

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020944
Article Type:	Protocol
Date Submitted by the Author:	05-Dec-2017
Complete List of Authors:	Tse, Choi Yeung Andy; Department of Health and Physical Education Lee, Paul; Hong Kong Polytechnic University, School of Nursing Zhang, Jihui; The Chinese University of Hong Kong, Department of Psychiatry Lai, Elvis, W.H.; The Hong Kong Castle Peak Hospital, Department of Psychiatry
Keywords:	Physical activity, Sleep, Children with autism spectrum disorders, Melatonin

SCHOLARONE™
Manuscripts

Peer Review Only

TITLE PAGE WITH AUTHOR INFORMATION

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 modelAndy C.Y. Tse¹, Paul H. Lee², J. Zhang³, Elvis W.H. Lai⁴¹Department of Health and Physical Education, The Education University of Hong Kong, Hong Kong China²School of Nursing, The Hong Kong Polytechnic University, Hong Kong China³Department of Psychiatry, The Chinese University of Hong Kong, Hong Kong China⁴Department of Psychiatry, The Hong Kong Castle Peak Hospital

Dr. Andy C.Y. Tse [Corresponding Author]

Rm D4-2/F-03, Block D4,

10 Lo Ping Road, Tai Po, N.T., Hong Kong.

Tel: (852) 29488074

Email: andytcy@eduhk.hk

Dr. Paul H. Lee

School of Nursing, PQ433

Hong Kong Polytechnic University, Hung Hom,

Kowloon, Hong Kong

Tel: (852) 3400 8275

Email: paul.h.lee@polyu.edu.hk

Dr. J. Zhang

Tel: (852) 26475321

Email: jihui.zhang@cuhk.edu.hk

G/F Multicentre

Tai Po Hospital, Tai Po,

N.T., Hong Kong

Dr. Elvis W.H Lai

15, Ching Chung Koon Road,

Tuen Mun, N.T., Hong Kong

Tel: (852)24567111

Email: lwh041@ha.org.hk

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-mediated 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 disorders¹ 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 ASD⁴⁻⁷ 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 performance¹² and memory consolidation¹³) and daily functioning (e.g., increased stereotypy¹⁴ and overall autistic behavior¹⁵) 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 al²⁵ examined the effects of acute and regular exercise on sleep. It indicates that both

1
2
3 acute and regular exercise increase total sleep time, improve sleep onset latency and
4 sleep efficiency, reduce rapid eye movement, and promote slow wave sleep.

5 While physical activity is shown to be beneficial to sleep quality, most studies
6 were conducted on healthy adults and good sleepers.^{24,26} A few similar studies were
7 conducted in a child population^{27,28} and positive association between physical activity
8 level and sleep quality were found. Recently, two small-scale studies have been
9 conducted to explore the impact of physical activity on sleep in children with ASD.
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
One of them (Wachob et al²⁹) 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 al³⁰)
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 theory³² and
melatonin-mediated mechanism³³) 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 al⁴¹
asked seven healthy participants to complete a moderate intensity cycling exercise in

1
2
3 the morning with their salivary melatonin concentration measured in at baseline,
4 during exercise, after exercise and exercise recovery. Results showed that
5 participants' melatonin levels significantly increased during and after exercise
6 compared with those at baseline and recovery. However, to our best knowledge, none
7 of the previous studies have examined the relationship between physical activity,
8 melatonin and sleep, particularly in children with ASD. Therefore, the mechanism of
9 how physical activity impacts on sleep in children with ASD remains in question.
10 Here, we present the study protocol which will begin enrolment in January 2018.
11
12

13 14 **Objectives**

15 The purpose of this study is twofold: (1) to examine the associations between physical
16 activity, melatonin, and sleep quality in children with ASD, which ultimately lead to
17 an answer of how physical activity impacts on sleep in children with ASD from the
18 perspective of melatonin-mediated mechanism model; and (2) to examine the possible
19 sustained effect of physical activity on improved melatonin secretion and sleep
20 quality in children with ASD. It is hypothesized that physical activity can improve
21 sleep quality in children with ASD by increasing their endogenous melatonin level,
22 and these beneficial effects can be sustained.
23
24

25 26 **Methods/design**

27 **Study design**

28 The purposed study will be a parallel, two-group randomized controlled trial (RCT)
29 design with equal allocation ratio to the intervention group and control group (1:1). A
30 flow diagram of the study is given in Fig. 1.
31
32

33 <Fig. 1 is inserted here>
34
35

36 **Data collection**

37 Each participant will attend 3 one-week assessments, where we will assess their
38 habitual sleep patterns: before the intervention (T1: baseline), immediately after the
39 12 weeks of physical activity or regular treatment (T2: post-intervention), and 12
40 weeks after post-intervention (T3: follow-up). T1 and T2 represent the pre-and
41 post-intervention. T3 serves as the 12-week follow-up.
42
43

44 **Participant**

45 Children will be screened with the following inclusion criteria.

46 The inclusion criteria are: (1) age 9 –12 years; (2) pre-puberty or early puberty as
47 indicated by Tanner stage I or II ; (3) ASD diagnosis from a physician based on
48 Diagnostic and Statistical Manual of Mental Disorders, 5th edition, (DSM-V)⁴²; (4)
49 non-verbal IQ over 40; (5) the ability to follow instructions; (6) physically able to
50 participate in the intervention; (7) no additional regular participation in physical
51 exercise other than school physical education classes for at least 6 months prior to the
52 study; (8) no concurrent medication for at least 6 months before the study or any prior
53 melatonin treatment; and (9) have sleep difficulties, including sleep onset insomnia
54
55
56
57
58
59
60

1
2
3 and frequent and prolonged nightwaking and/or early morning awakenings (see
4 Giannotti et al⁴³ for definitions) reported by parents.

5 Exclusion criteria are: (1) with one or co-morbid psychiatric disorders as established
6 by a structured interview based on DSM-V; (2) with other medical conditions that
7 limit their physical activity capacities (e.g., asthma, seizure, cardiac disease); and (3)
8 with a complex neurologic disorder (e.g., epilepsy, phenylketonuria, fragile X
9 syndrome, tuberous sclerosis). All screening will be carried out by a psychologist and
10 a physician.
11
12

13 **Intervention**

14 Intervention group: The intervention is a 12-week jogging program consisting of 24
15 sessions (two sessions per week, 30 min per session) in a hall/gymnasium of each
16 participating school. Each intervention session will be conducted in the morning by a
17 trained research assistant assisted by student helpers. The staff-to-participant ratio for
18 both groups is 1:2 to 1:1 depending on the attendance. The research assistant and
19 student helpers will be majoring in physical education or adapted physical education
20 and have experience with children with ASD. Each intervention session will be
21 conducted in an identical format, comprising three activities: warm-up (5 min),
22 jogging (20 min), and cool-down (5 min). In the jogging activity, participants will be
23 asked to jog side-by-side with the research staff around an activity circuit (57m x 50m)
24 marked with 4 red cones. Participants are required to run at a moderate intensity level.
25 The intensity level of jogging will be objectively measured by asking participants to
26 wear heart rate monitor (Polar H1) during each jogging session. According to the
27 Center for Disease Control and Prevention⁴⁴, MVPA is achieved with a target heart
28 rate above 60% of the maximum heart rate (subtracting the participant's age from
29 220⁴⁵). Considering the low physical activity level of children with ASD⁴⁶, physical
30 activity with a heart rate above 50% of the maximum heart rate should be considered
31 MVPA. Meanwhile, jogging was chosen because it is one of the most common
32 exercises studied with regards to ASD⁴⁷ and can serve as endurance training, which is
33 shown to be beneficial for sleep²⁶. Participants will be positively reinforced verbally
34 with compliments for their efforts in jogging and their daily and weekly
35 improvements will be visualized through graphs and scales kept at home in the child's
36 bedroom³⁰. After the intervention, the participants will be required to follow their
37 normal daily routine without participating in any additional physical activity/exercise
38 program throughout the follow-up period (T2-T3).
39
40
41
42
43
44

45 Control group: Participants in the control group will receive no physical intervention
46 (i.e. jogging program) and will be required to follow their daily routine without
47 participating in any additional physical activity/exercise program throughout the
48 whole study period (T1-T3).
49
50

51 **Outcomes**

52 Before the initial assessment, participants' parents will be asked to provide
53 demographic data and a brief developmental history. Both participants and their
54
55
56
57
58
59
60

1
2
3 parents will undertake T1, T2, and T3, where the following measurements will be
4 carried out.

