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Opinion Paper

Insights into exercise timing to regulate circadian clocks and phenotypes

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SUMMARY

Circadian clocks consist of the central clock in the suprachiasmatic nucleus (SCN) and the peripheral clocks in the peripheral tissues and provide endogenous control of physiological functions. Zeitgebers, such as light-dark cycles, exercise, and feeding, entrain the circadian clocks, and proper exposure to such zeitgebers is important for health. Exercise has substantial effects on circadian rhythms. Mouse studies strongly argue that exercise affects the peripheral clock but not the central clock. On the other hand, human studies have shown that exercise affects the melatonin rhythm, which is generally considered to reflect the central clock. From these results, it is plausible that exercise may cause the melatonin rhythm to deviate from the oscillations of the central clock. The regulatory mechanism is unknown. Here, we propose that exercise directly affects the pineal gland, which lies outside the blood-brain barrier, resulting in exercise-induced changes in the melatonin rhythm independent of the central clock.

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Introduction

Circadian clocks provide endogenous control of physiological functions, such as the sleep/wake cycle, blood pressure, glucose control, hormone secretion, body temperature, and physical activity. In mammals, the circadian clock consists of a central clock in the suprachiasmatic nucleus (SCN) and peripheral clocks in each tissue of the body [1–4]. The central clock provides rhythmic cues to all peripheral clocks through hormones, such as melatonin and adrenocorticotropic hormone, and the autonomic nervous system, thereby synchronizing the body's circadian clocks [5]. Zeitgebers, such as light-dark cycles, exercise, and feeding, entrain the circadian clocks to 24 h to promote a species-specific response to changes in time [6–9]. However, inappropriate exposure to zeitgebers causes circadian misalignment, which negatively affects health. For instance, shift workers have typical circadian misalignment and a high risk of developing symptoms of various diseases, including cancer [10], type 2 diabetes [11], and depression [12] compared to full-time day workers. Therefore, management of the timing of exposure to zeitgebers can potentially reduce the health disadvantages associated with circadian misalignment. Although bright light is regarded the most potent zeitgeber, the phase-shift efficacy of bright light treatment for improving jet lag may be less than that predicted at the laboratory level [13]. Furthermore, bright light treatment can induce adverse side effects, such as headache and nausea, in susceptible individuals [14]. Therefore, the possibility of phase-shift effects due to zeitgebers other than bright lights is worth exploring. Exercise has been shown to have a strong phase-shift effect on the peripheral clocks without mediating the central clock [15–18]. On the other hand, inconsistent results have been obtained in that the central clock is affected by exercise [19–24]. In this paper, we discuss the possibility of exercise-induced phase shifts in the circadian clocks, focusing on the effects of exercise on the central and peripheral clocks, and propose that exercise directly affects the pineal gland, which lies outside the blood-brain barrier.

Entrainment of circadian clocks

Light-dark cycles entrain the oscillations in the central clock, which in turn entrains the oscillations in the peripheral clocks. As a result, the function of each tissue is activated according to time [25]. However, contradictory results have been obtained regarding the effects of exercise, a nonphotic zeitgeber, on the central clock. Mouse studies have shown that exercise either entrains the central clock [19–21], or it does not [15–17]. In studies examining the effects of exercises on both the SCN and several peripheral tissues [15–17,20], the latter argument is stronger [26]. However, the majority of human studies investigating the phase-shift effects of exercise timing have evaluated either the rhythm of melatonin secretion [22–24] or of core body temperature [18,23], which are usually used as indicators to reflect the oscillations of the central clock [27,28]. These studies could not assess the effects of exercise on the oscillations of the peripheral clocks.

Clock genes

To investigate the rhythms of peripheral clocks, it is necessary to evaluate the expression of clock genes, which are transcriptional regulators that have been elucidated as the basic molecular mechanisms of circadian clocks and are present in almost all tissues [29]. A complex of transcriptional activators of the brain and muscle ARNT-like protein 1 (BMAL1) and circadian locomotor output cycle kaput (CLOCK) activates the transcription of various genes, including *period 1-3* (*Per1-3*) and *cryptochrome 1-2* (*Cry1-2*), which are transcriptional repressors [25]. In contrast, the PER1-3 and CRY1-2 complex suppresses the activity of the BMAL1 and CLOCK complex [25]. The resulting transcription–translation core loop has a cycle of approximately 24 h, creating a circadian rhythm in transcriptional regulation. The core loop is also closely involved in the regulation of energy metabolism-related factors, such as glucose control, oxidative phosphorylation, and mitochondrial biosynthesis. Thus, circadian misalignment is associated with lifestyle diseases, such as obesity and diabetes [25]. Conversely, factors related to energy metabolism have been reported to affect clock genes located in the transcription–translation core loop. *Bmal1* expression is promoted by peroxisome proliferator-activated receptor α (PPARα), a member of the nuclear receptor superfamily of ligand-

activated transcription factors that regulate the expression of genes related to lipid metabolism [30]. PPARγ coactivator-1α (PGC-1α), a transcriptional coactivator of peroxisome proliferator-activated receptors and major regulator of exercise-induced phenotype adaptation and substrate utilization, also promotes *Bmal1* expression [31]. Additionally, adenosine monophosphate-activated protein kinase (AMPK), which is activated by energy expenditure, promotes phosphorylation and degradation of CRY1 [32]. Therefore, it is plausible that exercise strongly stimulates energy metabolism and modulates the circadian clock through changes in PPARα, PGC-1α, and AMPK in peripheral tissues.

