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A study protocol for a randomized controlled trial examining the association between physical activity and sleep quality in children with autism spectrum disorder using melatonin-mediated mechanism model

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Abstract

Introduction: Sleep disturbance is commonly found in children with autism spectrum disorders (ASD). Disturbed sleep may exacerbate the core symptoms of ASD, including stereotypic behaviors, social interactions, and health problems. Behavioral interventions and supplemental melatonin medication are traditionally used to improve sleep quality. However, the poor sustainability of behavioral intervention effects and use of other medications (e.g., antidepressants and stimulants) that metabolize melatonin may degrade the effectiveness of these interventions. Alternatively, previous research supported physical activity as an effective intervention for treating sleep disturbance in typically developing children. It is therefore natural to extend the study to examine whether such intervention is also effective in children with ASD. We present a protocol (date 4 December 2017) for a jogging intervention trial, which is a parallel and two-group randomized controlled trial (RCT) that uses an objective actigraphic assessment and 6-sulfatoxymelatonin measurement to determine if the 12-week physical activity intervention given to the participants would elicit any changes of sleep quality and melatonin level.

Methods and analysis: All eligible participants will be randomly allocated to either a jogging intervention group or a control group receiving standard care. Changes of their sleep quality as depicted by four sleep parameters (sleep onset latency, sleep efficiency, wake after sleep onset and sleep duration) will be monitored through actigraphic assessment and parental sleep logs. All participants will also be instructed to collect a 24-h urine sample. 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin representative of the participant's melatonin levels, will be measured from the sample. All assessments will be carried out before the intervention (T1), immediately after the 12-week intervention or regular treatment (T2), and 12 weeks after the intervention (T3) to examine the sustainability of the intervention effects. The first enrolment will be started in January 2017.

Trial registration number NCT03348982.

Keywords: Physical activity; Sleep; Children with autism spectrum disorders; Melatonin

Strengths and limitations of this study

- This study will be conducted as a large randomized controlled trial examining the relationship between physical activity and sleep quality in children with autism spectrum disorders (ASD); it will provide clinicians and educators with important information about the impact of physical activity on sleep quality in children with ASD.
- It is the first step of studying the melatonin-medicated mechanism of the possible underlying causal pathways by which physical activity impacts on sleep in children with ASD.
- The melatonin-mediated mechanism could inform further investigation of the interactions between physical activity and melatonin in any population suffering from sleep disturbance.
- The lack of blinding of the research staff collecting data on implementing the physical activity intervention is a limitation to the study design.

Introduction

According to the World Health Organization, autism spectrum disorder (ASD) is defined as a group of complex brain development disorders[1] and often presents at a young age.[1,2] It is characterized, in varying degrees, by significant impairments in social interaction and communication accompanied by restricted and repetitive patterns of behavior.[2] In Hong Kong, an estimate of 90 per 10,000 individuals suffered from ASD. Compared with TD children, preference of sleep disturbance is higher in children with ASD,[3,4] evident in 40% to 80 % of children with ASD[4-7] compared with 20% to 45% in TD children.[8,9] The number of reports from parents on sleep disturbance is also shown to be consistently higher from parents of children with ASD (between 50% to 80%) than those of TD children (between 10% to 25%).[8,10] The most frequent sleep disturbances reported include delayed sleep onset, difficulty in sleep maintenance and insufficient sleep duration.[3,10,11] Sleep disturbance has detrimental effects on the cognitive development (e.g., impairments in learning performance[12] and memory consolidation[13]) and daily functioning (e.g., increased stereotypy[14] and overall autistic behavior[15]) of children with ASD. Together with cognitive deficits and behavior problems associated with ASD, the negative impacts of sleep disturbance may further worsen the general well-being of the affected children. In addition, poor sleep patterns in children with ASD has been associated with adverse sleep quality and higher levels of stress in their parents.[16,17]

Given the aforementioned high prevalence rate and negative consequences of sleep disturbances in children with ASD, effective intervention strategies are required. Currently, behavioral intervention and medication are the two main strategies for ameliorating sleep disturbances in children with ASD.[18,19] Considering the undesirable side effects of medication, such as morning downiness and increased enuresis, parents generally do not prefer to use drugs to treat their children,[20]; therefore behavior intervention is recommended as first-line therapy.[17,19] There are various forms of behavioral interventions (e.g., extinction, fade bedtime) developed to treat sleep disturbances of children with ASD, the most common of which is extinction (removal of reinforcement to reduce a behavior).[21,22] It is well-documented that extinction is an effective technique to treat sleep disturbances (to initiate and maintain sleep in children with ASD).[21,22] However, it is very stressful for children and parents, as it may result in a temporary increase in negative behavior (i.e., extinction burst).[21,23] In addition, the extinction intervention is intensive and its implementation requires tremendous support and resources and therefore may not be cost-effective.[22] Conversely, research on these interventions was usually limited to small-case or single-subject design studies and the procedures were difficult to follow.[21] Therefore, efficacy of these interventions remains in question.

Meanwhile, a significant body of research has investigated the effects of physical activity on sleep in a normal population. Several meta-analytical review studies provided compelling evidence that exercise has a positive impact on sleep quality.[24-26] For example, the recent meta-analysis of 66 studies (n = 284) by Kredlow et al [25] examined the effects of acute and regular exercise on sleep. It indicates that both

acute and regular exercise increase total sleep time, improve sleep onset latency and sleep efficiency, reduce rapid eye movement, and promote slow wave sleep.

While physical activity is shown to be beneficial to sleep quality, most studies were conducted on healthy adults and good sleepers.[24,26] A few similar studies were conducted in a child population[27,28] and positive association between physical activity level and sleep quality were found. Recently, two small-scale studies have been conducted to explore the impact of physical activity on sleep in children with ASD. [29,30] One of them (Wachob et al[29]) used accelerometers to objectively measure the subjects' physical activity level and their sleeping quality (i.e., sleep efficiency (SE) and wake after sleep onset (WASO)) in ten children with ASD. Results revealed a negative relationship between average time spent in moderate-to-vigorous physical activity (MVPA) and average WASO time, and a positive relationship between average sedentary time and average SE percentages. The other study (Brand et al[30]) also found a similar association between PA and sleep quality. In the study, researchers asked ten children with ASD to participate in thrice-weekly 30-min sessions of bicycle workout followed by 30 min of training in coordination and balance. A sleep-encephalography (sleep-EEG) device was applied to objectively measure several sleep parameters (e.g., total sleep time, sleeping period, and sleep efficiency). Results showed that the physical exercises had increased sleep efficiency, shortened sleep onset latency, and decreased WASO time.[30]

Given the aforementioned benefits of physical activity on sleep, it is suggested that physical activity may be an alternative treatment for sleep disturbances in children with ASD.[29,30] However, whether the intervention effects can be sustained remains unknown. Also, precautions should be taken when interpreting the results because of the small sample sizes and lack of control conditions.[29,30] More importantly, the mechanism of how physical activity impacts on sleep remains unclear, particularly in children with ASD. Indeed, it is important to understand such mechanisms to design an effective physical activity intervention for sleep disturbances among children with ASD. In the normal population, several mechanism models (e.g., thermoregulatory hypothesis,[31] body restoration theory[32] and melatonin-mediated mechanism[33]) were proposed. Of particular interest to this study is the melatonin-mediated mechanism. In this mechanism model, it is suggested that physical activity could affect circadian rhythm by altering melatonin level.[34]

Melatonin, a natural hormone produced by the pineal gland, serves as a key regulator of the circadian rhythm and promotes sleep onset and sleep maintenance.[35] Secretion of melatonin normally increases shortly after darkness, peaks in the middle of the night and falls slowly during early morning hours.[36] This hormonal response allows for maintaining a normal circadian rhythm and sleeping through night. Compared with TD children, melatonin levels appeared to be lower[4,37] in some children with ASD, although no such difference was shown in other studies.[38,39] To counter this melatonin deficit, supplemental melatonin is commonly used to treat insomnia in children with ASD.[40] Recently, researchers suggested that melatonin levels could also be moderated by physical activity.[41] In an experiment, Marrin et al [41] asked seven healthy participants to complete a moderate intensity cycling exercise in

the morning with their salivary melatonin concentration measured in at baseline, during exercise, after exercise and exercise recovery. Results showed that participants' melatonin levels significantly increased during and after exercise compared with those at baseline and recovery. However, to our best knowledge, none of the previous studies have examined the relationship between physical activity, melatonin and sleep, particularly in children with ASD. Therefore, the mechanism of how physical activity impacts on sleep in children with ASD remains in question. Here, we present the study protocol which will begin enrolment in January 2018.

Objectives

The purpose of this study is twofold: (1) to examine the associations between physical activity, melatonin, and sleep quality in children with ASD, which ultimately lead to an answer of how physical activity impacts on sleep in children with ASD from the perspective of melatonin-mediated mechanism model; and (2) to examine the possible sustained effect of physical activity on improved melatonin secretion and sleep quality in children with ASD. It is hypothesized that physical activity can improve sleep quality in children with ASD by increasing their endogenous melatonin level, and these beneficial effects can be sustained.

Methods/design

Study design

The purposed study will be a parallel, two-group randomized controlled trial (RCT) design with equal allocation ratio to the intervention group and control group (1:1). A flow diagram of the study is given in Fig. 1.

<Fig. 1 is inserted here>

Data collection

Each participant will attend 3 one-week assessments, where we will assess their habitual sleep patterns: before the intervention (T1: baseline), immediately after the 12 weeks of physical activity or regular treatment (T2: post-intervention), and 12 weeks after post-intervention (T3: follow-up). T1 and T2 represent the pre-and post-intervention. T3 serves as the 12-week follow-up.

Participant

Children will be screened with the following inclusion criteria.

The inclusion criteria are: (1) age 9 –12 years; (2) pre-puberty or early puberty as indicated by Tanner stage I or II ; (3) ASD diagnosis from a physician based on Diagnostic and Statistical Manual of Mental Disorders, 5th edition, (DSM-V)[42]; (4) non-verbal IQ over 40; (5) the ability to follow instructions; (6) physically able to participate in the intervention; (7) no additional regular participation in physical exercise other than school physical education classes for at least 6 months prior to the study; (8) no concurrent medication for at least 6 months before the study or any prior melatonin treatment; and (9) have sleep difficulties, including sleep onset insomnia

and frequent and prolonged nightwaking and/or early morning awakenings (see Giannotti et al[43] for definitions) reported by parents.

Exclusion criteria are: (1) with one or co-morbid psychiatric disorders as established by a structured interview based on DSM-V; (2) with other medical conditions that limit their physical activity capacities (e.g., asthma, seizure, cardiac disease); and (3) with a complex neurologic disorder (e.g., epilepsy, phenylketonuria, fragile X syndrome, tuberous sclerosis). All screening will be carried out by a psychologist and a physician.