5 Sleep: Four sleep parameters including sleep onset latency (length of time taken to
6 fall asleep, expressed in minutes, SOL); sleep efficiency (actual sleep time divided by
7 time in bed, expressed as a percent, SE); wake after sleep onset (length of time they
8 were awake after sleep onset, expressed in minutes, WASO) and sleep duration (total
9 sleep in hours and minutes, SD) will be objectively measured by a GT3X
10 accelerometer²⁹. Participants will be asked to wear the device on the non-dominant
11 wrist for seven consecutive days (Monday to Sunday). Non-wear time is defined as 60
12 min of consecutive zeros with a 2-min spike tolerance²⁹. The night (2200–0700) will
13 be considered invalid if the wear time is less than 8 h and will be excluded from the
14 analysis. In addition, participants' sleep patterns will be logged by their parents using
15 Children's Sleep Habits Questionnaire (CSHQ), which is a validated 45-item
16 parent-administered questionnaire to examine sleep patterns of young children⁴⁸
17 including children with ASD²⁹. Parents will be asked to recall specific sleep patterns
18 of their children over the assessment weeks (T1, T2, and T3). Finally, parents will
19 also be asked to log the bedtime, sleep latency, sleep start, sleep end, wake-up time
20 and assumed sleep length in a sleep log for the whole assessment week. The sleep log
21 is used as part of the refined sleep algorithm (RSA) in Actigraph data analysis to
22 identify nocturnal sleep and to exclude non-wear time/wakefulness.⁴⁹

23 Physical activity level: The physical activity level of the participants will also be
24 measured as secondary data to examine its relationship to sleep as suggested by
25 previous studies^{4,29}. It will be measured using the same accelerometer (i.e. GT3X).
26 The data used for analysis are the times spent in sedentary activity and MVPA based
27 on the default energy expenditure algorithm in the accelerometer device⁵⁰. The day
28 (0700–2100) will be considered invalid if the wear time is less than 10 h and will then
29 be excluded from the analysis.

30 Melatonin level: All participants will be instructed to collect a 24-h urine sample.
31 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin and
32 representative of melatonin level, will be measured from the sample⁵¹. The weekend
33 has been chosen to allow the participants to stay at home for sample collection. All
34 urine samples will be collected using 24-h urine bottles containing 0.1L of 0.5M
35 hydrochloric acid as a preservative. Upon completion, the research assistant will
36 immediately collect the urine sample from the participants' apartments and bring it to
37 the Sleep Assessment Unit of Sha Tin Hospital. The urine sample will be stored at
38 –80° before analysis.

47 **Sample size**

48 A pilot study on improving sleeping quality among children with ASD³⁰ showed that
49 physical activity had notable effect (corresponding to a Cohen's *d* of about 1.0) on
50 improving sleeping quality. Given this effect size, a sample of 16 participants per
51 group is required to achieve a power of 80% and a level of significance of 5%.
52 Assuming 20% attrition, 20 participants will be recruited per group. They will be
53
54
55
56
57
58
59

1
2
3 recruited from 3 local special schools with existing research links to the PI and his
4 collaborators.
5

6 7 **Randomization**

8 After screening, all the eligible participants will be randomly assigned to either the
9 intervention or control group. To ensure equal allocation ratios for the intervention
10 and control groups, block randomization⁵² will be used. A block size of 8 will be used
11 in the proposed study (i.e., 4 in one group and 4 in the other group for every 8
12 consecutively entered participants). The block randomization process will be done by
13 a trained research assistant.
14
15

16 17 **Blinding**

18 The person responsible for analyzing the sleep parameters and melatonin level will be
19 blinded for the group assignment.
20

21 22 **Ethics and dissemination**

23 Prior to the study, information about the study will be provided to all participants and
24 their parents. Written consent will be obtained from the parents and children. They
25 will be informed that withdrawal at any time will not result in any adverse
26 consequences. All sets of data will be encrypted with passwords. To prevent any
27 leakage of sensitive information, only the PI and his collaborators will have access to
28 the datasets. Ethical approval was obtained from the Human Research Ethics
29 Committee, The Education University of Hong Kong (reference number
30 2016-2017-0155). Results of the study will be published in a peer-reviewed journal.
31 Findings of the study will also be shared with other university and non-governmental
32 organizations in HK that specialize in Autism by means of a formal dissemination
33 seminar.
34
35

36 37 **Statistical methods**

38 All statistical analyses will be conducted using SPSS (version 23.0) for Windows
39 (SPSS Inc., Chicago, IL, USA) to assess sleep; multilevel regression or a generalized
40 estimating equation (GEE) will be used to assess the effects of the physical activity
41 intervention, time effect, and their interaction on sleep outcomes and melatonin level
42 outcomes. Two potential confounding variables (i.e., average time spent in daily
43 sedentary activity and average time spent in daily MVPA) will be used as covariates
44 because they may be closely related to sleep quality.⁵³ The effect size will be reported
45 as a Cohen's *d*. Bonferroni adjustment will be used to control for possible type I error
46 inflation caused by multiple comparisons.
47
48
49

50 51 **Discussion**

52 This study is the first randomized control trial to investigate the effectiveness of
53 physical activity intervention on sleep disorder among children with ASD. In addition,
54 it is also the first step of studying the melatonin-mediated mechanism of the possible
55 underlying causal pathways by which physical activity impacts on sleep in children
56
57
58
59

1
2
3 with ASD. The results obtained in this study have two significant impacts. First, if the
4 intervention is effective, doctors can then prescribe physical activity to children with
5 ASD who are not able to take drugs based on the concept of 'exercise is medicine'.
6 Second, the melatonin-mediated mechanism investigated in this study could lead to
7 further investigation of the interactions between physical activity and melatonin in
8 any population suffering from sleep disturbance. Such research could include
9 comparison of the effectiveness of physical activity and supplemental melatonin
10 interventions on sleep quality in populations suffering from sleep disorders and the
11 manipulation of different physical activity intervention parameters (e.g., intensity,
12 frequency, and time) on melatonin level and sleep quality.
13
14
15

16 **Acknowledgement**

17 This study is funded by the Early Career Scheme of Research Grants Council,
18 University Grants Committee of HKSAR Government (#28602517).
19
20

21 **Contributorship Statement**

22 AT and JZ conceived of the study and designed the study protocol, PL assisted in
23 defining the statistical analysis and provided input for the manuscript. EL provided
24 critical comment on implementation of participant's screening protocol. All authors
25 contributed to, read drafts of and approved the final manuscript.
26
27

28 **Competing interests**

29 The authors declare there is no competing interests with respect to the research,
30 authorship, and/or publication of this article.
31
32

33 **Funding**

34 This work was supported by the Early Career Scheme of Research Grant Council
35 (Grant number: 28602517).
36
37
38
39
40

41 **References**

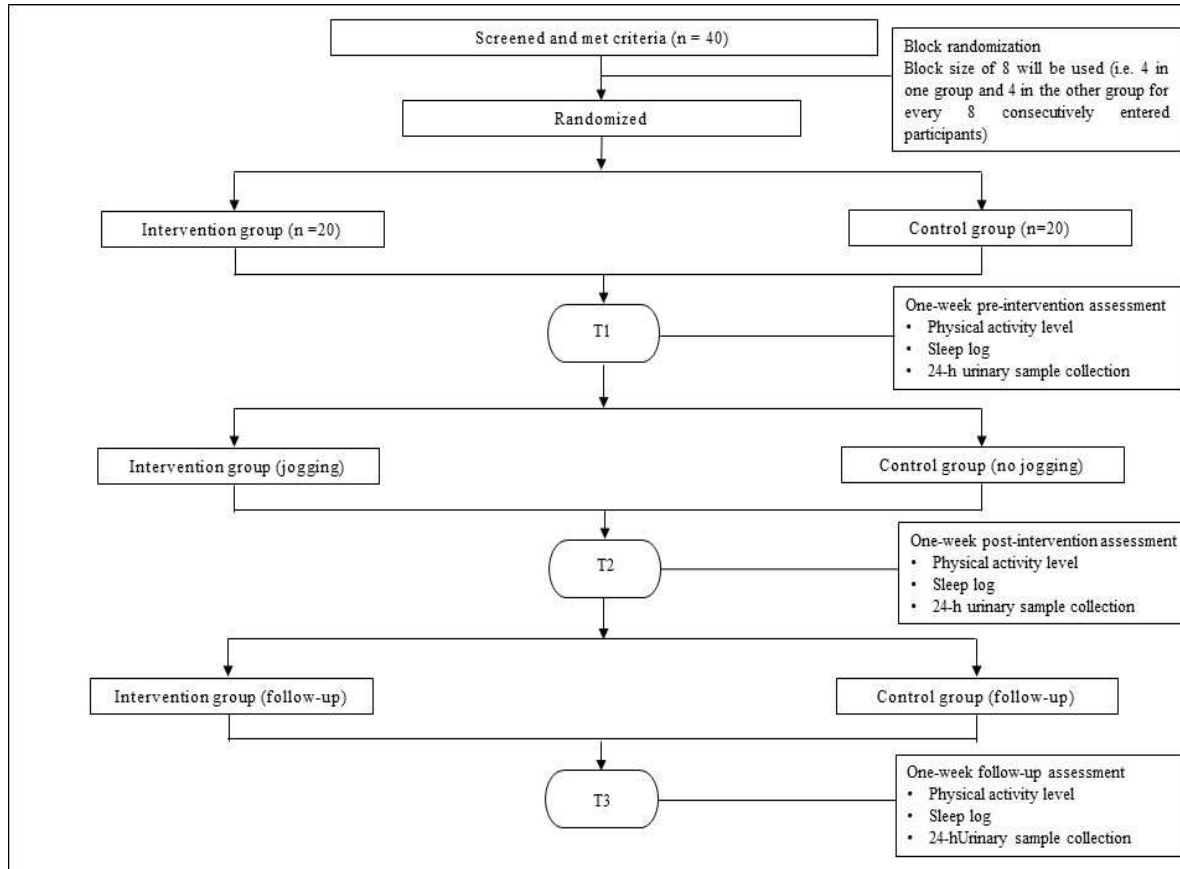
- 42 1. World Health Organization. Questions and answers about autism spectrum disorders
43 (ASD). 2016; <http://www.who.int/features/qa/85/en/>. Accessed 12th August, 2016.
- 44 2. Rapin I, Tuchman RF. Autism: definition, neurobiology, screening, diagnosis. *Pediatr*
45 *Clin North Am.* 2008;55(5):1129-1146.
- 46 3. Cortesi F, Giannotti F, Ivanenko A, Johnson K. Sleep in children with autistic spectrum
47 disorder. *Sleep Med.* 2010;11(7):659-664.
- 48 4. Liu X, Hubbard JA, Fabes RA, Adam JB. Sleep disturbances and correlates of children
49 with Autism Spectrum Disorders. *Child Psy Human Dev.* 2006;37(2):179-191.
- 50 5. Sivertsen B, Posserud M-B, Gillberg C, Lundervold AJ, Hysing M. Sleep problems in
51 children with autism spectrum problems: a longitudinal population-based study. *Autism.*
52 2012;16(2):139-150.
53
54
55
56
57
58
59
60