Discussion

We provide evidence for our hypothesis in Fig. 1. Leise et al. [21] reported that free exercise on a running wheel over 9 days entrained PER2:LUC in the SCN. These studies explain that exercise suppresses Per mRNA expression by increasing the firing rate of neurons in the SCN. On the other hand, mouse studies examining the effects of free running exercise over 2, 4 [15], or 28 days [16] and forced treadmill exercise over 3 days [17] showed phase-shifts in the PER1 and PER2 rhythms in the skeletal muscles, liver, and kidney but not in the SCN. According to these studies, strong exercise stimuli in terms of intensity [17] and period [16] entrain only the rhythm of clock gene expression in the peripheral clocks but not in the SCN. In general, the liver and kidney clocks are sensitive to energy metabolism and stress, respectively [33,34]. Therefore, exercise affects the peripheral clocks in the tissues associated with the exercise and exercise-induced physiological responses, including energy metabolism and stress, regardless of the central clock.

A human study [18] investigated the effects of cycle ergometer exercise performed in the morning (7:00–8:00) or afternoon (16:00–17:00) at 60% maximal oxygen uptake ($\dot{V}O_2$ max) on both rhythms of clock gene expression in leukocytes, an accessible tissue to the multi-organ transcriptome [35,36], and

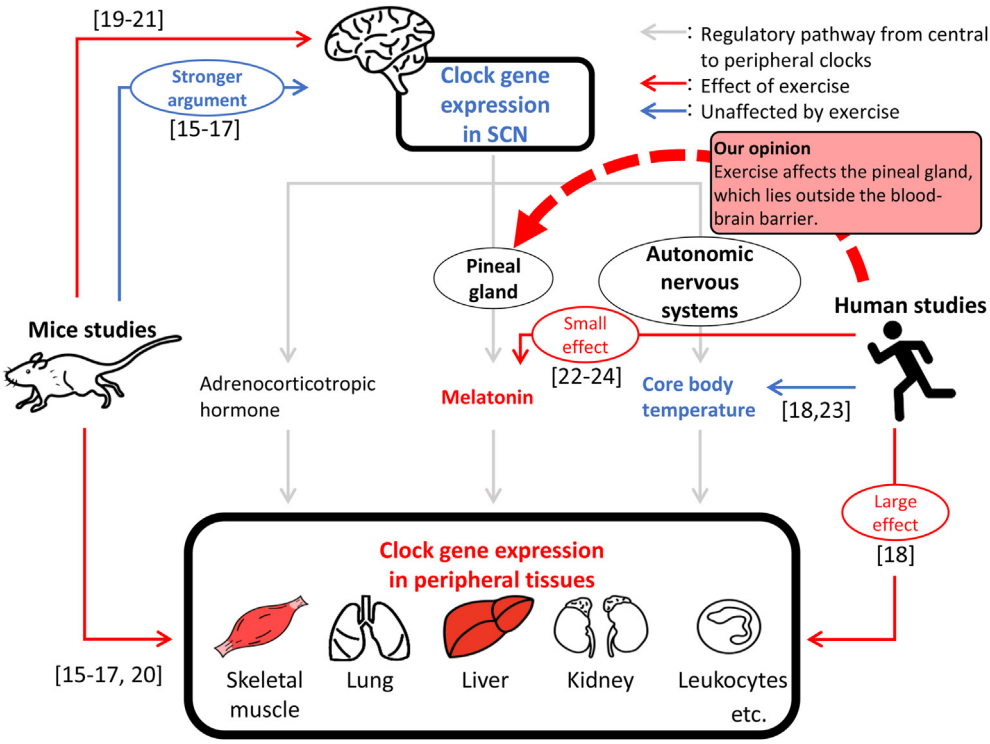

Fig. 1. Effect of exercise on the central and peripheral clock regulation pathway.

core body temperature. Compared to not exercising, the peak timing of *Bmal1* expression was advanced by morning exercise (amount of phase-shift, mean ± SD; 147 ± 185 min) and delayed by afternoon exercise (151 ± 184 min). Morning exercise also tended to advance the peak timing of *Cry1* expression (254 ± 257 min). The exercise did not affect the rhythms of other clock genes (*Clock*, *Per1-3*, and *Cry2*) and temporarily increased the core body temperature, but it did not affect its rhythm. Since the transcriptional regulation of clock genes forms a loop, the changes in the expression rhythms of some clock genes due to exercise might have affected other clock genes at the end of the measurement periods, suggesting that exercise tends to affect the peripheral clocks both in humans and in mice.