Intervention

Intervention group: The intervention is a 12-week jogging program consisting of 24 sessions (two sessions per week, 30 min per session) in a hall/gymnasium of each participating school. Each intervention session will be conducted in the morning by a trained research assistant assisted by student helpers. The staff-to-participant ratio for both groups is 1:2 to 1:1 depending on the attendance. The research assistant and student helpers will be majoring in physical education or adapted physical education and have experience with children with ASD. Each intervention session will be conducted in an identical format, comprising three activities: warm-up (5 min), jogging (20 min), and cool-down (5 min). In the jogging activity, participants will be asked to jog side-by-side with the research staff around an activity circuit (57m x 50m) marked with 4 red cones. Participants are required to run at a moderate intensity level. The intensity level of jogging will be objectively measured by asking participants to wear heart rate monitor (Polar H1) during each jogging session. According to the Center for Disease Control and Prevention [44], MVPA is achieved with a target heart rate above 60% of the maximum heart rate (subtracting the participant's age from 220[45]). Considering the low physical activity level of children with ASD [46], physical activity with a heart rate above 50% of the maximum heart rate should be considered MVPA. Meanwhile, jogging was chosen because it is one of the most common exercises studied with regards to ASD[47] and can serve as endurance training, which is shown to be beneficial for sleep [26]. Participants will be positively reinforced verbally with compliments for their efforts in jogging and their daily and weekly improvements will be visualized through graphs and scales kept at home in the child's bedroom [30]. After the intervention, the participants will be required to follow their normal daily routine without participating in any additional physical activity/exercise program throughout the follow-up period (T2-T3).

Control group: Participants in the control group will receive no physical intervention (i.e. jogging program) and will be required to follow their daily routine without participating in any additional physical activity/exercise program throughout the whole study period (T1-T3).

Outcomes

Before the initial assessment, participants' parents will be asked to provide demographic data and a brief developmental history. Both participants and their

parents will undertake T1, T2, and T3, where the following measurements will be carried out.

Sleep: Four sleep parameters including sleep onset latency (length of time taken to fall asleep, expressed in minutes, SOL); sleep efficiency (actual sleep time divided by time in bed, expressed as a percent, SE); wake after sleep onset (length of time they were awake after sleep onset, expressed in minutes, WASO) and sleep duration (total sleep in hours and minutes, SD) will be objectively measured by a GT3X accelerometer [29]. Participants will be asked to wear the device on the non-dominant wrist for seven consecutive days (Monday to Sunday). Non-wear time is defined as 60 min of consecutive zeros with a 2-min spike tolerance [29]. The night (2200–0700) will be considered invalid if the wear time is less than 8 h and will be excluded from the analysis. In addition, participants' sleep patterns will be logged by their parents using Children's Sleep Habits Questionnaire (CSHQ), which is a validated 45-item parent-administered questionnaire to examine sleep patterns of young children [48] including children with ASD [29]. Parents will be asked to recall specific sleep patterns of their children over the assessment weeks (T1, T2, and T3). Finally, parents will also be asked to log the bedtime, sleep latency, sleep start, sleep end, wake-up time and assumed sleep length in a sleep log for the whole assessment week. The sleep log is used as part of the refined sleep algorithm (RSA) in Actigraph data analysis to identify nocturnal sleep and to exclude non-wear time/wakefulness.[49]

Physical activity level: The physical activity level of the participants will also be measured as secondary data to examine its relationship to sleep as suggested by previous studies [4,29]. It will be measured using the same accelerometer (i.e. GT3X). The data used for analysis are the times spent in sedentary activity and MVPA based on the default energy expenditure algorithm in the accelerometer device [50]. The day (0700–2100) will be considered invalid if the wear time is less than 10 h and will then be excluded from the analysis.

Melatonin level: All participants will be instructed to collect a 24-h urine sample. 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin and representative of melatonin level, will be measured from the sample [51]. The weekend has been chosen to allow the participants to stay at home for sample collection. All urine samples will be collected using 24-h urine bottles containing 0.1L of 0.5M hydrochloric acid as a preservative. Upon completion, the research assistant will immediately collect the urine sample from the participants' apartments and bring it to the Sleep Assessment Unit of Sha Tin Hospital. The urine sample will be stored at −80° before analysis.

Sample size

A pilot study on improving sleeping quality among children with ASD[30] showed that physical activity had notable effect (corresponding to a Cohen's *d* of about 1.0) on improving sleeping quality. Given this effect size, a sample of 16 participants per group is required to achieve a power of 80% and a level of significance of 5%. Assuming 20% attrition, 20 participants will be recruited per group. They will be

recruited from 3 local special schools with existing research links to the PI and his collaborators.

Randomization

After screening, all the eligible participants will be randomly assigned to either the intervention or control group. To ensure equal allocation ratios for the intervention and control groups, block randomization[52] will be used. A block size of 8 will be used in the proposed study (i.e., 4 in one group and 4 in the other group for every 8 consecutively entered participants). The block randomization process will be done by a trained research assistant.

Blinding

The person responsible for analyzing the sleep parameters and melatonin level will be blinded for the group assignment.

Ethics and dissemination

Prior to the study, information about the study will be provided to all participants and their parents. Written consent will be obtained from the parents and children. They will be informed that withdrawal at any time will not result in any adverse consequences. All sets of data will be encrypted with passwords. To prevent any leakage of sensitive information, only the PI and his collaborators will have access to the datasets. Ethical approval was obtained from the Human Research Ethics Committee, The Education University of Hong Kong (reference number 2016-2017-0155). Results of the study will be published in a peer-reviewed journal. Findings of the study will also be shared with other university and non-governmental organizations in HK that specialize in Autism by means of a formal dissemination seminar.

Statistical methods

All statistical analyses will be conducted using SPSS (version 23.0) for Windows (SPSS Inc., Chicago, IL, USA) to assess sleep; multilevel regression or a generalized estimating equation (GEE) will be used to assess the effects of the physical activity intervention, time effect, and their interaction on sleep outcomes and melatonin level outcomes. Two potential confounding variables (i.e., average time spent in daily sedentary activity and average time spent in daily MVPA) will be used as covariates because they may be closely related to sleep quality.[53] The effect size will be reported as a Cohen's *d*. Bonferroni adjustment will be used to control for possible type I error inflation caused by multiple comparisons.

Discussion

This study is the first randomized control trial to investigate the effectiveness of physical activity intervention on sleep disorder among children with ASD. In addition, it is also the first step of studying the melatonin-mediated mechanism of the possible underlying causal pathways by which physical activity impacts on sleep in children

with ASD. The results obtained in this study have two significant impacts. First, if the intervention is effective, doctors can then prescribe physical activity to children with ASD who are not able to take drugs based on the concept of 'exercise is medicine'. Second, the melatonin-mediated mechanism investigated in this study could lead to further investigation of the interactions between physical activity and melatonin in any population suffering from sleep disturbance. Such research could include comparison of the effectiveness of physical activity and supplemental melatonin interventions on sleep quality in populations suffering from sleep disorders and the manipulation of different physical activity intervention parameters (e.g., intensity, frequency, and time) on melatonin level and sleep quality.

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Contributorship Statement
AT and JZ conceived of the study and designed the study protocol, PL assisted in defining the statistical analysis and provided input for the manuscript. EL provided critical comment on implementation of participant's screening protocol. All authors contributed to, read drafts of and approved the final manuscript.

Competing interests
The authors declare there is no competing interests with respect to the research, authorship, and/or publication of this article.

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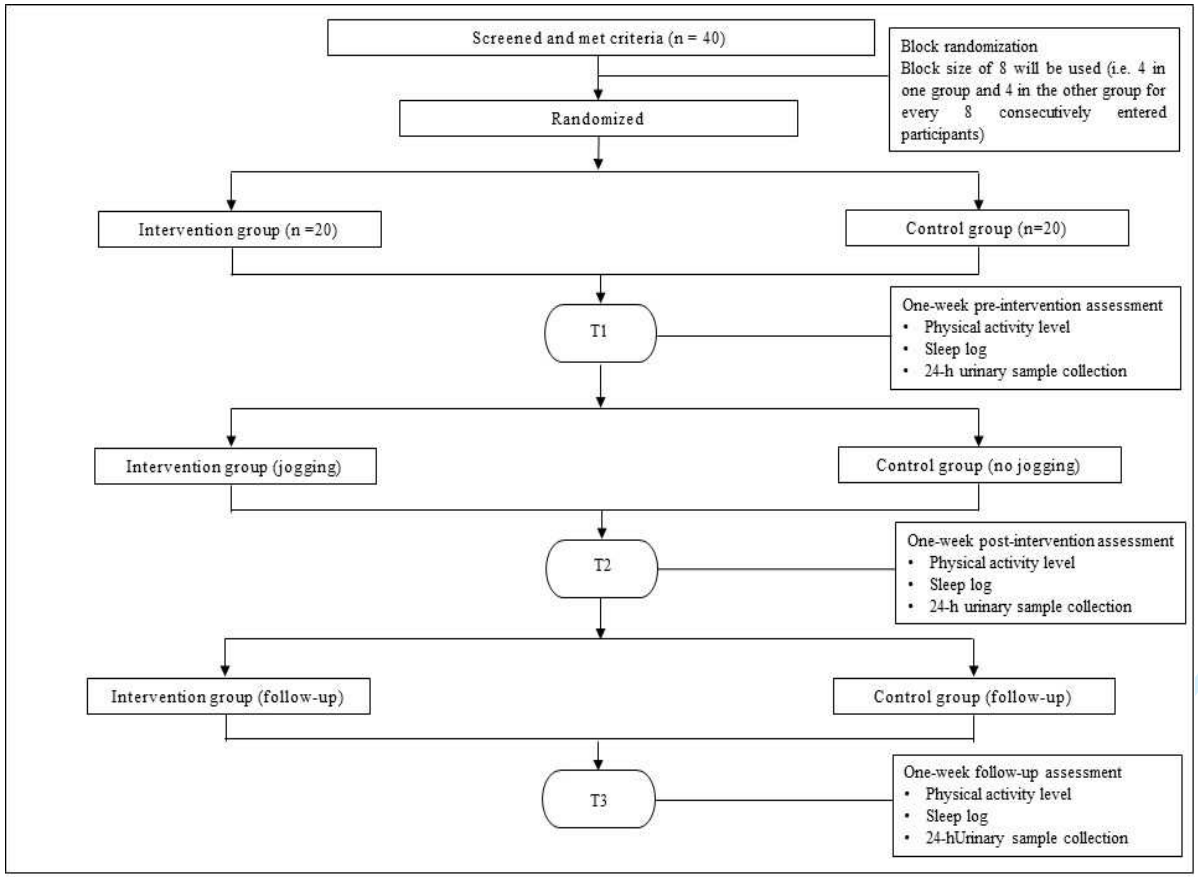
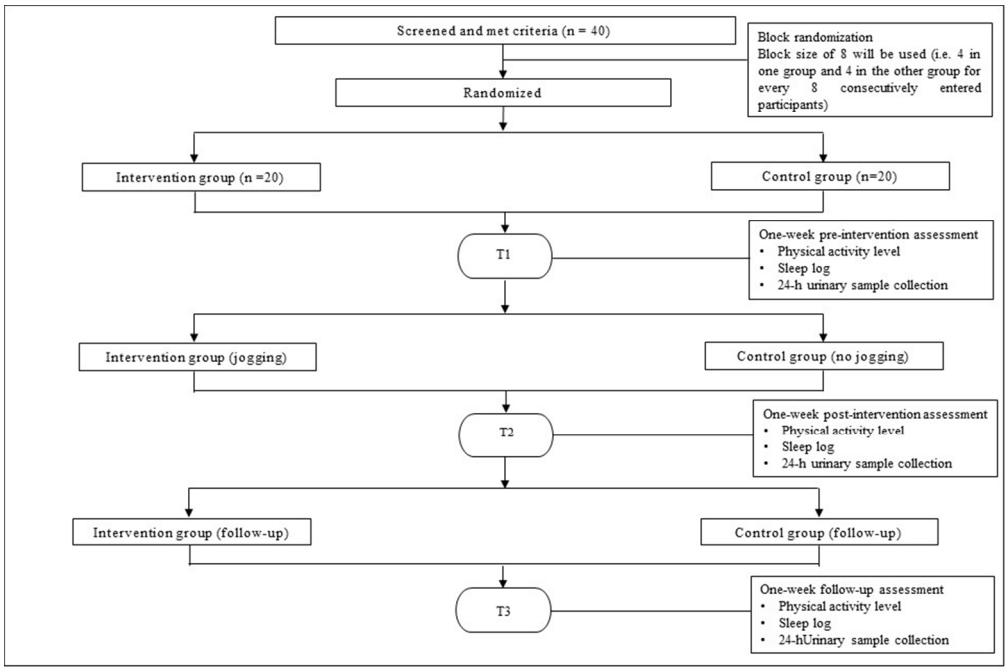
Figure 1. Flow chart of the study





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A study protocol for a randomized controlled trial examining the association between physical activity and sleep quality in children with autism spectrum disorder using a melatonin-mediated mechanism model

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Abstract

Introduction: Sleep disturbance is commonly found in children with autism spectrum disorders (ASD). Disturbed sleep may exacerbate the core symptoms of ASD. Behavioral interventions and supplemental melatonin medication are traditionally used to improve sleep quality. However, poor sustainability of behavioral intervention effects and use of other medications that metabolize melatonin may degrade the effectiveness of these interventions. Alternatively, previous research supported physical activity as an effective intervention for treating sleep disturbance in typically developing children. It is therefore natural to extend the study to examine whether such intervention is also effective in children with ASD. We present a protocol (date 4 December 2017) for a jogging intervention trial, which is a parallel and two-group randomized controlled trial (RCT) that uses an objective actigraphic assessment and 6-sulfatoxymelatonin measurement to determine if the 12-week physical activity intervention given to the participants would elicit any changes of sleep quality and melatonin level.