- 1
- 2
- 3 6. Orsmond GI, Seltzer MM. Siblings of individuals with autism spectrum disorders across
- 4 the life course. *Ment Retard Dev Disabil Res Rev*. 2007;13(4):313-320.
- 5 7. Goldman SE, Richdale AL, Clemons T, Malow BA. Parental sleep concerns in Autism
- 6 Spectrum Disorders: Variations from childhood to adolescence. *J Autism and Dev Disord*.
- 7 2012;42(4):531-538.
- 8 8. Fricke-Oerkermann L, Pluck J, Schredl M, et al. Prevalence and course of sleep problems
- 9 in childhood. *Sleep*. 2007;30(10):1371-1377.
- 10 9. Calhoun SL, Fernandez-Mendoza J, Vgontzas AN, Liao D, Bixler EO. Prevalence of
- 11 insomnia symptoms in a general population sample of young children and preadolescents:
- 12 gender effects. *Sleep Med*. 2014;15(1):91-95.
- 13 10. Souders MC, Mason TB, Valladares O, et al. Sleep behaviors and sleep quality in
- 14 children with autism spectrum disorders. *Sleep*. 2009;32(12):1566-1578.
- 15 11. Richdale AL, Schreck KA. Sleep problems in autism spectrum disorders: prevalence,
- 16 nature, & possible biopsychosocial aetiologies. *Sleep Med Rev*. 2009;13(6):403-411.
- 17 12. Dewald JF, Meijer AM, Oort FJ, Kerkhof GA, Bögels SM. The influence of sleep quality,
- 18 sleep duration and sleepiness on school performance in children and adolescents: A
- 19 meta-analytic review. *Sleep Med Rev*. 2010;14(3):179-189.
- 20 13. Curcio G, Ferrara M, De Gennaro L. Sleep loss, learning capacity and academic
- 21 performance. *Sleep Med Rev*. 2006;10(5):323-337.
- 22 14. Mazurek MO, Sohl K. Sleep and behavioral problems in children with Autism Spectrum
- 23 Disorder. *J Autism Dev Disord*. 2016;46(6):1906-1915.
- 24 15. Schreck KA, Mulick JA, Smith AF. Sleep problems as possible predictors of intensified
- 25 symptoms of autism. *Res Dev Disabil*. 2004;25(1):57-66.
- 26 16. Doo S, Wing YK. Sleep problems of children with pervasive developmental disorders:
- 27 correlation with parental stress. *Dev Med & Child Neuro*. 2006;48(8):650-655.
- 28 17. Yu XT, Lam HS, Au CT, Chan SHY, Chan DFY, Li AM. Extended parent-based
- 29 behavioural education improves sleep in children with Autism Spectrum Disorder. *Hong*
- 30 *Kong J Ped*. 2015;20(4):219-225.
- 31 18. Richdale AL. Sleep problems in autism: prevalence, cause, and intervention. *Dev Child*
- 32 *Neuro*. 1999;41(01):60-66.
- 33 19. Malow BA, Byars K, Johnson K, et al. A practice pathway for the identification,
- 34 evaluation, and management of insomnia in children and adolescents with Autism
- 35 Spectrum Disorders. *Pediatrics*. 2012;130(2):106-124.
- 36 20. Bramble D. Consumer opinion concerning the treatment of a common sleep problem.
- 37 *Child Care Health Dev*. 1996;22(6):355-366.
- 38 21. Vriend JL, Corkum PV, Moon EC, Smith IM. Behavioral interventions for sleep problems
- 39 in children with Autism Spectrum Disorders: Current findings and future directions. *J*
- 40 *Ped Psycho*. 2011;36(9):1017-1029.
- 41 22. Weiskop S, Richdale A, Matthews J. Behavioural treatment to reduce sleep problems in
- 42 children with autism or fragile X syndrome. *Dev Med Child Neuro*. 2005;47(2):94-104.
- 43 23. Lancioni GE, O'Reilly MF, Basili G. Review of strategies for treating sleep problems in
- 44 persons with severe or profound mental retardation or multiple handicaps. *Am J Mental*
- 45 *Retard*. 1999;104(2):170-186.
- 46 24. Driver HS, Taylor SR. Exercise and sleep. *Sleep Med Rev*. 2000;4(4):387-402.
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

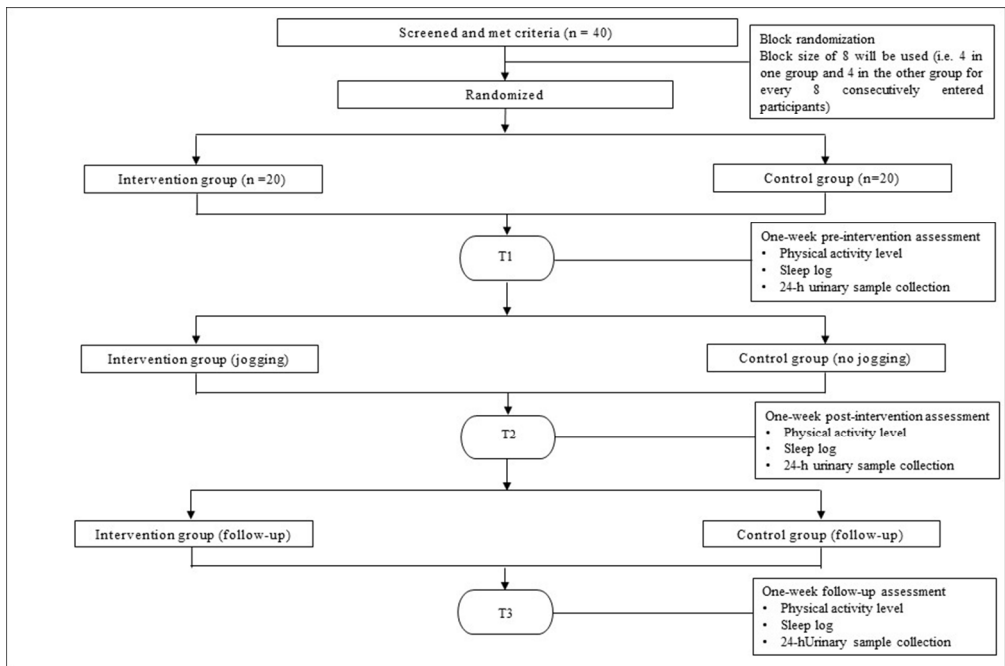
25. Kredlow MA, Capozzoli MC, Hearon BA, Calkins AW, Otto MW. The effects of physical activity on sleep: a meta-analytic review. *J Behav Med.* 2015;38(3):427-449.
26. Youngstedt SD, O'Connor PJ, Dishman RK. The effects of acute exercise on sleep: a quantitative synthesis. *Sleep.* 1997;20(3):203-214.
27. Lundahl A, Nelson TD, Van Dyk TR, West T. Psychosocial Stressors and Health Behaviors: Examining Sleep, Sedentary Behaviors, and Physical Activity in a Low-Income Pediatric Sample. *Clinical Pediatrics.* 2013;52(8):721-729.
28. Stone MR, Stevens D, Faulkner GEJ. Maintaining recommended sleep throughout the week is associated with increased physical activity in children. *Prev Med.* 2013;56(2):112-117.
29. Wachob D, Lorenzi DG. Brief Report: Influence of Physical Activity on Sleep Quality in Children with Autism. *J Autism Dev Disord.* 2015;45(8):2641-2646.
30. Brand S, Jossen S, Holsboer-Trachsler E, Pühse U, Gerber M. Impact of aerobic exercise on sleep and motor skills in children with autism spectrum disorders – a pilot study. *Neuropsychiatric Disease and Treatment.* 2015;11:1911-1920.
31. McGinty D, Szymusiak R. Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. *Trends Neurosci.* 1990;13(12):480-487.
32. Adam K, Oswald I. Protein synthesis, bodily renewal and the sleep-wake cycle. *Clinical Science (London, England : 1979).* 1983;65(6):561-567.
33. Atkinson G, Edwards B, Reilly T, Waterhouse J. Exercise as a synchroniser of human circadian rhythms: an update and discussion of the methodological problems. *Eur J Appl Physiol.* 2007;99(4):331-341.
34. Lee H, Kim S, Kim D. Effects of exercise with or without light exposure on sleep quality and hormone responses. *J Exerc Nutr & Biochem.* 2014;18(3):293-299.
35. Wirojanan J, Jacquemont S, Diaz R, et al. The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X syndrome. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine.* 2009;5(2):145-150.
36. Rognigni DA, Frye RE. Melatonin in autism spectrum disorders: a systematic review and meta-analysis. *Dev Med & Child Neuro.* 2011;53(9):783-792.
37. Krakowiak P, Goodlin-Jones B, Hertz-Picciotto I, Croen LA, Hansen RL. Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study. *J Sleep Res.* 2008;17(2):197-206.
38. Veatch OJ, Goldman SE, Adkins KW, Malow BA. Melatonin in Children with Autism Spectrum Disorders: How Does the Evidence Fit Together? *J Nat and Sci.* 2015;1(7):125.
39. Goldman SE, Adkins KW, Calcutt MW, et al. Melatonin in children with autism spectrum disorders: endogenous and pharmacokinetic profiles in relation to sleep. *J Autism Dev Disord.* 2014;44(10):2525-2535.
40. Arendt J. Importance and relevance of melatonin to human biological rhythms. *J Neuroendocrinol.* 2003;15(4):427-431.
41. Marrin K, Drust B, Gregson W, Morris CJ, Chester N, Atkinson G. Diurnal variation in the salivary melatonin responses to exercise: relation to exercise-mediated tachycardia. *Eur J of Appl Physiol.* 2011;111(11):2707-2714.

