise were associated with changes in metabolic profile (e.g., glucose metabolism and Krebs cycle) (Ra et al. 2014; Berton et al. 2017), suggesting that using metabolomics in athletes is expected to be useful for physical conditioning. Another study has demonstrated that urea cycle metabolites, including ornithine and citrulline, can be considered as markers of chronic fatigue syndrome (Yamano et al. 2016). On the other hand, sleep deprivation increased some metabolite-related depression markers such as tryptophan, serotonin, and taurine, suggesting that some sleep-related metabolites are associated with brain function (Davies et al. 2014). In this study, we found that lysine, proline, tyrosine, ornithine, citrulline, urea, 2-oxoglutaric acid, myo-inositol 3-phosphate, and caffeine levels were different between the two groups of volleyball players with different sleep efficiency. Ornithine, citrulline, and arginine are part of the urea cycle and play an important role in removal of ammonia and urea excretion. As expected, ornithine, citrulline, and arginine levels were greater and urea levels were lower in the better sleep quality group. Therefore, higher sleep quality may be associated with enhanced ammonia excretion and fatigue.

Table 2 Relationships between sleep efficiency, cognitive function, and selected metabolites

	Sleep efficiency	Response time		
		Rest	Light intensity	Heavy intensity
Lysine	0.695*	−0.035	−0.315	−0.497
Proline	0.491	−0.161	−0.127	−0.341
Tyrosine	0.646*	0.115	0.019	−0.247
Ornithine	0.611*	0.091	−0.035	−0.280
Citrulline	0.674*	0.049	−0.322	−0.734*
Arginine	0.589*	−0.026	−0.045	−0.364
Urea	−0.314	0.049	0.110	0.204
2-Oxoglutaric acid	0.636*	−0.151	−0.124	−0.444
Myo-inositol 3-phosphate	0.811*	−0.046	−0.068	−0.371
Caffeine	−0.754*	0.384	0.407	0.607

*$P < 0.05$

Lysine and tyrosine are metabolized in the Krebs cycle via aceto-acetyl-coenzyme A. Proline is converted to glutamate by deamination and also metabolized into 2-oxoglutaric acid, which is an intermediate of Krebs cycle and used as an energy source. 2-Oxoglutaric acid is known to synthesize neurotransmitters (glutamate and γ-aminobutyric acid cycle) in astrocytes (Bak et al. 2006). It has been reported that glutamate/glutamine levels in the prefrontal cortex are associated with cognitive performance (Huang et al. 2015). Myo-inositol 3-phosphate is known as the second messenger of calcium metabolism in the brain and is associated with depressive disorder (Levine et al. 1995). Caffeine also increases energy expenditure, neuromuscular coordination, and cognitive function (Glade 2010). It was interesting to note that the metabolites related to brain function were detected by sleep quality analysis in the athletes in this study.

There are multiple studies investigating the effect of caffeine intake on sleep duration and quality (Walsh et al. 1990; Watson et al. 2016). Drapeau et al. (2006) demonstrated that caffeine intake 3 h and 1 h before sleep elevated saliva caffeine concentrations next morning with reduction in sleep efficiency via polysomnography. Several cross-sectional studies have shown that greater habitual caffeine intake was associated with shorter time in bed and total sleep time (Kant and Graubard 2014; Watson et al. 2016). However, other studies found that increased caffeine intake reduced time in bed, but did not total sleep time, then increase sleep efficiency, while sleep quality is seen as evaluated by Pittsburgh Sleep Quality Index (Sanchez-ortuno et al. 2005; Kant and Graubard, 2014). We did not measure the caffeine consumption throughout our current experimental study. In this regard, we instructed the subjects to fast before saliva collection, which would not induce an acute effect. Therefore, the effect of sleep on caffeine seems to be remains equivocal and further studies are necessary.

Among the metabolites that were detected to be related with sleep efficiency, citrulline was also correlated with response time in the Stroop test (Table 2). There was also a significant correlation between citrulline levels and both sleep efficiency and cognitive function during heavy intensity exercise. Citrulline is known not only as an intermediate amino acid in the urea cycle, but also as a product of nitric oxide (NO) synthesized from arginine (Bahri et al. 2008). Citrulline is also recycled into arginine and plays an important role in NO regulation (Romero et al. 2006). It has been reported that citrulline exerts its effect on vascular function and exercise performance (Bailey et al. 2015; Figueroa et al. 2015). In addition, an animal study has supported the possibility that citrulline has a protective effect in cerebrovascular injury and neuronal cell death (Yabuki et al. 2013). Reduced hepatic capacity results in hyperammonaemia and neuropsychiatric disturbances including impaired memory and reduced attention (Bosoi and Rose 2009). These results suggest that citrulline's role in urea cycle and ammonia metabolism is an important mediator between sleep and cognitive function during exercise.