Methods and analysis: All eligible participants will be randomly allocated to either a jogging intervention group or a control group receiving standard care. Changes of their sleep quality will be monitored through actigraphic assessment and parental sleep logs. All participants will also be instructed to collect a 24-h urine sample. 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin representative of the participant's melatonin levels, will be measured from the sample. All assessments will be carried out before the intervention (T1), immediately after the 12-week intervention or regular treatment (T2), 6 weeks after the intervention (T3) and 12 weeks after the intervention (T4) to examine the sustainability of the intervention effects. The first enrolment will be started in February 2018.

Ethics and dissemination: Ethical approval was obtained through Human Research Ethics Committee, Education University of Hong Kong. Results of this trial will be submitted for publication in peer-reviewed journals.

Trial registration number NCT03348982.

Keywords: Physical activity; Sleep; Children with autism spectrum disorders; Melatonin

Strengths and limitations of this study

- It is the first randomized controlled trial in examining the relationship between physical activity and sleep quality in children with autism spectrum disorders (ASD).
- Integrates bioanalysis to examine the relationship between physical activities and sleep quality.
- Significant engagement with special schools in developing and implementing study.
- The study is limited to children with high functioning autism.

Introduction

According to the World Health Organization, autism spectrum disorder (ASD) is defined as a group of complex brain development disorders[1] and often presents at a young age.[1,2] It is characterized, in varying degrees, by significant impairments in social interaction and communication accompanied by restricted and repetitive patterns of behavior.[2] In Hong Kong, an estimate of 90 per 10,000 individuals suffer from ASD. Compared with TD children, preference of sleep disturbance is higher in children with ASD,[3,4] evident in 40% to 80 % of children with ASD[4-7] compared with 20% to 45% in TD children.[8,9] The number of reports from parents on sleep disturbance is also shown to be consistently higher from parents of children with ASD (between 50% to 80%) than those of TD children (between 10% to 25%).[8,10] The most frequent sleep disturbances reported include delayed sleep onset, difficulty in sleep maintenance and insufficient sleep duration.[3,10,11] Sleep disturbance has detrimental effects on the cognitive development (e.g., impairments in learning performance[12] and memory consolidation[13]) and daily functioning (e.g., increased stereotypy[14] and overall autistic behavior[15]) of children with ASD. Together with cognitive deficits and behavior problems associated with ASD, the negative impacts of sleep disturbance may further worsen the general well-being of the affected children. In addition, poor sleep patterns in children with ASD have been associated with adverse sleep quality and higher levels of stress in their parents.[16,17]

Given the aforementioned high prevalence rate and negative consequences of sleep disturbances in children with ASD, effective intervention strategies are required. Currently, behavioral intervention and medication are the two main strategies for ameliorating sleep disturbances in children with ASD.[18,19] Considering the undesirable side effects of medication, such as morning drowsiness and increased enuresis, parents generally do not prefer to use drugs to treat their children.[20] Therefore, behavior intervention is recommended as first-line therapy.[17,19] There are various forms of behavioral interventions (e.g., extinction, fade bedtime) developed to treat sleep disturbances of children with ASD, the most common of which is extinction (removal of reinforcement to reduce a behavior).[21,22] It is well-documented that extinction is an effective technique to treat sleep disturbances (to initiate and maintain sleep in children with ASD).[21,22] However, it is very stressful for children and parents, as it may result in a temporary increase in negative behavior (i.e. extinction burst).[21,23] In addition, the extinction intervention is intensive and its implementation requires tremendous support and resources and therefore may not be cost-effective.[22] Conversely, research on these interventions was usually limited to small-case or single-subject design studies and the procedures were difficult to follow.[21] Therefore, efficacy of these interventions remains in question.

Meanwhile, a significant body of research has investigated the effects of physical activity on sleep in a normal population. Several meta-analytical review studies provided compelling evidence that exercise has a positive impact on sleep quality.[24-26] For example, the recent meta-analysis of 66 studies (n = 284) by Kredlow et al [25] examined the effects of acute and regular exercise on sleep. It indicates that both

acute and regular exercise increase total sleep time, improve sleep onset latency and sleep efficiency, reduce rapid eye movement, and promote slow wave sleep.

While physical activity is shown to be beneficial to sleep quality, most studies were conducted on healthy adults and good sleepers.[24,26] A few similar studies were conducted in a child population[27,28] and positive association between physical activity level and sleep quality were found. Recently, two small-scale studies have been conducted to explore the impact of physical activity on sleep in children with ASD. [29,30] One of them (Wachob et al[29]) used accelerometers to objectively measure the subjects' physical activity level and their sleeping quality (i.e., sleep efficiency (SE) and wake after sleep onset (WASO)) in ten children with ASD. Results revealed a negative relationship between average time spent in moderate-to-vigorous physical activity (MVPA) and average WASO time, and a positive relationship between average sedentary time and average SE percentages. The other study (Brand et al[30]) also found a similar association between PA and sleep quality. In the study, researchers asked ten children with ASD to participate in thrice-weekly 30-min sessions of bicycle workout followed by 30 min of training in coordination and balance. A sleep-encephalography (sleep-EEG) device was applied to objectively measure several sleep parameters (e.g., total sleep time, sleeping period, and sleep efficiency). Results showed that the physical exercises had increased sleep efficiency, shortened sleep onset latency, and decreased WASO time.[30]

Given the aforementioned benefits of physical activity on sleep, it is suggested that physical activity may be an alternative treatment for sleep disturbances in children with ASD.[29,30] However, whether the intervention effects can be sustained remains unknown. Also, precautions should be taken when interpreting the results because of the small sample sizes and lack of control conditions.[29,30] More importantly, the mechanism of how physical activity impacts on sleep remains unclear, particularly in children with ASD. Indeed, it is important to understand such mechanisms to design an effective physical activity intervention for sleep disturbances among children with ASD. In the normal population, several mechanism models (e.g., thermoregulatory hypothesis,[31] body restoration theory[32] and melatonin-mediated mechanism[33]) were proposed. Of particular interest to this study is the melatonin-mediated mechanism. In this mechanism model, it is suggested that physical activity could affect circadian rhythm by altering melatonin level.[34]

Melatonin, a natural hormone produced by the pineal gland, serves as a key regulator of the circadian rhythm and promotes sleep onset and sleep maintenance.[35] Secretion of melatonin normally increases shortly after darkness, peaks in the middle of the night and falls slowly during early morning hours.[36] This hormonal response allows for maintaining a normal circadian rhythm and sleeping through night. Compared with TD children, melatonin levels appeared to be lower[4,37] in some children with ASD, although no such difference was shown in other studies.[38,39] To counter this melatonin deficit, supplemental melatonin is commonly used to treat insomnia in children with ASD.[40] Recently, researchers suggested that melatonin levels could also be moderated by physical activity.[41] In an experiment, Marrin et al [41] asked seven healthy participants to complete a moderate intensity cycling exercise in

the morning with their salivary melatonin concentration measured in at baseline, during exercise, after exercise and exercise recovery. Results showed that participants' melatonin levels significantly increased during and after exercise compared with those at baseline and recovery. However, to our best knowledge, none of the previous studies have examined the relationship between physical activity, melatonin and sleep, particularly in children with ASD. Therefore, the mechanism of how physical activity impacts on sleep in children with ASD remains in question. Here, we present the study protocol which will begin enrolment in February 2018.

Objectives

The purpose of this study is twofold: (1) to examine the associations between physical activity, melatonin, and sleep quality in children with ASD, which ultimately lead to an answer of how physical activity impacts on sleep in children with ASD from the perspective of melatonin-mediated mechanism model; and (2) to examine the possible sustained effect of physical activity on improved melatonin secretion and sleep quality in children with ASD. It is hypothesized that physical activity can improve sleep quality in children with ASD by increasing their endogenous melatonin level, and these beneficial effects can be sustained.

Methods/design

Study design

The purposed study will be a parallel, two-group randomized controlled trial (RCT) design with equal allocation ratio to the intervention group and control group (1:1). A flow diagram of the study is given in Fig. 1.

<Fig. 1 is inserted here>

Data collection

Each participant will attend 4 one-week assessments, where we will assess their habitual sleep patterns: before the intervention (T1: baseline), immediately after the 12 weeks of physical activity or regular treatment (T2: post-intervention), 6 week after and 12 weeks after post-intervention (T3- T4: follow-up). T1 and T2 represent the pre-and post-intervention. T3 serves as the 6-week follow-up and T4 serves as the 12-week follow-up.

Participant

Children will be screened with the following inclusion criteria.

The inclusion criteria are: (1) age 9 –12 years; (2) pre-puberty or early puberty as indicated by Tanner stage I or II ; (3) ASD diagnosis from a physician based on Diagnostic and Statistical Manual of Mental Disorders, 5th edition, (DSM-V)[42]; (4) non-verbal IQ over 40; (5) the ability to follow instructions; (6) physically able to participate in the intervention; (7) no additional regular participation in physical exercise other than school physical education classes for at least 6 months prior to the study; (8) no concurrent medication for at least 6 months before the study or any prior

melatonin treatment; and (9) have sleep difficulties, including sleep onset insomnia and frequent and prolonged nightwaking and/or early morning awakenings (see Giannotti et al[43] for definitions) reported by parents.

Exclusion criteria are: (1) with one or co-morbid psychiatric disorders as established by a structured interview based on DSM-V; (2) with other medical conditions that limit their physical activity capacities (e.g., asthma, seizure, cardiac disease); and (3) with a complex neurologic disorder (e.g., epilepsy, phenylketonuria, fragile X syndrome, tuberous sclerosis). All screening will be carried out by a psychologist and a physician.