- 1
2
3 42. Association AP. *Diagnostic and statistical manual of mental disorders: DSM-5*
4 Washington, DC: American Psychiatric Association; 2016.
- 5 43. Giannotti F, Cortesi F, Cerquiglini A, Bernabei P. An Open-Label Study of
6 Controlled-Release Melatonin in Treatment of Sleep Disorders in Children with Autism.
7 *J Autism and Dev Disord.* 2006;36(6):741-752.
- 8 44. Prevention CfDcA. Measuring Physical Activity Intensity: Target Heart Rate and
9 Estimated Maximum Heart Rate. 2015;
10 <http://www.cdc.gov/physicalactivity/basics/measuring/hearttrate.htm>. Accessed 26th
11 March, 2016, 2016.
- 12 45. Londeree BR, Moeschberger ML. Effect of Age and Other Factors on Maximal Heart
13 Rate. *Res Q Exerc Sport.* 1982;53(4):297-304.
- 14 46. Pan CY, Chu CH, Tsai CL, Sung MC, Huang CY, Ma WY. The impacts of physical
15 activity intervention on physical and cognitive outcomes in children with autism
16 spectrum disorder. *Autism.* 2016.
- 17 47. Lang R, Koegel LK, Ashbaugh K, Register A, Ence W, Smith W. Physical exercise and
18 individuals with autism spectrum disorders: A systematic review. *Res Autism Spectr*
19 *Disord.* 2010;4(4):565-576.
- 20 48. Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire (CSHQ):
21 psychometric properties of a survey instrument for school-aged children. *Sleep.*
22 2000;23(8):1043-1051.
- 23 49. Barreira TV, Schuna JM, Jr., Mire EF, et al. Identifying children's nocturnal sleep using
24 24-h waist accelerometry. *Med Sci Sports Exerc.* 2015;47(5):937-943.
- 25 50. ActiGraph. What is MVPA and how I can view in ActiLife. 2016;
26 [https://help.theactigraph.com/entries/22148365-What-is-MVPA-and-how-can-I-view-it-i](https://help.theactigraph.com/entries/22148365-What-is-MVPA-and-how-can-I-view-it-in-ActiLife-)
27 [n-ActiLife-](https://help.theactigraph.com/entries/22148365-What-is-MVPA-and-how-can-I-view-it-in-ActiLife-). Accessed 2nd September,2016, 2016.
- 28 51. Schernhammer ES, Kroenke CH, Dowsett M, Folkard E, Hankinson SE. Urinary
29 6-sulfatoxymelatonin levels and their correlations with lifestyle factors and steroid
30 hormone levels. *J Pineal Res.* 2006;40(2):116-124.
- 31 52. Efird J. Blocked Randomization with Randomly Selected Block Sizes. *International J*
32 *Enviro Res Public Health.* 2011;8(1):15-20.
- 33 53. Kline CE, Crowley EP, Ewing GB, et al. The effect of exercise training on obstructive
34 sleep apnea and sleep quality: a randomized controlled trial. *Sleep.*
35 2011;34(12):1631-1640.
- 36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59

Figure 1. Flow chart of the study



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



254x190mm (96 x 96 DPI)

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020944.R1
Article Type:	Protocol
Date Submitted by the Author:	17-Jan-2018
Complete List of Authors:	Tse, Choi Yeung Andy; Department of Health and Physical Education Lee, Paul; Hong Kong Polytechnic University, School of Nursing Zhang, Jihui; The Chinese University of Hong Kong, Department of Psychiatry Lai, Elvis, W.H.; The Hong Kong Castle Peak Hospital, Department of Psychiatry
Primary Subject Heading:	Research methods
Secondary Subject Heading:	Sports and exercise medicine
Keywords:	Physical activity, Sleep, Children with autism spectrum disorders, Melatonin

SCHOLARONE™
Manuscripts

Only

TITLE PAGE WITH AUTHOR INFORMATION

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 modelAndy C.Y. Tse¹, Paul H. Lee², J. Zhang³, Elvis W.H. Lai⁴¹Department of Health and Physical Education, The Education University of Hong Kong, Hong Kong China²School of Nursing, The Hong Kong Polytechnic University, Hong Kong China³Department of Psychiatry, The Chinese University of Hong Kong, Hong Kong China⁴Department of Psychiatry, The Hong Kong Castle Peak Hospital

Dr. Andy C.Y. Tse [Corresponding Author]

Rm D4-2/F-03, Block D4,
10 Lo Ping Road, Tai Po, N.T., Hong Kong.

Tel: (852) 29488074

Email: andytcy@eduhk.hk

Dr. J. Zhang

G/F Multicentre, Tai Po Hospital, Tai Po, N.T.,

Hong Kong

Tel: (852) 26475321

Email: jihui.zhang@cuhk.edu.hk

Dr. Paul H. Lee

School of Nursing, GH527
Hong Kong Polytechnic University, Hung Hom,
Kowloon, Hong Kong

Tel: (852) 3400 8275

Email: paul.h.lee@polyu.edu.hk

Dr. Elvis W.H Lai

15, Ching Chung Koon Road,
Tuen Mun, N.T., Hong Kong

Tel: (852)24567111

Email: lwh041@ha.org.hk

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.

For peer review only

Introduction

According to the World Health Organization, autism spectrum disorder (ASD) is defined as a group of complex brain development disorders¹ 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 ASD⁴⁻⁷ 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 performance¹² and memory consolidation¹³) and daily functioning (e.g., increased stereotypy¹⁴ and overall autistic behavior¹⁵) 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 al²⁵ examined the effects of acute and regular exercise on sleep. It indicates that both

1
2
3 acute and regular exercise increase total sleep time, improve sleep onset latency and
4 sleep efficiency, reduce rapid eye movement, and promote slow wave sleep.

5 While physical activity is shown to be beneficial to sleep quality, most studies
6 were conducted on healthy adults and good sleepers.^{24,26} A few similar studies were
7 conducted in a child population^{27,28} and positive association between physical activity
8 level and sleep quality were found. Recently, two small-scale studies have been
9 conducted to explore the impact of physical activity on sleep in children with ASD.
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
One of them (Wachob et al²⁹) 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 al³⁰)
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 theory³² and
melatonin-mediated mechanism³³) 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 al⁴¹
asked seven healthy participants to complete a moderate intensity cycling exercise in

1
2
3 the morning with their salivary melatonin concentration measured in at baseline,
4 during exercise, after exercise and exercise recovery. Results showed that
5 participants' melatonin levels significantly increased during and after exercise
6 compared with those at baseline and recovery. However, to our best knowledge, none
7 of the previous studies have examined the relationship between physical activity,
8 melatonin and sleep, particularly in children with ASD. Therefore, the mechanism of
9 how physical activity impacts on sleep in children with ASD remains in question.
10 Here, we present the study protocol which will begin enrolment in February 2018.
11
12

13 14 **Objectives**

15 The purpose of this study is twofold: (1) to examine the associations between physical
16 activity, melatonin, and sleep quality in children with ASD, which ultimately lead to
17 an answer of how physical activity impacts on sleep in children with ASD from the
18 perspective of melatonin-mediated mechanism model; and (2) to examine the possible
19 sustained effect of physical activity on improved melatonin secretion and sleep
20 quality in children with ASD. It is hypothesized that physical activity can improve
21 sleep quality in children with ASD by increasing their endogenous melatonin level,
22 and these beneficial effects can be sustained.
23
24