Notably, Zambon et al. [37] showed that *Bmal1*, *Per2*, and *Cry1* expression levels in the exercised leg muscle increased 6 h after unilateral leg exercise (beginning at 13:30), which comprised 10 sets of eight repetitions of isotonic knee extension using a Cybex leg extension machine at 80% of 1-repetition maximum, compared to the non-exercised leg. The difference in clock gene expression levels between the exercised and non-exercised legs (i.e., tissues not involved in the exercise and its physiological response) suggests that exercise-induced changes in the peripheral clocks are tissue specific.

Many human studies have used the rhythms of melatonin, its metabolites, and of core body temperature, which are generally considered to reflect the oscillations of the central clock, since it is impossible or ethically challenging to collect skeletal muscle and SCN samples over time. Buxton et al. [22] investigated the effects of a single bout of stepping exercise performed in the morning (beginning at 9:36 ± 0:33), afternoon (beginning at 12:56 ± 0:53), evening (beginning at 18:24 ± 0:24), or at night (beginning at 0:33 ± 0:01) for 1 h, comprising 25% $\dot{V}O_2$ max for 10 min, 75% $\dot{V}O_2$ max for 40 min, and 25% $\dot{V}O_2$ max for 10 min on the plasma melatonin rhythm. The plasma melatonin onset (timing of evening rise) was advanced by exercise performed in the evening (30 ± 15 min) and delayed by exercise performed in the morning (20 ± 14 min), afternoon (43 ± 12 min), and at night (49 ± 13 min). Yamanaka et al. [23] investigated a 4-day cycle ergometer exercise for 2 h (consisting of warm-up for 10 min, exercise at 65–75% HRmax for 45 min, rest on a chair for 10 min, exercise at 65–75% HRmax for 45 min, and cooling for 10 min) in the morning (beginning 3 h after waking) or evening (beginning 10 h after waking) on both rhythms of plasma melatonin secretion and core body temperature. Compared to no exercise, the plasma melatonin offset (timing of morning decline) was delayed by evening exercise (60 ± 48 min) but not by morning exercise. In contrast, both the plasma melatonin onset and core body temperature rhythms were unaffected by both morning and evening exercise. Youngstedt et al. [24] investigated the effects of 3-day treadmill exercise (65–75% heart rate reserve) performed at one of eight times within a 24-h period for 1 h on the excretory rhythm of the urinary melatonin metabolite. The excretion onset and peak timing of the urinary melatonin metabolite were advanced when exercise was performed at 05:00–17:00 (approximately 0–60 min) and delayed when exercise was performed at 17:00–05:00 (approximately 15–60 min). In contrast, the excretion offset of the urinary melatonin metabolite was not affected by exercise. Although the results of these studies are inconsistent, the rhythm of melatonin secretion was affected by the exercise timing.

To summarize the results of previous studies, exercise strongly affects clock gene expression in peripheral tissues involved in exercise and its physiological responses without affecting clock gene expression in the SCN. Interestingly, the melatonin rhythm, which is influenced by the central clock, is also affected by exercise, even if the effects are small. Since clock gene expression in the SCN was unaffected by 28-day exercise in a mouse study [16], even in humans, it may not be affected by exercise for1–4 days [22–24]. Considering that the melatonin rhythm of blind individuals with limited light-induced central clock entrainment has a 24-h cycle, it is plausible that non-photic stimulations, including exercise, may cause melatonin rhythms to deviate from the oscillations of the central clock. Furthermore, time cues of hormone secretion affected by exercise (e.g., of melatonin) may entrain the peripheral clocks in tissues that are not involved in exercise and its physiological response. Melatonin receptors are present in the pancreas and are regarded as regulatory of the insulin secretion rhythm [38]. The regulatory mechanism through which exercise directly affects the peripheral clocks has not been identified. We believe that exercise-induced change in humoral factors (hormone secretion, cytokines, and metabolites) directly affects the pineal gland, which lies outside the blood-brain barrier, resulting in the melatonin rhythm being uncoupled from the central clock in the SCN. On the other hand, it is unlikely that the autonomic nervous system is involved in the regulation of the circadian clock by exercise since the core body temperature rhythm has not been reported to change with

exercise [18,23]. Moreover, exercise might have no or insufficient effect on the central clock since exercise does not affect the rhythm of core body temperature in humans [18,23] and inconsistent results have been obtained from mouse studies regarding the effects of exercise on clock gene expression in the SCN [15–17,20,21].

Social jet lag, in which the sleep/wake cycle is forcefully determined by social schedules, such as work and school, is considered a serious problem. This induces circadian misalignment due to inconsistencies between sleep timing and endogenous control of circadian clocks and carries high risks of symptoms related to circadian misalignment. The management of exercise timing affects the circadian clocks and may be an effective tool for improving circadian misalignment and associated symptoms. However, the subjects in human studies that have investigated the effects of exercise timing on circadian clocks were healthy. To generalize the potential efficacy of exercise, further research on subjects with circadian misalignment should be conducted. Additionally, further research is required to elucidate the effects of exercise timing on the peripheral clocks and whether the peripheral clocks affect the central clock. In the future, it would be desirable to determine the exercise conditions that could be useful for exercise training and treatment of circadian rhythm disorders by affecting phenotypes such as the sleep–wake cycle and feeding rhythms.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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