Intervention

Intervention group: The intervention is a 12-week jogging program consisting of 24 sessions (two sessions per week, 30 min per session) in a hall/gymnasium of each participating school. 24 sessions are selected based on previous studies involving jogging in this population.[44,45] Each intervention session will be conducted in the morning by a trained research assistant assisted by student helpers. The staff-to-participant ratio for both groups is 1:2 to 1:1 depending on the attendance. The research assistant and student helpers will be majoring in physical education or adapted physical education and have experience with children with ASD. Each intervention session will be conducted in an identical format, comprising three activities: warm-up (5 min), jogging (20 min), and cool-down (5 min). In the jogging activity, participants will be asked to jog side-by-side with the research staff around an activity circuit (57m x 50m) marked with 4 red cones. The activity circuit will be set up in an outdoor sports ground or indoor gymnasium depending on the weather and the arrangement of the participating schools. Participants are required to run at a moderate intensity level. The intensity level of jogging will be objectively measured by asking participants to wear heart rate monitor (Polar H1) during each jogging session. According to the Center for Disease Control and Prevention [46], MVPA is achieved with a target heart rate above 60% of the maximum heart rate (subtracting the participant's age from 220[47]). Considering the low physical activity level of children with ASD[48], physical activity with a heart rate (HR) above 50% of the maximum heart rate should be considered MVPA. The intervention is considered successful if the participants can maintain their target heart rate (THR) for 15 minutes or above throughout the jogging session. The Polar devices will also be used to calculate how long the participants are within their THR (i.e. 50% or above the maximum HR). Meanwhile, jogging is chosen because it is one of the most common exercises studied with regards to ASD[49] and can serve as endurance training, which is shown to be beneficial for sleep[26]. Participants will be positively reinforced verbally with compliments for their efforts in jogging and their daily and weekly improvements will be visualized through graphs and scales kept at home in the child's bedroom.[30] After the intervention, the participants will be required to follow their normal daily routine without participating in any additional physical activity/exercise program (except the 60-minute weekly physical education (PE) classes provided by school) throughout the follow-up period (T2-T4).

Control group: Participants in the control group will receive no physical intervention (i.e. jogging program) and will be required to follow their daily routine without participating in any additional physical activity/exercise program except the regular PE classes throughout the whole study period (T1-T4).

Study measures

Before the initial assessment, participants' parents will be asked to provide demographic data and a brief developmental history. Both participants and their parents will undertake T1, T2, T3 and T4, where the following measurements will be carried out.

Primary outcome measures

Sleep: Four sleep parameters including sleep onset latency (length of time taken to fall asleep, expressed in minutes, SOL); sleep efficiency (actual sleep time divided by time in bed, expressed as a percent, SE); wake after sleep onset (length of time they were awake after sleep onset, expressed in minutes, WASO) and sleep duration (total sleep in hours and minutes, SD) will be objectively measured by a GT3X accelerometer[29]. Participants will be asked to wear the device on the non-dominant wrist for seven consecutive days (Monday to Sunday). Non-wear time is defined as 60 min of consecutive zeros with a 2-min spike tolerance.[29] The night (2200–0700) will be considered invalid if the wear time is less than 8 h and will be excluded from the analysis. In addition, participants' sleep patterns will be logged by their parents using Children's Sleep Habits Questionnaire (CSHQ), which is a validated 45-item parent-administered questionnaire to examine sleep patterns of young children,[50] including children with ASD.[29] Parents will be asked to recall specific sleep patterns of their children over the assessment weeks (T1, T2, T3 and T4). Finally, parents will also be asked to log the bedtime, sleep latency, sleep start, sleep end, wake-up time and assumed sleep length in a sleep log for the whole assessment week. The sleep log is used as part of the refined sleep algorithm (RSA) in Actigraph data analysis to identify nocturnal sleep and to exclude non-wear time/wakefulness.[51]

Melatonin level: All participants will be instructed to collect a 24-h urine sample. 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin and representative of melatonin level, will be measured from the sample.[52] The weekend has been chosen to allow the participants to stay at home for sample collection. All urine samples will be collected using 24-h urine bottles containing 0.1L of 0.5M hydrochloric acid as a preservative. Upon completion, the research assistant will immediately collect the urine sample from the participants' apartments and bring it to the Sleep Assessment Unit of Sha Tin Hospital. The urine sample will be stored at −80° before analysis.

Secondary outcome measure

Physical activity level: The physical activity level of the participants will also be measured as secondary data to examine its relationship to sleep as suggested by previous studies.[4,29] It will be measured using the same accelerometer (i.e. GT3X).

The data used for analysis are the times spent in sedentary activity and MVPA based on the default energy expenditure algorithm in the accelerometer device.[53] The day (0700–2100) will be considered invalid if the wear time is less than 10 h and will then be excluded from the analysis.

Sample size

A pilot study on improving sleeping quality among children with ASD[30] showed that physical activity had notable effect (corresponding to a Cohen's *d* of about 1.0) on improving sleeping quality. Given this effect size, a sample of 16 participants per group is required to achieve a power of 80% and a level of significance of 5%. Assuming 20% attrition, 20 participants will be recruited per group. They will be recruited from 3 local special schools with existing research links to the PI and his collaborators.

Randomization

After screening, all the eligible participants will be randomly assigned to either the intervention or control group. To ensure equal allocation ratios for the intervention and control groups, block randomization[54] will be used. A block size of 8 will be used in the proposed study (i.e., 4 in one group and 4 in the other group for every 8 consecutively entered participants). The block randomization process will be done by a trained research assistant.

Blinding

The person responsible for analyzing the sleep parameters and melatonin level will be blinded for the group assignment.

Ethics and dissemination

Prior to the study, information about the study will be provided to all participants and their parents. Written consent (see Appendix I) will be obtained from the parents and children. They will be informed that withdrawal at any time will not result in any adverse consequences. All sets of data will be encrypted with passwords. To prevent any leakage of sensitive information, only the PI and his collaborators will have access to the datasets. Ethical approval was obtained from the Human Research Ethics Committee, The Education University of Hong Kong (reference number 2016-2017-0155). Results of the study will be published in a peer-reviewed journal. Findings of the study will also be shared with other university and non-governmental organizations in HK that specialize in Autism by means of a formal dissemination seminar.

Statistical methods

All statistical analyses will be conducted using SPSS (version 23.0) for Windows (SPSS Inc., Chicago, IL, USA) to assess sleep; multilevel regression or a generalized estimating equation (GEE) will be used to assess the effects of the physical activity intervention, time effect, and their interaction on sleep outcomes and melatonin level

outcomes. Two potential confounding variables (i.e., average time spent in daily sedentary activity and average time spent in daily MVPA) will be used as covariates because they may be closely related to sleep quality.[55] The effect size will be reported as a Cohen's *d*. Bonferroni adjustment will be used to control for possible type I error inflation caused by multiple comparisons. Intention to treat approach will be used to handle any missing data.

Discussion

This study is the first randomized control trial to investigate the effectiveness of physical activity intervention on sleep disorder among children with ASD. In addition, it is also the first step of studying the melatonin-mediated mechanism of the possible underlying causal pathways by which physical activity impacts on sleep in children with ASD. The results obtained in this study have two significant impacts. First, if the intervention is effective, doctors can then prescribe physical activity to children with ASD who are not able to take drugs based on the concept of 'exercise is medicine'. Second, the melatonin-mediated mechanism investigated in this study could lead to further investigation of the interactions between physical activity and melatonin in any population suffering from sleep disturbance. Such research could include comparison of the effectiveness of physical activity and supplemental melatonin interventions on sleep quality in populations suffering from sleep disorders and the manipulation of different physical activity intervention parameters (e.g., intensity, frequency, and time) on melatonin level and sleep quality.

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Contributorship Statement

AT and JZ conceived of the study and designed the study protocol, PL assisted in defining the statistical analysis and provided input for the manuscript. EL provided critical comment on implementation of participant's screening protocol. All authors contributed to, read drafts of and approved the final manuscript.

Competing interests

The authors declare there is no competing interests with respect to the research, authorship, and/or publication of this article.

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Figure 1. Flow chart of the proposed study

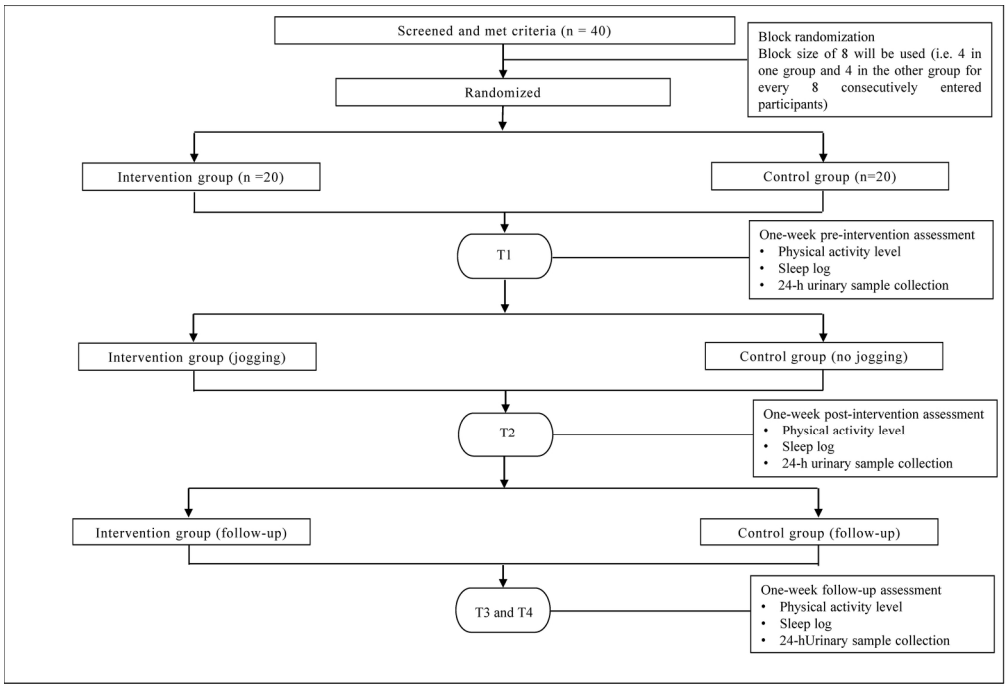

Figure 1. Flow chart of the proposed study

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page Number on which item is reported
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P.1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P.2
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	NA
Funding	4	Sources and types of financial, material, and other support	P.10
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P.1 and P.10
	5b	Name and contact information for the trial sponsor	NA
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P.4-5
	6b	Explanation for choice of comparators	NA
Objectives	7	Specific objectives or hypotheses	P.6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P.6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P.9
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P.6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P.7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P.7
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P.8-9

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P.15
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P.9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	NA
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P.9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P.9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P.9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P.8-9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P.9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P.9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P.9-10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P.9-10
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P.10
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	NA
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P.9
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P.9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P.9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P.10
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P.9
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P.9

	31b	Authorship eligibility guidelines and any intended use of professional writers	P.10
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	P.16
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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A study protocol for a randomized controlled trial examining the association between physical activity and sleep quality in children with autism spectrum disorder using a melatonin-mediated mechanism model

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A study protocol for a randomized controlled trial examining the association between physical activity and sleep quality in children with autism spectrum disorder using a melatonin-mediated mechanism model

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Abstract

Introduction: Sleep disturbance is commonly found in children with autism spectrum disorders (ASD). Disturbed sleep may exacerbate the core symptoms of ASD. Behavioral interventions and supplemental melatonin medication are traditionally used to improve sleep quality. However, poor sustainability of behavioral intervention effects and use of other medications that metabolize melatonin may degrade the effectiveness of these interventions. Alternatively, previous research supported physical activity as an effective intervention for treating sleep disturbance in typically developing children. It is therefore natural to extend the study to examine whether such intervention is also effective in children with ASD. We present a protocol (date 4 December 2017) for a jogging intervention trial, which is a parallel and two-group randomized controlled trial (RCT) that uses an objective actigraphic assessment and 6-sulfatoxymelatonin measurement to determine if the 12-week physical activity intervention given to the participants would elicit any changes of sleep quality and melatonin level.