25 26 **Methods/design**

27 **Study design**

28 The purposed study will be a parallel, two-group randomized controlled trial (RCT)
29 design with equal allocation ratio to the intervention group and control group (1:1). A
30 flow diagram of the study is given in Fig. 1.
31
32

33 <Fig. 1 is inserted here>
34
35

36 **Data collection**

37 Each participant will attend 4 one-week assessments, where we will assess their
38 habitual sleep patterns: before the intervention (T1: baseline), immediately after the
39 12 weeks of physical activity or regular treatment (T2: post-intervention), 6 week
40 after and 12 weeks after post-intervention (T3- T4: follow-up). T1 and T2 represent
41 the pre-and post-intervention. T3 serves as the 6-week follow-up and T4 serves as the
42 12-week follow-up.
43
44

45 46 **Participant**

47 Children will be screened with the following inclusion criteria.

48 The inclusion criteria are: (1) age 9 –12 years; (2) pre-puberty or early puberty as
49 indicated by Tanner stage I or II ; (3) ASD diagnosis from a physician based on
50 Diagnostic and Statistical Manual of Mental Disorders, 5th edition, (DSM-V)⁴²; (4)
51 non-verbal IQ over 40; (5) the ability to follow instructions; (6) physically able to
52 participate in the intervention; (7) no additional regular participation in physical
53 exercise other than school physical education classes for at least 6 months prior to the
54 study; (8) no concurrent medication for at least 6 months before the study or any prior
55
56
57
58
59

1
2
3 melatonin treatment; and (9) have sleep difficulties, including sleep onset insomnia
4 and frequent and prolonged nightwaking and/or early morning awakenings (see
5 Giannotti et al⁴³ for definitions) reported by parents.

6 Exclusion criteria are: (1) with one or co-morbid psychiatric disorders as established
7 by a structured interview based on DSM-V; (2) with other medical conditions that
8 limit their physical activity capacities (e.g., asthma, seizure, cardiac disease); and (3)
9 with a complex neurologic disorder (e.g., epilepsy, phenylketonuria, fragile X
10 syndrome, tuberous sclerosis). All screening will be carried out by a psychologist and
11 a physician.
12
13

14 **Intervention**

15 Intervention group: The intervention is a 12-week jogging program consisting of 24
16 sessions (two sessions per week, 30 min per session) in a hall/gymnasium of each
17 participating school. 24 sessions are selected based on previous studies involving
18 jogging in this population.^{44,45} Each intervention session will be conducted in the
19 morning by a trained research assistant assisted by student helpers. The
20 staff-to-participant ratio for both groups is 1:2 to 1:1 depending on the attendance.
21 The research assistant and student helpers will be majoring in physical education or
22 adapted physical education and have experience with children with ASD. Each
23 intervention session will be conducted in an identical format, comprising three
24 activities: warm-up (5 min), jogging (20 min), and cool-down (5 min). In the jogging
25 activity, participants will be asked to jog side-by-side with the research staff around
26 an activity circuit (57m x 50m) marked with 4 red cones. The activity circuit will be
27 set up in an outdoor sports ground or indoor gymnasium depending on the weather
28 and the arrangement of the participating schools. Participants are required to run at a
29 moderate intensity level. The intensity level of jogging will be objectively measured
30 by asking participants to wear heart rate monitor (Polar H1) during each jogging
31 session. According to the Center for Disease Control and Prevention⁴⁶, MVPA is
32 achieved with a target heart rate above 60% of the maximum heart rate (subtracting
33 the participant's age from 220⁴⁷). Considering the low physical activity level of
34 children with ASD⁴⁸, physical activity with a heart rate (HR) above 50% of the
35 maximum heart rate should be considered MVPA. The intervention is considered
36 successful if the participants can maintain their target heart rate (THR) for 15 minutes
37 or above throughout the jogging session. The Polar devices will also be used to
38 calculate how long the participants are within their THR (i.e. 50% or above the
39 maximum HR). Meanwhile, jogging is chosen because it is one of the most common
40 exercises studied with regards to ASD⁴⁹ and can serve as endurance training, which is
41 shown to be beneficial for sleep²⁶. Participants will be positively reinforced verbally
42 with compliments for their efforts in jogging and their daily and weekly
43 improvements will be visualized through graphs and scales kept at home in the child's
44 bedroom.³⁰ After the intervention, the participants will be required to follow their
45 normal daily routine without participating in any additional physical activity/exercise
46 program (except the 60-minute weekly physical education (PE) classes provided by
47 school) throughout the follow-up period (T2-T4).
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Control group: Participants in the control group will receive no physical intervention
4 (i.e. jogging program) and will be required to follow their daily routine without
5 participating in any additional physical activity/exercise program except the regular
6 PE classes throughout the whole study period (T1-T4).
7

8 9 **Study measures**

10 Before the initial assessment, participants' parents will be asked to provide
11 demographic data and a brief developmental history. Both participants and their
12 parents will undertake T1, T2, T3 and T4, where the following measurements will be
13 carried out.
14

15 16 *Primary outcome measures*

17 Sleep: Four sleep parameters including sleep onset latency (length of time taken to
18 fall asleep, expressed in minutes, SOL); sleep efficiency (actual sleep time divided by
19 time in bed, expressed as a percent, SE); wake after sleep onset (length of time they
20 were awake after sleep onset, expressed in minutes, WASO) and sleep duration (total
21 sleep in hours and minutes, SD) will be objectively measured by a GT3X
22 accelerometer²⁹. Participants will be asked to wear the device on the non-dominant
23 wrist for seven consecutive days (Monday to Sunday). Non-wear time is defined as 60
24 min of consecutive zeros with a 2-min spike tolerance.²⁹ The night (2200–0700) will
25 be considered invalid if the wear time is less than 8 h and will be excluded from the
26 analysis. In addition, participants' sleep patterns will be logged by their parents using
27 Children's Sleep Habits Questionnaire (CSHQ), which is a validated 45-item
28 parent-administered questionnaire to examine sleep patterns of young children,⁵⁰
29 including children with ASD.²⁹ Parents will be asked to recall specific sleep patterns
30 of their children over the assessment weeks (T1, T2, T3 and T4). Finally, parents will
31 also be asked to log the bedtime, sleep latency, sleep start, sleep end, wake-up time
32 and assumed sleep length in a sleep log for the whole assessment week. The sleep log
33 is used as part of the refined sleep algorithm (RSA) in Actigraph data analysis to
34 identify nocturnal sleep and to exclude non-wear time/wakefulness.⁵¹
35

36 Melatonin level: All participants will be instructed to collect a 24-h urine sample.
37 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin and
38 representative of melatonin level, will be measured from the sample.⁵² The weekend
39 has been chosen to allow the participants to stay at home for sample collection. All
40 urine samples will be collected using 24-h urine bottles containing 0.1L of 0.5M
41 hydrochloric acid as a preservative. Upon completion, the research assistant will
42 immediately collect the urine sample from the participants' apartments and bring it to
43 the Sleep Assessment Unit of Sha Tin Hospital. The urine sample will be stored at
44 –80° before analysis.
45
46
47
48
49
50

51 52 *Secondary outcome measure*

53 Physical activity level: The physical activity level of the participants will also be
54 measured as secondary data to examine its relationship to sleep as suggested by
55 previous studies.^{4,29} It will be measured using the same accelerometer (i.e. GT3X).
56
57
58
59
60

1
2
3 The data used for analysis are the times spent in sedentary activity and MVPA based
4 on the default energy expenditure algorithm in the accelerometer device.⁵³ The day
5 (0700–2100) will be considered invalid if the wear time is less than 10 h and will then
6 be excluded from the analysis.
7

8 9 **Sample size**

10 A pilot study on improving sleeping quality among children with ASD³⁰ showed that
11 physical activity had notable effect (corresponding to a Cohen's *d* of about 1.0) on
12 improving sleeping quality. Given this effect size, a sample of 16 participants per
13 group is required to achieve a power of 80% and a level of significance of 5%.
14 Assuming 20% attrition, 20 participants will be recruited per group. They will be
15 recruited from 3 local special schools with existing research links to the PI and his
16 collaborators.
17
18

19 20 **Randomization**

21 After screening, all the eligible participants will be randomly assigned to either the
22 intervention or control group. To ensure equal allocation ratios for the intervention
23 and control groups, block randomization⁵⁴ will be used. A block size of 8 will be used
24 in the proposed study (i.e., 4 in one group and 4 in the other group for every 8
25 consecutively entered participants). The block randomization process will be done by
26 a trained research assistant.
27
28

29 30 **Blinding**

31 The person responsible for analyzing the sleep parameters and melatonin level will be
32 blinded for the group assignment.
33
34

35 36 **Ethics and dissemination**

37 Prior to the study, information about the study will be provided to all participants and
38 their parents. Written consent (see Appendix I) will be obtained from the parents and
39 children. They will be informed that withdrawal at any time will not result in any
40 adverse consequences. All sets of data will be encrypted with passwords. To prevent
41 any leakage of sensitive information, only the PI and his collaborators will have
42 access to the datasets. Ethical approval was obtained from the Human Research Ethics
43 Committee, The Education University of Hong Kong (reference number
44 2016-2017-0155). Results of the study will be published in a peer-reviewed journal.
45 Findings of the study will also be shared with other university and non-governmental
46 organizations in HK that specialize in Autism by means of a formal dissemination
47 seminar.
48
49