In this study, we performed a comprehensive metabolomics analysis, which allowed us to identify and quantify the specific biomarkers through a large number of biological compounds at a small molecular level (Heaney et al. 2019; Takeda et al. 2009). The analysis of key metabolites provides new insight into the biophysiological mechanisms of response to disease, exercise, and sleep (Zhang et al. 2012). The chemical composition of saliva is considered to the levels present in blood, since blood metabolites are diffused and transported into saliva through salivary glands and gingival sulcus (Spielmann and Wong 2011). In addition, saliva collection is a non-invasive and easy procedure. Using a saliva metabolomics approach, Ra et al. (2014) identified physical fatigue-related metabolites (glucose phosphate isomers and several amino acids). Another study by Santone et al. (2014) found that cellular energy of metabolites was associated with aerobic capacity. These results imply that saliva metabolite analysis would be able to detect changes in

physical condition before athletic performance is decreased. We aimed to find the sleep efficiency-related metabolites using an untargeted approach. The technique allowed us to analyse a few hundreds of salivary metabolites and identify differences in urea cycle and energy metabolism-related amino acids between groups in the present study. A previous study has demonstrated that ornithine intake improved sleep quality (Miyake et al. 2014). Ornithine has been reported to regulate serotonin and melatonin in the striatum (Kurata et al. 2012). Although the precise mechanism explaining the relationship between sleep quality- and urea cycle-related metabolite was not determined in this study, our results suggest that sleep regulation may influence physical conditioning through urea cycle and other identified metabolites.

Sleep duration and quality are considered critical not only to physiological homeostasis, cognitive function, and mood state, but also to metabolic process regulation (Fullagar et al. 2015; Kayaba et al. 2017). Benedict et al. (2011) have reported that sleep deprivation reduced energy expenditure of resting metabolic rate on the next day morning. Another study by Hursel et al. (2011) demonstrated that sleep fragmentation by waking up hourly decreased sleep quality and activity-related energy expenditure of carbohydrate and fat oxidation in the next day. Furthermore, sleep loss was reported to be associated with cognitive dysfunction and mood disturbance (Axelsson et al. 2008; Fullagar et al. 2015), suggesting that sleep-related-psychological changes prevent optimal cognitive performance. However, we did not measure related to mood status in the present study, which should be done.

The present study had some limitations. First, this study was conducted using a small sample size and no control group, because we recruited participants from only one team. The use of a control group investigation would strengthen the results of the present study. Furthermore, we focused only on female volleyball players. Therefore, the results of present study may limit our ability to generalise our findings to other sports. Second, the effect of exercise intensity and duration on cognitive function has been reported (Tsukamoto et al. 2017); these authors investigated the post-exercise cognitive function in response to acute aerobic exercise at 30% peak oxygen consumption (VO_{2peak}) for 20 min, 60% VO_{2peak} for 20 min, and 30% VO_{2peak} for 40 min. Interaction effects were observed in cognitive function immediately after exercise when comparing 30% and 60% VO_{2peak}, but not 20 min and 40 min. These results support our findings and imply that the cognitive function response to exercise is intensity—rather than volume dependent. Third, this study has a cross-sectional design. Sleep deprivation affects blood metabolite such as tryptophan, serotonin, taurine, and depression-related metabolites (Davies et al. 2014). Recently, it was reported that bright light exposure before sleep, which induces decrease in melatonin concentration and delay in body temperature drop, affects urine metabolites in the general sedentary population (Nakamura et al. 2019). However, we could not carry out any similar intervention in a sport team due to ethical concerns. Therefore, we cannot establish a causal relationship between sleep efficiency, metabolite levels, and cognitive function in this study. Sleep interventional researches are warranted in future studies.

Conclusions

In conclusion, we have identified some metabolites (mainly in the urea cycle and Krebs cycles) with significantly differences between the two sleep efficiency groups. Furthermore, we demonstrated that response time in a Stroop task was faster in the better sleep quality group at heavy exercise intensity. The results of the present study suggest that sleep quality is associated with ammonia metabolism and cognitive performance during exercise in female volleyball players.

Author contributions NA and SM conceived and designed research. NA and NK conducted experiments. NA, NK, YN, HK, and YC collected and analyzed data. NA wrote the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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