Methods and analysis: All eligible participants will be randomly allocated to either a jogging intervention group or a control group receiving standard care. Changes of their sleep quality will be monitored through actigraphic assessment and parental sleep logs. All participants will also be instructed to collect a 24-h urine sample. 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin representative of the participant's melatonin levels, will be measured from the sample. All assessments will be carried out before the intervention (T1), immediately after the 12-week intervention or regular treatment (T2), 6 weeks after the intervention (T3) and 12 weeks after the intervention (T4) to examine the sustainability of the intervention effects. The first enrolment will be started in February 2018.

Ethics and dissemination: Ethical approval was obtained through the Human Research Ethics Committee, Education University of Hong Kong. Results of this trial will be submitted for publication in peer-reviewed journals.

Trial registration number NCT03348982.

Keywords: Physical activity; Sleep; Children with autism spectrum disorders; Melatonin

Strengths and limitations of this study

- It is the first randomized controlled trial examining the relationship between physical activity and sleep quality in children with autism spectrum disorders (ASD).
- Integrates bioanalysis to examine the relationship between physical activities and sleep quality.
- Heavy involvement of local special schools in implementing the study.
- The findings of the study cannot be generalized to children with typically development.

Introduction

According to the World Health Organization, autism spectrum disorder (ASD) is defined as a group of complex brain development disorders[1] and often presents at a young age.[1,2] It is characterized, in varying degrees, by significant impairments in social interaction and communication accompanied by restricted and repetitive patterns of behavior.[2] In Hong Kong, an estimate of 90 per 10,000 individuals was diagnosed with ASD[3]. Compared with TD children, preference of sleep disturbance is higher in children with ASD,[4,5] evident in 40% to 80 % of children with ASD[5-8] compared with 20% to 45% in TD children.[9,10] The number of reports from parents on sleep disturbance is also shown to be consistently higher from parents of children with ASD (between 50% to 80%) than those of TD children (between 10% to 25%).[9,11] The most frequent sleep disturbances reported include delayed sleep onset, difficulty in sleep maintenance and insufficient sleep duration.[4,11,12] Sleep disturbance has detrimental effects on cognitive development (e.g., impairments in learning performance[13] and memory consolidation[14]) and daily functioning (e.g., increased stereotypy[15] and overall autistic behavior[16]) of children with ASD. Together with cognitive deficits and behavior problems associated with ASD, the negative impacts of sleep disturbance may further worsen the general well-being of the affected children. In addition, poor sleep patterns in children with ASD have been associated with adverse sleep quality and higher levels of stress in their parents.[17,18]

Given the aforementioned high prevalence rate and negative consequences of sleep disturbances in children with ASD, effective intervention strategies are required. Currently, behavioral intervention and medication are the two main strategies for ameliorating sleep disturbances in children with ASD.[19,20] Considering the undesirable side effects of medication, such as morning drowsiness and increased enuresis, parents generally do not prefer to use drugs to treat their children.[21] Therefore, behavior intervention is recommended as first-line therapy.[18,20] There are various forms of behavioral interventions (e.g., extinction, fade bedtime) developed to treat sleep disturbances of children with ASD, the most common of which is extinction (removal of reinforcement to reduce a behavior).[22,23] It is well-documented that extinction is an effective technique to treat sleep disturbances (to initiate and maintain sleep in children with ASD).[22,23] However, it is very stressful for children and parents, as it may result in a temporary increase in negative behavior (i.e. extinction burst).[22,24] In addition, the extinction intervention is intensive and its implementation requires tremendous support and resources and therefore may not be cost-effective.[23] Conversely, research on these interventions was usually limited to small-case or single-subject design studies and the procedures were difficult to follow.[22] Therefore, the efficacy of these interventions remains in question.

Meanwhile, a significant body of research has investigated the effects of physical activity on sleep in a normal population. Several meta-analytical review studies provided compelling evidence that exercise has positive impacts on sleep quality.[25-27] For example, the recent meta-analysis of 66 studies (n = 284) by Kredlow et al [26] examined the effects of acute and regular exercise on sleep. It indicated that both

acute and regular exercise could increase total sleep time, improve sleep onset latency and sleep efficiency, reduce rapid eye movement, and promote slow wave sleep.

While physical activity is shown to be beneficial to sleep quality, most studies were conducted on healthy adults and good sleepers[25,27] and only a few similar studies were conducted in the child population.[28,29] Recently, two small-scale studies have been conducted to explore the impact of physical activity on sleep in children with ASD.[30,31] One of them (Wachob et al[30]) used accelerometers to objectively measure the subjects' physical activity level and their sleeping quality (i.e., sleep efficiency (SE) and wake after sleep onset (WASO)) in ten children with ASD. Results revealed a negative relationship between average time spent in moderate-to-vigorous physical activity (MVPA) and average WASO time, and a positive relationship between average sedentary time and average SE percentages. The other study (Brand et al[31]) also found a similar association between PA and sleep quality. In the study, researchers asked ten children with ASD to participate in thrice-weekly 30-min sessions of bicycle workout followed by 30 min of training in coordination and balance. A sleep-encephalography (sleep-EEG) device was applied to objectively measure several sleep parameters (e.g., total sleep time, sleeping period, and sleep efficiency). Results showed that the physical exercises had increased sleep efficiency, shortened sleep onset latency, and decreased WASO time.[31]

Given the aforementioned benefits of physical activity on sleep, it is suggested that physical activity may be an alternative treatment for sleep disturbances in children with ASD.[30,31] However, whether the intervention effect can be sustained remains unknown. Also, precautions should be taken when interpreting the results because of the small sample sizes and lack of control conditions.[30,31] More importantly, the mechanism of how physical activity impacts on sleep remains unclear, particularly in children with ASD. Indeed, it is important to understand such mechanisms to design an effective physical activity intervention for sleep disturbances among children with ASD. In the normal population, several mechanism models (e.g., thermoregulatory hypothesis,[32] body restoration theory[33] and melatonin-mediated mechanism[34]) were proposed. Of particular interest to this study is the melatonin-mediated mechanism. In this mechanism model, it is suggested that physical activity could affect circadian rhythm by altering melatonin level.[35]

Melatonin, a natural hormone produced by the pineal gland, serves as a key regulator of the circadian rhythm and promotes sleep onset and sleep maintenance.[36] Secretion of melatonin normally increases shortly after darkness, peaks in the middle of the night and falls slowly during early morning hours.[37] This hormonal response allows for maintaining a normal circadian rhythm and sleeping through night. Compared with TD children, melatonin levels appeared to be lower[5,38] in some children with ASD, although no such difference was shown in other studies.[39,40] To counter this melatonin deficit, supplemental melatonin is commonly used to treat insomnia in children with ASD.[41] Recently, researchers suggested that melatonin levels could also be moderated by physical activity.[42] In an experiment, Marrin et al [42] asked seven healthy participants to complete a moderate intensity morning cycling exercise with their salivary melatonin concentration measured at different time points:

baseline, during exercise, after exercise and recovery. Results showed that participants' melatonin levels significantly increased during and after exercise compared with those at baseline and recovery. However, to our best knowledge, none of the previous studies have examined the relationship between physical activity, melatonin, and sleep, particularly in children with ASD. Therefore, the mechanism of how physical activity impacts on sleep in children with ASD remains a question. Here, we present the study protocol which will begin enrolment in February 2018.

Objectives

The purpose of this study is twofold: (1) to examine the associations between physical activity, melatonin, and sleep quality in children with ASD, which ultimately lead to an answer of how physical activity impacts on sleep in children with ASD from the perspective of melatonin-mediated mechanism model; and (2) to examine the possible sustained effect of physical activity on improved melatonin secretion and sleep quality in children with ASD. It is hypothesized that physical activity could improve sleep quality in children with ASD by increasing their endogenous melatonin level, and these beneficial effects could be sustained.

Methods/design

Study design

The purposed study will be a parallel, two-group randomized controlled trial (RCT) design with equal allocation ratio to the intervention group and control group (1:1). A flow diagram of the study is given in Fig. 1.

<Fig. 1 is inserted here>

Data collection

Each participant will attend 4 one-week assessments, where we will assess their habitual sleep patterns: before the intervention (T1), immediately after the intervention (T2), 6 weeks after and 12 weeks after the intervention (T3 and T4). T1 and T2 represent the pre-and post-intervention. T3 serves as the 6-week follow-up and T4 serves as the 12-week follow-up.

Participant

Children will be screened with the following inclusion criteria.

The inclusion criteria are: (1) age 9 –12 years; (2) pre-puberty or early puberty as indicated by Tanner stage I or II ; (3) ASD diagnosis from a physician based on Diagnostic and Statistical Manual of Mental Disorders, 5th edition, (DSM-V);[43] (4) non-verbal IQ over 40; (5) the ability to follow instructions; (6) physically able to participate in the intervention; (7) no additional regular participation in physical exercise other than school physical education classes for at least 6 months prior to the study; (8) no concurrent medication for at least 6 months before the study or any prior melatonin treatment; and (9) have sleep difficulties, including sleep onset insomnia

and frequent and prolonged nightwaking and/or early morning awakenings (see Giannotti et al[44] for definitions) reported by parents.

Exclusion criteria are: (1) with one or co-morbid psychiatric disorders as established by a structured interview based on DSM-V; (2) with other medical conditions that limit their physical activity capacities (e.g., asthma, seizure, cardiac disease); and (3) with a complex neurologic disorder (e.g., epilepsy, phenylketonuria, fragile X syndrome, tuberous sclerosis). All screening will be carried out by a psychologist and a physician.

Intervention

Intervention group: The intervention is a 12-week jogging program consisting of 24 sessions (two sessions per week, 30 min per session) in a hall/gymnasium of each participating school. 24 sessions are selected based on previous studies involving jogging in this population.[45,46] Each intervention session will be conducted in the morning by a trained research assistant and student helpers. The staff-to-participant ratio for both groups is 1:2 to 1:1 depending on the attendance. The research assistant and student helpers must be majoring in physical education or adapted physical education and must have experience with children with ASD. Each intervention session will be conducted in an identical format, comprising three activities: warm-up (5 min), jogging (20 min), and cool-down (5 min). In the jogging activity, participants will be asked to jog around an activity circuit (57m x 50m) marked with 4 red cones together with the research staff. The activity circuit will be set up in an outdoor sports ground or indoor gymnasium depending on the weather and the arrangement of the participating schools. Participants are required to run at a moderate intensity level. The intensity level of jogging will be objectively measured by asking participants to wear heart rate monitor (Polar H1) during each jogging session. According to the Center for Disease Control and Prevention,[47] MVPA is achieved with a target heart rate above 60% of the maximum heart rate (subtracting the participant's age from 220[48]). Considering the low physical activity level of children with ASD,[49], physical activity with a heart rate (HR) above 50% of the maximum heart rate should be considered MVPA. The intervention is considered successful if the participants can maintain their target heart rate (THR) for 15 minutes or above throughout the jogging session. The Polar devices will also be used to calculate how long the participants are within their THR (i.e. 50% or above the maximum HR). Meanwhile, jogging is chosen because it is one of the most common exercises studied with regards to ASD[50] and can serve as endurance training, which is shown to be beneficial for sleep.[27] Participants will be positively reinforced verbally with compliments for their efforts in jogging and their daily and weekly improvements will be visualized through graphs and scales kept at home in the child's bedroom.[31] After the intervention, the participants will be required to follow their normal daily routine without participating in any additional physical activity/exercise program (except the 60-minute weekly physical education (PE) classes provided by school) throughout the follow-up period (T2-T4).