50 51 **Statistical methods**

52 All statistical analyses will be conducted using SPSS (version 23.0) for Windows
53 (SPSS Inc., Chicago, IL, USA) to assess sleep; multilevel regression or a generalized
54 estimating equation (GEE) will be used to assess the effects of the physical activity
55 intervention, time effect, and their interaction on sleep outcomes and melatonin level
56
57
58
59

1
2
3 outcomes. Two potential confounding variables (i.e., average time spent in daily
4 sedentary activity and average time spent in daily MVPA) will be used as covariates
5 because they may be closely related to sleep quality.⁵⁵ The effect size will be reported
6 as a Cohen's *d*. Bonferroni adjustment will be used to control for possible type I error
7 inflation caused by multiple comparisons. Intention to treat approach will be used to
8 handle any missing data.
9

11 Discussion

12 This study is the first randomized control trial to investigate the effectiveness of
13 physical activity intervention on sleep disorder among children with ASD. In addition,
14 it is also the first step of studying the melatonin-mediated mechanism of the possible
15 underlying causal pathways by which physical activity impacts on sleep in children
16 with ASD. The results obtained in this study have two significant impacts. First, if the
17 intervention is effective, doctors can then prescribe physical activity to children with
18 ASD who are not able to take drugs based on the concept of 'exercise is medicine'.
19 Second, the melatonin-mediated mechanism investigated in this study could lead to
20 further investigation of the interactions between physical activity and melatonin in
21 any population suffering from sleep disturbance. Such research could include
22 comparison of the effectiveness of physical activity and supplemental melatonin
23 interventions on sleep quality in populations suffering from sleep disorders and the
24 manipulation of different physical activity intervention parameters (e.g., intensity,
25 frequency, and time) on melatonin level and sleep quality.
26
27
28
29
30

31 Acknowledgement

32 This study is funded by the Early Career Scheme of Research Grants Council,
33 University Grants Committee of HKSAR Government (#28602517).
34
35

36 Contributorship Statement

37 AT and JZ conceived of the study and designed the study protocol, PL assisted in
38 defining the statistical analysis and provided input for the manuscript. EL provided
39 critical comment on implementation of participant's screening protocol. All authors
40 contributed to, read drafts of and approved the final manuscript.
41
42

43 Competing interests

44 The authors declare there is no competing interests with respect to the research,
45 authorship, and/or publication of this article.
46
47

48 Funding

49 This work was supported by the Early Career Scheme of Research Grant Council
50 (Grant number: 28602517).
51
52
53
54
55
56
57
58
59
60

References

1. World Health Organization. Questions and answers about autism spectrum disorders (ASD). 2016; <http://www.who.int/features/qa/85/en/>. Accessed 12th August, 2016.
2. Rapin I, Tuchman RF. Autism: definition, neurobiology, screening, diagnosis. *Pediatr Clin North Am*. 2008;55(5):1129-1146.
3. Cortesi F, Giannotti F, Ivanenko A, Johnson K. Sleep in children with autistic spectrum disorder. *Sleep Med*. 2010;11(7):659-664.
4. Liu X, Hubbard JA, Fabes RA, Adam JB. Sleep disturbances and correlates of children with Autism Spectrum Disorders. *Child Psy Human Dev*. 2006;37(2):179-191.
5. Sivertsen B, Posserud M-B, Gillberg C, Lundervold AJ, Hysing M. Sleep problems in children with autism spectrum problems: a longitudinal population-based study. *Autism*. 2012;16(2):139-150.
6. Orsmond GI, Seltzer MM. Siblings of individuals with autism spectrum disorders across the life course. *Ment Retard Dev Disabil Res Rev*. 2007;13(4):313-320.
7. Goldman SE, Richdale AL, Clemons T, Malow BA. Parental sleep concerns in Autism Spectrum Disorders: Variations from childhood to adolescence. *J Autism and Dev Disord*. 2012;42(4):531-538.
8. Fricke-Oerkermann L, Pluck J, Schredl M, et al. Prevalence and course of sleep problems in childhood. *Sleep*. 2007;30(10):1371-1377.
9. Calhoun SL, Fernandez-Mendoza J, Vgontzas AN, Liao D, Bixler EO. Prevalence of insomnia symptoms in a general population sample of young children and preadolescents: gender effects. *Sleep Med*. 2014;15(1):91-95.
10. Souders MC, Mason TB, Valladares O, et al. Sleep behaviors and sleep quality in children with autism spectrum disorders. *Sleep*. 2009;32(12):1566-1578.
11. Richdale AL, Schreck KA. Sleep problems in autism spectrum disorders: prevalence, nature, & possible biopsychosocial aetiologies. *Sleep Med Rev*. 2009;13(6):403-411.
12. Dewald JF, Meijer AM, Oort FJ, Kerkhof GA, Bögels SM. The influence of sleep quality, sleep duration and sleepiness on school performance in children and adolescents: A meta-analytic review. *Sleep Med Rev*. 2010;14(3):179-189.
13. Curcio G, Ferrara M, De Gennaro L. Sleep loss, learning capacity and academic performance. *Sleep Med Rev*. 2006;10(5):323-337.
14. Mazurek MO, Sohl K. Sleep and behavioral problems in children with Autism Spectrum Disorder. *J Autism Dev Disord*. 2016;46(6):1906-1915.
15. Schreck KA, Mulick JA, Smith AF. Sleep problems as possible predictors of intensified symptoms of autism. *Res Dev Disabil*. 2004;25(1):57-66.
16. Doo S, Wing YK. Sleep problems of children with pervasive developmental disorders: correlation with parental stress. *Dev Med & Child Neuro*. 2006;48(8):650-655.
17. Yu XT, Lam HS, Au CT, Chan SHY, Chan DFY, Li AM. Extended parent-based behavioural education improves sleep in children with Autism Spectrum Disorder. *Hong Kong J Ped*. 2015;20(4):219-225.

18. Richdale AL. Sleep problems in autism: prevalence, cause, and intervention. *Dev Child Neuro*. 1999;41(01):60-66.
19. Malow BA, Byars K, Johnson K, et al. A practice pathway for the identification, evaluation, and management of insomnia in children and adolescents with Autism Spectrum Disorders. *Pediatrics*. 2012;130(2):106-124.
20. Bramble D. Consumer opinion concerning the treatment of a common sleep problem. *Child Care Health Dev*. 1996;22(6):355-366.
21. Vriend JL, Corkum PV, Moon EC, Smith IM. Behavioral interventions for sleep problems in children with Autism Spectrum Disorders: Current findings and future directions. *J Ped Psycho*. 2011;36(9):1017-1029.
22. Weiskop S, Richdale A, Matthews J. Behavioural treatment to reduce sleep problems in children with autism or fragile X syndrome. *Dev Med Child Neuro*. 2005;47(2):94-104.
23. Lancioni GE, O'Reilly MF, Basili G. Review of strategies for treating sleep problems in persons with severe or profound mental retardation or multiple handicaps. *Am J Mental Retard*. 1999;104(2):170-186.
24. Driver HS, Taylor SR. Exercise and sleep. *Sleep Med Rev*. 2000;4(4):387-402.
25. Kredlow MA, Capozzoli MC, Hearon BA, Calkins AW, Otto MW. The effects of physical activity on sleep: a meta-analytic review. *J Behav Med*. 2015;38(3):427-449.
26. Youngstedt SD, O'Connor PJ, Dishman RK. The effects of acute exercise on sleep: a quantitative synthesis. *Sleep*. 1997;20(3):203-214.
27. Lundahl A, Nelson TD, Van Dyk TR, West T. Psychosocial Stressors and Health Behaviors: Examining Sleep, Sedentary Behaviors, and Physical Activity in a Low-Income Pediatric Sample. *Clinical Pediatrics*. 2013;52(8):721-729.
28. Stone MR, Stevens D, Faulkner GEJ. Maintaining recommended sleep throughout the week is associated with increased physical activity in children. *Prev Med*. 2013;56 (2):112-117.
29. Wachob D, Lorenzi DG. Brief Report: Influence of Physical Activity on Sleep Quality in Children with Autism. *J Autism Dev Disord*. 2015;45(8):2641-2646.
30. Brand S, Jossen S, Holsboer-Trachsler E, Pühse U, Gerber M. Impact of aerobic exercise on sleep and motor skills in children with autism spectrum disorders – a pilot study. *Neuropsychiatric Disease and Treatment*. 2015;11:1911-1920.
31. McGinty D, Szymusiak R. Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. *Trends Neurosci*. 1990;13(12):480-487.
32. Adam K, Oswald I. Protein synthesis, bodily renewal and the sleep-wake cycle. *Clinical Science (London, England : 1979)*. 1983;65(6):561-567.
33. Atkinson G, Edwards B, Reilly T, Waterhouse J. Exercise as a synchroniser of human circadian rhythms: an update and discussion of the methodological problems. *Eur J Appl Physiol*. 2007;99(4):331-341.
34. Lee H, Kim S, Kim D. Effects of exercise with or without light exposure on sleep quality and hormone responses. *J Exerc Nutr & Biochem*. 2014;18(3):293-299.
35. Wirojanan J, Jacquemont S, Diaz R, et al. The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X