Control group: Participants in the control group will receive no physical intervention (i.e. jogging program) and will be required to follow their daily routine without participating in any additional physical activity/exercise program except the regular PE classes throughout the whole study period (T1-T4).

Study measures

Before the initial assessment, participants' parents will be asked to provide demographic data and a brief developmental history. Both participants and their parents will undertake T1, T2, T3, and T4, where the following measurements will be carried out.

Primary outcome measures

Sleep: Four sleep parameters including sleep onset latency (length of time taken to fall asleep, expressed in minutes, SOL); sleep efficiency (actual sleep time divided by time in bed, expressed as a percent, SE); wake after sleep onset (length of time they were awake after sleep onset, expressed in minutes, WASO) and sleep duration (total sleep in hours and minutes, SD) will be objectively measured by a GT3X accelerometer.[30] Participants will be asked to wear the device on their non-dominant wrist for seven consecutive days (Monday to Sunday). Non-wear time is defined as 60 min of consecutive zeros with a 2-min spike tolerance.[30] The night (2200–0700) will be considered invalid if the wear time is less than 8 h and will be excluded from the analysis. In addition, participants' sleep patterns will be logged by their parents using Children's Sleep Habits Questionnaire (CSHQ), which is a validated 45-item parent-administered questionnaire to examine sleep patterns of young children,[51] including children with ASD.[30] Parents will be asked to recall specific sleep patterns of their children over the assessment weeks (T1, T2, T3, and T4). Finally, parents will also be asked to log the bedtime, sleep latency, sleep start, sleep end, wake-up time and assumed sleep length in a sleep log for the whole assessment week. The sleep log is used as part of the refined sleep algorithm (RSA) in Actigraph data analysis to identify nocturnal sleep and to exclude non-wear time/wakefulness.[52]

Melatonin level: All participants will be instructed to collect a 24-h urine sample. 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin and representative of melatonin level, will be measured from the sample.[53] The weekend has been chosen to allow the participants to stay at home for sample collection. All urine samples will be collected using 24-h urine bottles containing 0.1L of 0.5M hydrochloric acid as a preservative. Upon completion, the research assistant will immediately collect the urine sample from the participants' apartments and bring it to the Sleep Assessment Unit of Sha Tin Hospital. The urine sample will be stored at −80° before analysis.

Secondary outcome measure

Physical activity level: The physical activity level of the participants will also be measured as secondary data to examine its relationship to sleep as suggested by previous studies.[5,30] It will be measured using the same accelerometer (i.e. GT3X).

The data used for analysis are the times spent in sedentary activity and MVPA based on the default energy expenditure algorithm in the accelerometer device.[54] The day (0700–2100) will be considered invalid if the wear time is less than 10 h and will then be excluded from the analysis.

Sample size

A pilot study on improving sleeping quality among children with ASD[31] showed that physical activity had a notable effect (corresponding to a Cohen's *d* of about 1.0) on improving sleeping quality. Given this effect size, a sample of 16 participants per group is required to achieve a power of 80% and a level of significance of 5%. Assuming 20% attrition, 20 participants will be recruited per group. They will be recruited from 3 local special schools with existing research links to the PI and his collaborators. More special schools will be invited to join the research project if there is inadequate participant enrolment among the 3 participating schools.

Randomization

After screening, all the eligible participants will be randomly assigned to either the intervention or control group. To ensure equal allocation ratios for the intervention and control groups, block randomization[55] will be used. A block size of 8 will be used in the proposed study (i.e. 4 in one group and 4 in the other group for every 8 consecutively entered participants). The block randomization process will be done by a trained research assistant.

Blinding

The person responsible for analyzing the sleep parameters and melatonin level will be blinded for the group assignment.

Ethics and dissemination

Prior to the study, information about the study will be provided to all participants and their parents with the distribution of written consent form (see Appendix I). The consent form will be collected from the participants and their parents by a physical education teacher of each participating school. All participants and their parents will be informed that withdrawal at any time will not result in any adverse consequences. All sets of data will be encrypted with passwords. To prevent any leakage of sensitive information, only the PI and his collaborators will have access to the datasets. Ethical approval was obtained from the Human Research Ethics Committee, The Education University of Hong Kong (reference number 2016-2017-0155). Results of the study will be published in a peer-reviewed journal. Findings of the study will also be shared with other university and non-governmental organizations in HK that specialize in Autism by means of a formal dissemination seminar.

Statistical methods

All statistical analyses will be conducted using SPSS (version 23.0) for Windows (SPSS Inc., Chicago, IL, USA) to assess sleep; multilevel regression or a generalized

estimating equation (GEE) will be used to assess the effects of the physical activity intervention, time effect, and their interaction effect on sleep outcomes and melatonin level outcomes. Two potential confounding variables (i.e., average time spent in daily sedentary activity and average time spent in daily MVPA) will be used as covariates because they may be closely related to sleep quality.[56] The effect size will be reported as a Cohen's *d*. Bonferroni adjustment will be used to control for possible type I error inflation caused by multiple comparisons. Intention to treat approach will be used to handle any missing data.

Discussion

This study is the first randomized control trial examining the effectiveness of physical activity intervention on sleep disorder among children with ASD. In addition, it is also the first study investigating whether the melatonin-mediated mechanism would be a possible underlying pathway by which physical activity impacts on sleep in children with ASD. The results obtained in this study have two significant impacts. First, if the intervention is effective, doctors can prescribe physical activity to children with ASD who are not able to take drugs based on the concept of 'exercise is medicine'. Second, the melatonin-mediated mechanism investigated in this study could lead to further investigation of the interaction between physical activity and melatonin in any population suffering from sleep disturbance. Such research could include the comparison of the effectiveness of physical activity and supplemental melatonin interventions on sleep quality in populations suffering from sleep disorders and the manipulation of different physical activity intervention parameters (e.g., intensity, frequency, and time) on melatonin level and sleep quality.

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Contributorship Statement

AT and JZ conceived of the study and designed the study protocol, PL assisted in defining the statistical analysis and provided input for the manuscript. EL provided critical comment on implementation of participant's screening protocol. All authors contributed to, read drafts of and approved the final manuscript.

Competing interests

The authors declare there is no competing interests with respect to the research, authorship, and/or publication of this article.

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Figure 1. Flow chart of the proposed study

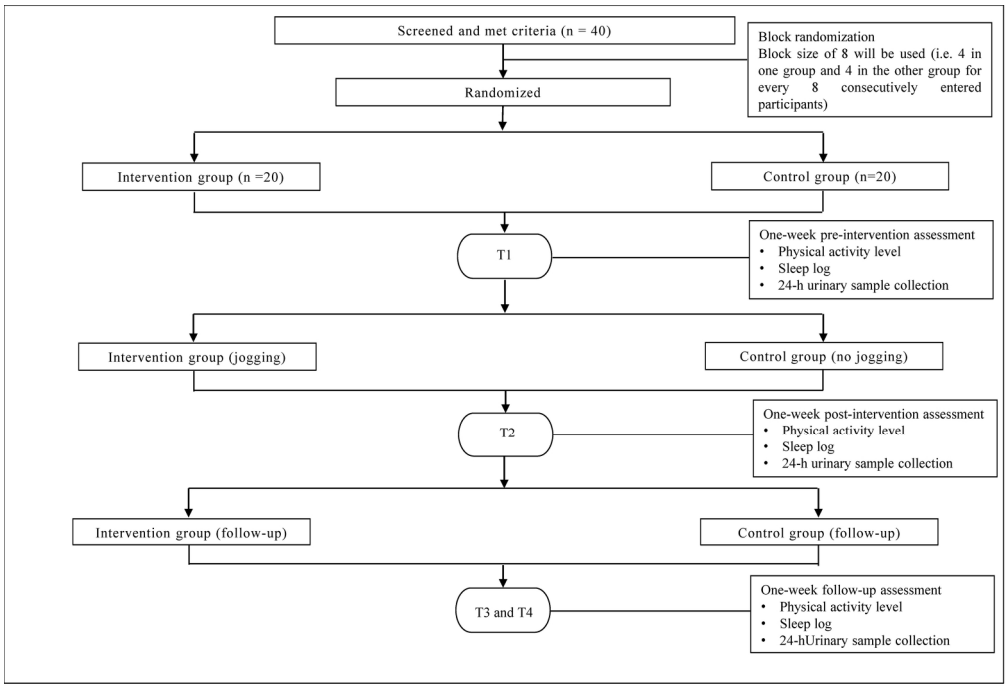

Figure 1. Flow chart of the proposed study

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page Number on which item is reported
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P.1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P.2
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	NA
Funding	4	Sources and types of financial, material, and other support	P.10
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P.1 and P.10
	5b	Name and contact information for the trial sponsor	NA
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P.4-5
	6b	Explanation for choice of comparators	NA
Objectives	7	Specific objectives or hypotheses	P.6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P.6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P.9
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P.6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P.7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P.7
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P.8-9

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P.15
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P.9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P.9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P.9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P.9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P.9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P.8-9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P.9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P.9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P.9-10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P.9-10
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P.10
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	NA
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P.9
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P.9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P.9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P.10
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P.9
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P.9

	31b	Authorship eligibility guidelines and any intended use of professional writers	P.10
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	P.16
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Study protocol for a randomized controlled trial examining the association between physical activity and sleep quality in children with autism spectrum disorder based on the melatonin-mediated mechanism model

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TITLE PAGE WITH AUTHOR INFORMATION

Study protocol for a randomized controlled trial examining the association between physical activity and sleep quality in children with autism spectrum disorder based on the melatonin-mediated mechanism model

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Abstract

Introduction: Sleep disturbance is commonly observed in children with autism spectrum disorders (ASD). Disturbed sleep may exacerbate the core symptoms of ASD. Behavioral interventions and supplemental melatonin medication are traditionally used to improve sleep quality, but poor sustainability of behavioral intervention effects and use of other medications that metabolize melatonin may degrade the effectiveness of these interventions. However, several studies have suggested that physical activity may provide an effective intervention for treating sleep disturbance in typically developing children. Thus, we designed a study to examine whether such an intervention is also effective in children with ASD. We present a protocol (date 4 December 2017) for a jogging intervention with a parallel and two-group randomized controlled trial (RCT) design using objective actigraphic assessment and 6-sulfatoxymelatonin measurement to determine whether a 12-week physical activity intervention elicits changes in sleep quality or melatonin levels.

Methods and analysis: All eligible participants will be randomly allocated to either a jogging intervention group or a control group receiving standard care. Changes of sleep quality will be monitored through actigraphic assessment and parental sleep logs. All participants will also be instructed to collect a 24-h urine sample. 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin representative of the participant's melatonin levels, will be measured from the sample. All assessments will be carried out before the intervention (T1), immediately after the 12-week intervention or regular treatment (T2), 6 weeks after the intervention (T3) and 12 weeks after the intervention (T4) to examine the sustainability of the intervention effects. The first enrolment began in February 2018.

Ethics and dissemination: Ethical approval was obtained through the Human Research Ethics Committee, Education University of Hong Kong. The results of this trial will be submitted for publication in peer-reviewed journals.

Trial registration number NCT03348982.

Keywords: Physical activity; Sleep; Children with autism spectrum disorders; Melatonin

Strengths and limitations of this study

- This protocol is the first randomized controlled trial examining the relationship between physical activity and sleep quality in children with autism spectrum disorders (ASD).
- The proposed study uses bioanalysis to examine the relationship between physical activity and sleep quality.
- The findings of the study cannot be generalized to children with typical development.

Introduction

The World Health Organization defines autism spectrum disorder (ASD) as a group of complex brain development disorders[1] that often presents at a young age.[1,2] ASD is characterized, in varying degrees, by significant impairments in social interaction and communication accompanied by restricted and repetitive patterns of behavior.[2] In Hong Kong, an estimated 0.3%–0.7% of children below the age of 15 years have been diagnosed with ASD by professionals.[3] Compared with typically developing (TD) children, the likelihood of sleep disturbance is higher in children with ASD,[4,5] evident in 40%–80% of children with ASD[5-8] compared with 20%–45% in TD children.[9,10] The number of reports of children's sleep disturbance from parents has also been shown to be consistently higher among parents of children with ASD (50%–80%) compared with those of TD children (10%–25%).[9,11] The most frequently reported sleep disturbances include delayed sleep onset, difficulty in sleep maintenance and insufficient sleep duration.[4,11,12] Sleep disturbance has detrimental effects on cognitive development (e.g., impairments in learning performance[13] and memory consolidation[14]) and daily functioning (e.g., increased stereotypy[15] and overall autistic behavior[16]) of children with ASD. Together with cognitive deficits and behavioral problems associated with ASD, the negative impacts of sleep disturbance may further worsen the general wellbeing of affected children. In addition, poor sleep patterns in children with ASD have been associated with adverse sleep quality and higher levels of stress for parents.[17,18]

Given the high prevalence rate and negative consequences of sleep disturbances in children with ASD, effective intervention strategies are required. Currently, behavioral intervention and medication are the two main strategies for ameliorating sleep disturbances in children with ASD.[19,20] Considering the undesirable side effects of medication, such as morning drowsiness and increased enuresis, parents generally prefer not to use drugs to treat their children.[21] Therefore, behavioral intervention is recommended as first-line therapy.[18,20] Various forms of behavioral intervention (e.g., extinction, faded bedtime) have been developed to treat sleep disturbances among children with ASD, the most common of which is extinction (removal of reinforcement to reduce a behavior).[22,23] Extinction has been well documented as an effective technique to treat sleep disturbances, potentially providing a method for initiating and maintaining sleep in children with ASD.[22,23] However, this method can be very stressful for children and parents, and can result in a temporary increase in negative behavior (i.e., extinction burst).[22,24] In addition, extinction interventions are intensive, and their implementation requires tremendous support and resources, and therefore may not be cost-effective.[23] In addition, research into these interventions has typically been limited to small-case or single-subject design studies, with procedures that are difficult to follow.[22] Therefore, the efficacy of these interventions remains unclear.

Meanwhile, a significant body of research has investigated the effects of physical activity on sleep in a normal population. Several meta-analytical review studies provided compelling evidence that exercise has positive impacts on sleep quality.[25-27] For example, a recent meta-analysis of 66 studies (n = 284) by

Kredlow et al.[26] examined the effects of acute and regular exercise on sleep. Their findings indicated that both acute and regular exercise could increase total sleep time, improve sleep onset latency and sleep efficiency, reduce rapid eye movement, and promote slow wave sleep.

Although physical activity has been shown to be beneficial to sleep quality, most studies have been conducted on healthy adults and good sleepers[25,27] and only a small number of studies have been conducted in the child population.[28,29] Recently, two small-scale studies were conducted to explore the impact of physical activity on sleep in children with ASD.[30,31] In one study, Wachob et al.[30] used accelerometers to objectively measure subjects' physical activity level and sleeping quality (i.e., sleep efficiency [SE] and wake after sleep onset [WASO]) in 10 children with ASD. The results revealed a negative relationship between the average time spent in moderate-to-vigorous physical activity (MVPA) and average WASO time, and a positive relationship between average sedentary time and average SE percentages. In another study Brand et al.[31] found a similar association between PA and sleep quality. In that study, researchers asked 10 children with ASD to participate in thrice-weekly 30-min sessions of bicycle workout followed by 30 min of coordination and balance training. A sleep-encephalography (sleep-EEG) device was used to objectively measure several sleep parameters (e.g., total sleep time, sleeping period, and SE). The results revealed that physical exercise was associated with increased SE, shortened sleep onset latency, and decreased WASO time.[31]

Given the benefits of physical activity on sleep, it has been suggested that physical activity may provide an alternative treatment for sleep disturbances in children with ASD.[30,31] However, it remains unclear whether the effect of exercise-based interventions can be sustained. Also, precautions should be taken when interpreting the results because of the small sample sizes and lack of control conditions.[30,31] More importantly, the mechanism of how physical activity impacts on sleep remains unclear, particularly in children with ASD. Indeed, it is important to understand such mechanisms to design an effective physical activity intervention for sleep disturbances among children with ASD. In the normal population, several mechanism models (e.g., the thermoregulatory hypothesis,[32] body restoration theory[33] and melatonin-mediated mechanism[34]) have been proposed. Of particular interest to the current study is the melatonin-mediated mechanism model, which suggests that physical activity affects the circadian rhythm by altering melatonin levels.[35]

Melatonin, a natural hormone produced by the pineal gland, functions as a key regulator of the circadian rhythm and promotes sleep onset and sleep maintenance.[36] Secretion of melatonin normally increases shortly after darkness, peaks in the middle of the night and falls slowly during the early morning hours.[37] This hormonal response allows for maintaining a normal circadian rhythm and sleeping through the night. Compared with TD children, melatonin levels have been reported to be lower[5,38] in some children with ASD, although no such difference was shown in two other studies.[39,40] To counter this melatonin deficit, supplemental melatonin is commonly used to treat insomnia in children with ASD.[41] Recently, researchers

suggested that melatonin levels could also be altered by physical activity.[42] In one experiment, Marrin et al.[42] asked seven healthy participants to complete a moderate intensity morning cycling exercise while measuring their salivary melatonin concentration at different time points: baseline, during exercise, after exercise and recovery. The results revealed that participants' melatonin levels significantly increased during and after exercise compared with those at baseline and recovery. However, to the best of our knowledge, no previous studies have examined the relationship between physical activity, melatonin, and sleep, particularly in children with ASD. Therefore, the mechanisms by which physical activity impacts on sleep in children with ASD remains unclear. To examine this question, here we propose a new study protocol, which began enrolment in February 2018.

Objectives

This study protocol has two objectives: (1) to examine the associations between physical activity, melatonin, and sleep quality in children with ASD, to ultimately elucidate how physical activity impacts on sleep in children with ASD, from the perspective of the melatonin-mediated mechanism model; and (2) to examine the possible sustained effect of physical activity on improved melatonin secretion and sleep quality in children with ASD. We hypothesize that physical activity can improve sleep quality in children with ASD by increasing their endogenous melatonin levels, and these beneficial effects can be sustained.

Methods/design

Study design

The proposed study will have a parallel, two-group randomized controlled trial (RCT) design, with equal allocation of participants to the intervention and control groups (1:1). A flow diagram of the study is shown in Fig. 1.

<Fig. 1 is inserted here>

Data collection

Each participant will attend four 1-week assessments, where we will assess their habitual sleep patterns: before the intervention (T1), immediately after the intervention (T2), 6 weeks after and 12 weeks after the intervention (T3 and T4, respectively). T1 and T2 represent the pre-and post-intervention, respectively. T3 serves as the 6-week follow-up and T4 serves as the 12-week follow-up.

Participants

Children will be screened using the following inclusion criteria.

The inclusion criteria are: (1) 9–12 years of age; (2) pre-puberty or early puberty as indicated by Tanner stage I or II ; (3) ASD diagnosis from a physician based on the Diagnostic and Statistical Manual of Mental Disorders, 5th edition, (DSM-V);[43] (4) non-verbal IQ over 40; (5) able to follow instructions; (6) physically able to participate in the intervention; (7) no additional regular participation in physical

exercise other than school physical education classes for at least 6 months prior to the study; (8) no concurrent medication for at least 6 months before the study or any prior melatonin treatment; and (9) sleep difficulties, including sleep onset insomnia and frequent and prolonged nightwaking and/or early morning awakening (see Giannotti et al.[44] for definitions) reported by parents.

The exclusion criteria are: (1) one or more co-morbid psychiatric disorders, as established by a structured interview based on DSM-V; (2) other medical conditions that limit physical activity capacity (e.g., asthma, seizure, cardiac disease); and (3) a complex neurological disorder (e.g., epilepsy, phenylketonuria, fragile X syndrome, tuberous sclerosis). All screening will be carried out by a psychologist and a physician.

Intervention

Intervention group: The intervention is a 12-week jogging program consisting of 24 sessions (two sessions per week, 30 min per session) in a hall/gymnasium of each participating school. The total of 24 sessions was selected based on previous studies involving jogging in this population.[45,46] Each intervention session will be conducted in the morning by a trained research assistant and student helpers. The staff-to-participant ratio for both groups is 1:2 to 1:1 depending on the attendance. The research assistant and student helpers must be majoring in physical education or adapted physical education and must have experience with children with ASD. Each intervention session will be conducted in an identical format, comprising three activities: warm-up (5 min), jogging (20 min), and cool-down (5 min). In the jogging activity group, participants will be asked to jog around an activity circuit (57 m × 50 m) marked with four red cones together with the research staff. The activity circuit will be set up in an outdoor sports ground or indoor gymnasium depending on the weather and the arrangement of the participating schools. Participants are required to run at a moderate intensity level. The intensity level of jogging will be objectively measured by asking participants to wear a heart rate monitor (Polar H1) during each jogging session. According to the Center for Disease Control and Prevention,[47] MVPA is achieved with a target heart rate above 60% of the maximum heart rate (subtracting the participant's age from 220)[48]. Considering the low physical activity level of children with ASD,[49] physical activity with a heart rate (HR) above 50% of the maximum heart rate should be considered MVPA. The intervention is considered successful if the participants can maintain their target heart rate (THR) for 15 minutes or above throughout the jogging session. A Polar device will also be used to calculate how long the participants are within their THR (i.e., 50% or above the maximum HR). Meanwhile, jogging is chosen because it is one of the most common exercises studied with regard to ASD[50] and can serve as endurance training, which is shown to be beneficial for sleep.[27] Participants will be positively reinforced verbally with compliments for their efforts in jogging and their daily and weekly improvements will be visualized through graphs and scales kept at home in the child's bedroom.[31] After the intervention, the participants will be required to follow their normal daily routine without participating in any additional physical activity/exercise program

(except the 60-minute weekly physical education [PE] classes provided by school) throughout the follow-up period (T2–T4).

Control group: Participants in the control group will receive no physical intervention (i.e., jogging program) and will be required to follow their daily routine without participating in any additional physical activity/exercise program except the regular PE classes throughout the whole study period (T1–T4).

Study measures

Before the initial assessment, participants' parents will be asked to provide demographic data and a brief developmental history. Both participants and their parents will undertake T1, T2, T3, and T4, where the following measurements will be carried out.