- 1
2
3 syndrome. *Journal of clinical sleep medicine : JCSM : official publication of the*
4 *American Academy of Sleep Medicine.* 2009;5(2):145-150.
- 5 36. Rossignol DA, Frye RE. Melatonin in autism spectrum disorders: a systematic
6 review and meta-analysis. *Dev Med & Child Neuro.* 2011;53(9):783-792.
- 7 37. Krakowiak P, Goodlin-Jones B, Hertz-Picciotto I, Croen LA, Hansen RL. Sleep
8 problems in children with autism spectrum disorders, developmental delays, and
9 typical development: a population-based study. *J Sleep Res.* 2008;17(2):197-206.
- 10 38. Veatch OJ, Goldman SE, Adkins KW, Malow BA. Melatonin in Children with
11 Autism Spectrum Disorders: How Does the Evidence Fit Together? *J Nat and Sci.*
12 2015;1(7):125.
- 13 39. Goldman SE, Adkins KW, Calcutt MW, et al. Melatonin in children with autism
14 spectrum disorders: endogenous and pharmacokinetic profiles in relation to sleep.
15 *J Autism Dev Disord.* 2014;44(10):2525-2535.
- 16 40. Arendt J. Importance and relevance of melatonin to human biological rhythms. *J*
17 *Neuroendocrinol.* 2003;15(4):427-431.
- 18 41. Marrin K, Drust B, Gregson W, Morris CJ, Chester N, Atkinson G. Diurnal
19 variation in the salivary melatonin responses to exercise: relation to
20 exercise-mediated tachycardia. *Eur J of Appl Physiol.* 2011;111(11):2707-2714.
- 21 42. American Psychiatric Association. *Diagnostic and statistical manual of mental*
22 *disorders: DSM-5* Washington, DC: American Psychiatric Association; 2016.
- 23 43. Giannotti F, Cortesi F, Cerquiglini A, Bernabei P. An Open-Label Study of
24 Controlled-Release Melatonin in Treatment of Sleep Disorders in Children with
25 Autism. *J Autism and Dev Disord.* 2006;36(6):741-752.
- 26 44. Petrus C, Adamson SR, Block L, Einarson SJ, Sharifnejad M, Harris SR. Effects
27 of exercise interventions on stereotypic behaviours in children with autism
28 spectrum disorder. *Physiothera Can* 2008;60(2):134-145.
- 29 45. Kern L, Koegel RL, Dyer K, Blew PA, Fenton LR. The effects of physical
30 exercise on self-stimulation and appropriate responding in autistic children. *J*
31 *Autism and Dev Disord.* 1982;12(4):399-419.
- 32 46. Centers of Disease Control and Prevention. Measuring physical activity intensity:
33 Target heart rate and estimated maximum heart rate;
34 <http://www.cdc.gov/physicalactivity/basics/measuring/hearttrate.htm>. Accessed
35 26th March, 2017.
- 36 47. Londeree BR, Moeschberger ML. Effect of Age and Other Factors on Maximal
37 Heart Rate. *Res Q Exerc Sport.* 1982;53(4):297-304.
- 38 48. Pan CY, Chu CH, Tsai CL, Sung MC, Huang CY, Ma WY. The impacts of
39 physical activity intervention on physical and cognitive outcomes in children with
40 autism spectrum disorder. *Autism.* 2016.
- 41 49. Lang R, Koegel LK, Ashbaugh K, Regester A, Ence W, Smith W. Physical
42 exercise and individuals with autism spectrum disorders: A systematic review.
43 *Res Autism Spectr Disord.* 2010;4(4):565-576.
- 44 50. Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire
45 (CSHQ): psychometric properties of a survey instrument for school-aged children.
46 *Sleep.* 2000;23(8):1043-1051.
- 47
48
49
50
51
52
53
54
55
56
57
58
59

- 1
2
3 51. Barreira TV, Schuna JM, Jr., Mire EF, et al. Identifying children's nocturnal sleep
4 using 24-h waist accelerometry. *Med Sci Sports Exerc.* 2015;47(5):937-943.
5
6 52. ActiGraph. What is MVPA and how I can view in ActiLife. 2016;
7 <https://help.theactigraph.com/entries/22148365-What-is-MVPA-and-how-can-I-view-it-in-ActiLife->. Accessed 2nd September,2017.
8
9 53. Schernhammer ES, Kroenke CH, Dowsett M, Folkerd E, Hankinson SE. Urinary
10 6-sulfatoxymelatonin levels and their correlations with lifestyle factors and
11 steroid hormone levels. *J Pineal Res.* 2006;40(2):116-124.
12
13 54. Efird J. Blocked Randomization with Randomly Selected Block Sizes.
14 *International J Enviro Res Public Health.* 2011;8(1):15-20.
15
16 55. Kline CE, Crowley EP, Ewing GB, et al. The effect of exercise training on
17 obstructive sleep apnea and sleep quality: a randomized controlled trial. *Sleep.*
18 2011;34(12):1631-1640.
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 1. Flow chart of the proposed study

For peer review only

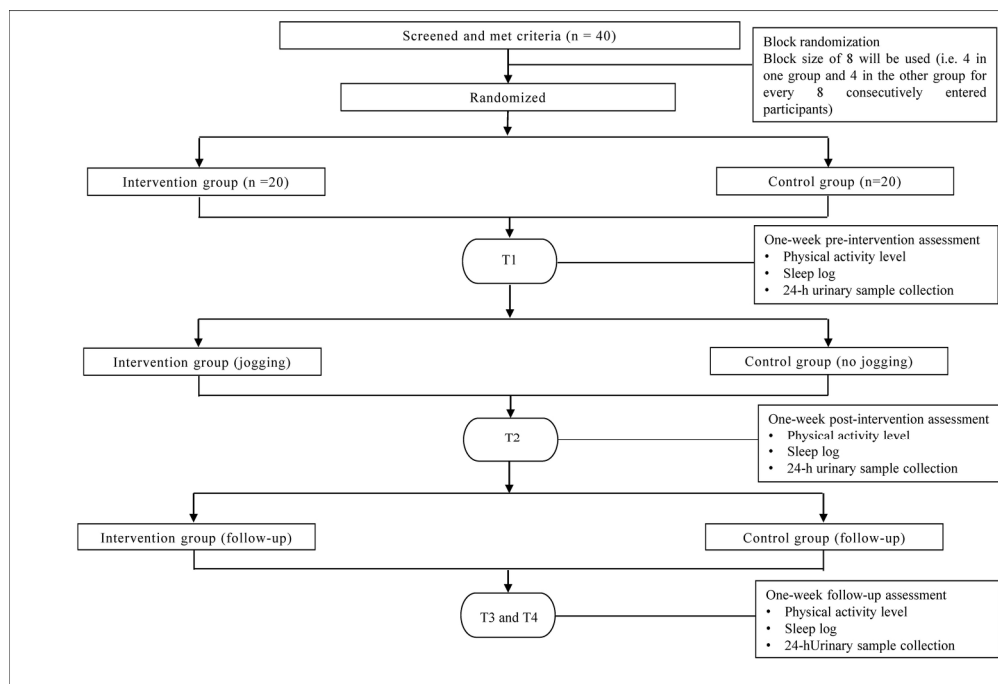


Figure 1. Flow chart of the proposed study

190x142mm (300 x 300 DPI)



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			

1 2 3 4 5 6 7 8 9 10	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
11 12 13 14 15 16		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
17 18 19 20 21 22 23 24	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
25 26 27 28 29	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
30 31 32		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P.9-10
33 34 35 36 37 38		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
39	Methods: Monitoring			
40 41 42 43 44 45 46 47 48	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
49 50 51 52 53 54 55 56 57 58 59 60		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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020944.R2
Article Type:	Protocol
Date Submitted by the Author:	08-Feb-2018
Complete List of Authors:	Tse, Choi Yeung Andy; Department of Health and Physical Education Lee, Paul; Hong Kong Polytechnic University, School of Nursing Zhang, Jihui; The Chinese University of Hong Kong, Department of Psychiatry Lai, Elvis, W.H.; The Hong Kong Castle Peak Hospital, Department of Psychiatry
Primary Subject Heading:	Research methods
Secondary Subject Heading:	Sports and exercise medicine
Keywords:	Physical activity, Sleep, Children with autism spectrum disorders, Melatonin

SCHOLARONE™
Manuscripts

Only

TITLE PAGE WITH AUTHOR INFORMATION

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 modelAndy C.Y. Tse¹, Paul H. Lee², J. Zhang³, Elvis W.H. Lai⁴¹Department of Health and Physical Education, The Education University of Hong Kong, Hong Kong China²School of Nursing, The Hong Kong Polytechnic University, Hong Kong China³Department of Psychiatry, The Chinese University of Hong Kong, Hong Kong China⁴Department of Psychiatry, The Hong Kong Castle Peak Hospital

Dr. Andy C.Y. Tse [Corresponding Author]

Rm D4-2/F-03, Block D4,
10 Lo Ping Road, Tai Po, N.T., Hong Kong.