Primary outcome measures

Sleep: Four sleep parameters including sleep onset latency (length of time taken to fall asleep, expressed in minutes, SOL); sleep efficiency (actual sleep time divided by time in bed, expressed as a percentage, SE); wake after sleep onset (length of time they were awake after sleep onset, expressed in minutes, WASO) and sleep duration (total sleep duration in hours and minutes, SD) will be objectively measured using a GT3X accelerometer.[30] Participants will be asked to wear the device on their non-dominant wrist for 7 consecutive days (Monday to Sunday). The non-wear time is defined as 60 min of consecutive zeros with a 2-min spike tolerance.[30] The night (2200–0700) will be considered invalid if the wear time is less than 8 h and will be excluded from the analysis. In addition, participants' sleep patterns will be logged by their parents using the Children's Sleep Habits Questionnaire (CSHQ), which is a validated 45-item parent-administered questionnaire to examine the sleep patterns of young children,[51] including children with ASD.[30] Parents will be asked to recall specific sleep patterns of their children over the assessment weeks (T1, T2, T3, and T4). Finally, parents will also be asked to log the bedtime, sleep latency, sleep start, sleep end, wake-up time and assumed sleep length in a sleep log for the whole assessment week. The sleep log is used as part of the refined sleep algorithm (RSA) in actigraph data analysis to identify nocturnal sleep and to exclude non-wear time/wakefulness.[52]

Melatonin level: All participants will be instructed to collect a 24-h urine sample. 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin that is considered representative of melatonin level, will be measured from the sample.[53] The weekend has been chosen to allow the participants to stay at home for sample collection. All urine samples will be collected using 24-h urine bottles containing 0.1 L of 0.5 M hydrochloric acid as a preservative. Upon completion, the research assistant will immediately collect the urine sample from the participants' homes and bring it to the Sleep Assessment Unit of Sha Tin Hospital. The urine sample will be stored at −80° before analysis.

Secondary outcome measure

Physical activity level: The physical activity level of the participants will also be measured as secondary data to examine its relationship to sleep, as suggested by previous studies.[5,30] This measurement will be conducted using the same accelerometer (i.e., GT3X). The data used for analysis are the times spent in sedentary activity and MVPA based on the default energy expenditure algorithm in the accelerometer device.[54] The day (0700–2100) will be considered invalid if the wear time is less than 10 h and will then be excluded from the analysis.

Sample size

A pilot study on improving sleeping quality among children with ASD[31] revealed that physical activity had a notable effect (corresponding to a Cohen's *d* of approximately 1.0) on improving sleeping quality. Given this effect size, a sample of 16 participants per group is required to achieve a power of 80% and a level of significance of 5%. Assuming a 20% attrition rate, 20 participants will be recruited per group. They will be recruited from three local special schools with existing research links to the principal investigator (PI) and collaborators. More special schools will be invited to join the research project if there is inadequate participant enrolment among the three participating schools.

Randomization

After screening, all eligible participants will be randomly assigned to either the intervention or control group. To ensure equal allocation ratios for the intervention and control groups, block randomization[55] will be used. A block size of eight will be used in the proposed study (i.e., four in one group and four in the other group for every eight consecutively entered participants). The block randomization process will be performed by a trained research assistant.

Patient and public involvement

Patients and public will not be involved in the study.

Blinding

The person responsible for analyzing the sleep parameters and melatonin level will be blinded to the group assignment.

Ethics and dissemination

Prior to the study, information about the study will be provided to all participants and their parents with the distribution of written consent forms (see Appendix I). The consent forms will be collected from the participants and their parents by a PE teacher at each participating school. All participants and their parents will be informed that withdrawal at any time will not result in any adverse consequences. All sets of data will be encrypted with passwords. To prevent any leakage of sensitive information, only the PI and collaborators will have access to the datasets. Ethical approval was obtained from the Human Research Ethics Committee, The Education University of Hong Kong (reference number 2016-2017-0155). The results of the study will be

published in a peer-reviewed journal. Findings of the study will also be shared with other university and non-governmental organizations in Hong Kong that specialize in autism by means of a formal dissemination seminar.

Statistical methods

All statistical analyses will be conducted using SPSS (version 23.0) for Windows (SPSS Inc., Chicago, IL, USA) to assess sleep; multilevel regression or a generalized estimating equation (GEE) will be used to assess the effect of the physical activity intervention, the effect of time, and their interaction effect on sleep outcomes and melatonin level outcomes. Two potential confounding variables (i.e., average time spent in daily sedentary activity and average time spent in daily MVPA) will be used as covariates because they may be closely related to sleep quality.[56] The effect size will be reported as a Cohen's *d*. Bonferroni adjustment will be used to control for possible type I error inflation caused by multiple comparisons. The intention to treat approach will be used to handle any missing data.

Discussion

This study is the first randomized control trial designed to examine the effectiveness of physical activity intervention on sleep disorder among children with ASD. In addition, it is the first study designed to investigate whether the melatonin-mediated mechanism is a potential underlying pathway by which physical activity impacts on sleep in children with ASD. The results obtained in this study will potentially have two significant implications. First, if the intervention is effective, doctors can prescribe physical activity to children with ASD who are not able to take drugs based on the notion of "exercise as medicine". Second, the melatonin-mediated mechanisms investigated in this study could lead to further investigation of the interaction between physical activity and melatonin in any population suffering from sleep disturbance. Such research could include a comparison of the effectiveness of physical activity and supplemental melatonin interventions on sleep quality in populations suffering from sleep disorders and the manipulation of different physical activity intervention parameters (e.g., intensity, frequency, and time) on melatonin level and sleep quality.

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Contributorship statement

ACYT and JZ conceived of the study and designed the study protocol, PHL assisted in defining the statistical analysis and provided input for the manuscript. EWHL provided critical comment on implementation of the participant screening protocol. All authors contributed to, read drafts of and approved the final manuscript.

Competing interests
The authors declare there is no competing interests with respect to the research, authorship, and/or publication of this article.

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Figure 1. Flow chart of the proposed study

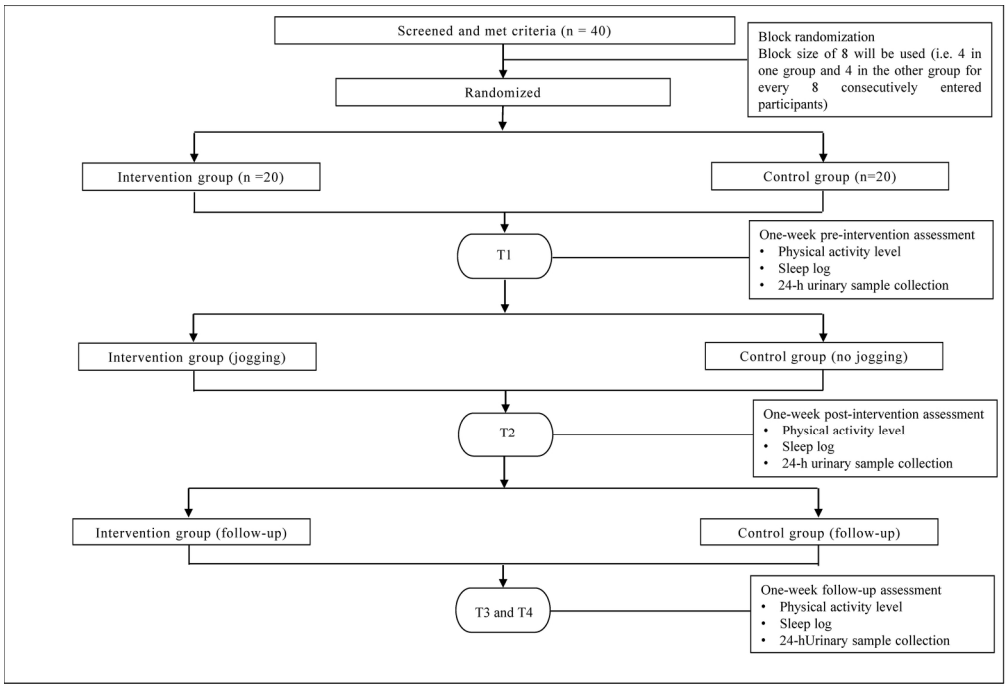

Figure 1. Flow chart of the proposed study

190x142mm (300 x 300 DPI)

同 意 書

「體力活動與自閉症小童睡眠質素及褪黑激素的關係」研究

研究目的

香港教育大學健康與體育學系現與香港中文大學醫學院精神科及香港理工大學護理學系正進行一項關於「體力活動與自閉症小童睡眠質素及褪黑激素的關係」的隨機對照試驗項目，目的是調查體力活動能否改變自閉症小童的褪黑激素分泌，從而改善他們的睡眠質素。

研究程序

是項研究對象為小三至小六的自閉症學童。他們會在學校課堂或任何在課堂以外在研究人員的安排下進行體力活動共三次評估，評估內容如下:

1. 填寫四份問卷表格；
2. 收集一天的兒童尿液樣本 (所需之尿液樣本收集，研究助理會直接聯絡家長約定交收時間)；
3. 讓每位兒童必須連續七天攜帶加速度計一個加速計。

其三次評估分別為: 前測(第一星期)，後測(第十三星期)，跟進測試 (第二十五星期)。

項目內的所有活動及各種評估將會由專業研究團隊負責，並且不會收取任何費用，而每位參加者的家長將可獲得超市現金券$500 元以表感謝。

研究地點

研究地點分別為: 運動技巧訓練會於 貴子女所屬學校進行，而睡眠質素評估及褪黑激素尿液測試會於閣下家中進行。

潛在風險

此研究並沒有其他潛在風險。在進行運動技巧訓練，如有需要，研究人員會提供食水。

研究裨益

參加者可以從中了解自己的體力活動及睡眠質素。你們的參與，將對於日常設計自閉症兒童的運動課程研究有極大的貢獻。

個人私隱

在研究過程中所收集的資料，只供作研究用途，個人資料將絕對保密。所有資料（包括個人及學校資料）將以代碼記錄及研究，以保障閣下的私隱。資料將會存放於研究員的辦公室，並鎖於櫃內，只有有關研究隊伍才可接觸該資料。你可隨時要求檢視錄影記錄，而我們可應你的要求刪除錄影記錄資料。所有資料將會在完全收集後六個月內進行分析，然後所有資料將會被銷毀。

參與及退出

參與純屬自願性質，參與與否將不會對閣下之學業成績構成任何影響。閣下可隨時提出終止，有關決定將不會引致任何不良後果。

疑問

如閣下想獲得更多有關這項研究的資料,請與首席研究員謝采揚博士聯絡 (電話：29488074) ，請閣下填妥以下回條，以表示是否同意參與是項研究。如對是項研究有任何疑問，請現在提

出。如日後對是項研究有任何查詢，敬請聯絡首席研究員謝采揚博士 (電話：29488074，電郵：andytcy@eduhk.hk) 。如閣下或 貴子女對這項研究的操守有任何意見,可隨時與香港教育大學人類實驗對象操守委員會聯絡(電郵: hrec@eduhk.hk; 地址:香港教育大學研究與發展事務處。在此多謝你的參與。

家長回條

本人 _____ (家長姓名) 已有足夠機會詢問清楚明白有關這項研究的內容，並同意參加這項研究。

此外，本人亦 ** 同意 / 不同意 本人之兒女_____ (學童名稱) _____(學童班別)_____(學童學號) 參與是項研究。[** 請刪去不適用者]

_____ _____

家長聯絡電話 日期

_____ _____

家長姓名 家長簽署

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page Number on which item is reported
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P.1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P.2
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	NA
Funding	4	Sources and types of financial, material, and other support	P.10
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P.1 and P.10
	5b	Name and contact information for the trial sponsor	NA
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P.4-5
	6b	Explanation for choice of comparators	NA
Objectives	7	Specific objectives or hypotheses	P.6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P.6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P.9
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P.6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P.7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P.7
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P.8-9

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P.15
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P.9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P.9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P.9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P.9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P.9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P.8-9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P.9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P.9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P.9-10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P.9-10
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P.10
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	NA
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P.9
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P.9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P.9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P.10
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P.9
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P.9

	31b	Authorship eligibility guidelines and any intended use of professional writers	P.10
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	P.16
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.