Tel: (852) 29488074

Email: andytscy@eduhk.hk

Dr. J. Zhang

G/F Multicentre, Tai Po Hospital, Tai Po, N.T.,
Hong Kong

Tel: (852) 26475321

Email: jihui.zhang@cuhk.edu.hk

Dr. Paul H. Lee

School of Nursing, GH527
Hong Kong Polytechnic University, Hung Hom,
Kowloon, Hong Kong

Tel: (852) 3400 8275

Email: paul.h.lee@polyu.edu.hk

Dr. Elvis W.H Lai

15, Ching Chung Koon Road,
Tuen Mun, N.T., Hong Kong

Tel: (852)24567111

Email: lwh041@ha.org.hk

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.

For peer review only

Introduction

According to the World Health Organization, autism spectrum disorder (ASD) is defined as a group of complex brain development disorders¹ 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 ASD³. Compared with TD children, preference of sleep disturbance is higher in children with ASD,^{4,5} evident in 40% to 80 % of children with ASD⁵⁻⁸ 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 performance¹³ and memory consolidation¹⁴) and daily functioning (e.g., increased stereotypy¹⁵ and overall autistic behavior¹⁶) 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 al²⁶ examined the effects of acute and regular exercise on sleep. It indicated that both

1
2
3 acute and regular exercise could increase total sleep time, improve sleep onset latency
4 and sleep efficiency, reduce rapid eye movement, and promote slow wave sleep.

5 While physical activity is shown to be beneficial to sleep quality, most studies
6 were conducted on healthy adults and good sleepers^{25,27} and only a few similar studies
7 were conducted in the child population.^{28,29} Recently, two small-scale studies have
8 been conducted to explore the impact of physical activity on sleep in children with
9 ASD.^{30,31} One of them (Wachob et al³⁰) used accelerometers to objectively measure
10 the subjects' physical activity level and their sleeping quality (i.e., sleep efficiency
11 (SE) and wake after sleep onset (WASO)) in ten children with ASD. Results revealed
12 a negative relationship between average time spent in moderate-to-vigorous physical
13 activity (MVPA) and average WASO time, and a positive relationship between
14 average sedentary time and average SE percentages. The other study (Brand et al³¹)
15 also found a similar association between PA and sleep quality. In the study,
16 researchers asked ten children with ASD to participate in thrice-weekly 30-min
17 sessions of bicycle workout followed by 30 min of training in coordination and
18 balance. A sleep-encephalography (sleep-EEG) device was applied to objectively
19 measure several sleep parameters (e.g., total sleep time, sleeping period, and sleep
20 efficiency). Results showed that the physical exercises had increased sleep efficiency,
21 shortened sleep onset latency, and decreased WASO time.³¹

22 Given the aforementioned benefits of physical activity on sleep, it is suggested
23 that physical activity may be an alternative treatment for sleep disturbances in
24 children with ASD.^{30,31} However, whether the intervention effect can be sustained
25 remains unknown. Also, precautions should be taken when interpreting the results
26 because of the small sample sizes and lack of control conditions.^{30,31} More
27 importantly, the mechanism of how physical activity impacts on sleep remains unclear,
28 particularly in children with ASD. Indeed, it is important to understand such
29 mechanisms to design an effective physical activity intervention for sleep
30 disturbances among children with ASD. In the normal population, several mechanism
31 models (e.g., thermoregulatory hypothesis,³² body restoration theory³³ and
32 melatonin-mediated mechanism³⁴) were proposed. Of particular interest to this study
33 is the melatonin-mediated mechanism. In this mechanism model, it is suggested that
34 physical activity could affect circadian rhythm by altering melatonin level.³⁵

35 Melatonin, a natural hormone produced by the pineal gland, serves as a key
36 regulator of the circadian rhythm and promotes sleep onset and sleep maintenance.³⁶
37 Secretion of melatonin normally increases shortly after darkness, peaks in the middle
38 of the night and falls slowly during early morning hours.³⁷ This hormonal response
39 allows for maintaining a normal circadian rhythm and sleeping through night.
40 Compared with TD children, melatonin levels appeared to be lower^{5,38} in some
41 children with ASD, although no such difference was shown in other studies.^{39,40} To
42 counter this melatonin deficit, supplemental melatonin is commonly used to treat
43 insomnia in children with ASD.⁴¹ Recently, researchers suggested that melatonin
44 levels could also be moderated by physical activity.⁴² In an experiment, Marrin et al⁴²
45 asked seven healthy participants to complete a moderate intensity morning cycling
46 exercise with their salivary melatonin concentration measured at different time points:
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 baseline, during exercise, after exercise and recovery. Results showed that
4 participants' melatonin levels significantly increased during and after exercise
5 compared with those at baseline and recovery. However, to our best knowledge, none
6 of the previous studies have examined the relationship between physical activity,
7 melatonin, and sleep, particularly in children with ASD. Therefore, the mechanism of
8 how physical activity impacts on sleep in children with ASD remains a question. Here,
9 we present the study protocol which will begin enrolment in February 2018.
10
11

12 **Objectives**

13 The purpose of this study is twofold: (1) to examine the associations between physical
14 activity, melatonin, and sleep quality in children with ASD, which ultimately lead to
15 an answer of how physical activity impacts on sleep in children with ASD from the
16 perspective of melatonin-mediated mechanism model; and (2) to examine the possible
17 sustained effect of physical activity on improved melatonin secretion and sleep
18 quality in children with ASD. It is hypothesized that physical activity could improve
19 sleep quality in children with ASD by increasing their endogenous melatonin level,
20 and these beneficial effects could be sustained.
21
22
23

24 **Methods/design**

25 **Study design**

26 The purposed study will be a parallel, two-group randomized controlled trial (RCT)
27 design with equal allocation ratio to the intervention group and control group (1:1). A
28 flow diagram of the study is given in Fig. 1.
29
30

31
32 <Fig. 1 is inserted here>
33

34 **Data collection**

35 Each participant will attend 4 one-week assessments, where we will assess their
36 habitual sleep patterns: before the intervention (T1), immediately after the
37 intervention (T2), 6 weeks after and 12 weeks after the intervention (T3 and T4). T1
38 and T2 represent the pre-and post-intervention. T3 serves as the 6-week follow-up and
39 T4 serves as the 12-week follow-up.
40
41
42

43 **Participant**

44 Children will be screened with the following inclusion criteria.

45 The inclusion criteria are: (1) age 9 –12 years; (2) pre-puberty or early puberty as
46 indicated by Tanner stage I or II ; (3) ASD diagnosis from a physician based on
47 Diagnostic and Statistical Manual of Mental Disorders, 5th edition, (DSM-V);⁴³ (4)
48 non-verbal IQ over 40; (5) the ability to follow instructions; (6) physically able to
49 participate in the intervention; (7) no additional regular participation in physical
50 exercise other than school physical education classes for at least 6 months prior to the
51 study; (8) no concurrent medication for at least 6 months before the study or any prior
52 melatonin treatment; and (9) have sleep difficulties, including sleep onset insomnia
53
54
55
56
57
58
59

1
2
3 and frequent and prolonged nightwaking and/or early morning awakenings (see
4 Giannotti et al⁴⁴ for definitions) reported by parents.

5 Exclusion criteria are: (1) with one or co-morbid psychiatric disorders as established
6 by a structured interview based on DSM-V; (2) with other medical conditions that
7 limit their physical activity capacities (e.g., asthma, seizure, cardiac disease); and (3)
8 with a complex neurologic disorder (e.g., epilepsy, phenylketonuria, fragile X
9 syndrome, tuberous sclerosis). All screening will be carried out by a psychologist and
10 a physician.
11
12

13 **Intervention**

14 Intervention group: The intervention is a 12-week jogging program consisting of 24
15 sessions (two sessions per week, 30 min per session) in a hall/gymnasium of each
16 participating school. 24 sessions are selected based on previous studies involving
17 jogging in this population.^{45,46} Each intervention session will be conducted in the
18 morning by a trained research assistant and student helpers. The staff-to-participant
19 ratio for both groups is 1:2 to 1:1 depending on the attendance. The research assistant
20 and student helpers must be majoring in physical education or adapted physical
21 education and must have experience with children with ASD. Each intervention
22 session will be conducted in an identical format, comprising three activities: warm-up
23 (5 min), jogging (20 min), and cool-down (5 min). In the jogging activity, participants
24 will be asked to jog around an activity circuit (57m x 50m) marked with 4 red cones
25 together with the research staff. The activity circuit will be set up in an outdoor sports
26 ground or indoor gymnasium depending on the weather and the arrangement of the
27 participating schools. Participants are required to run at a moderate intensity level.
28 The intensity level of jogging will be objectively measured by asking participants to
29 wear heart rate monitor (Polar H1) during each jogging session. According to the
30 Center for Disease Control and Prevention,⁴⁷ MVPA is achieved with a target heart
31 rate above 60% of the maximum heart rate (subtracting the participant's age from
32 220⁴⁸). Considering the low physical activity level of children with ASD,⁴⁹ physical
33 activity with a heart rate (HR) above 50% of the maximum heart rate should be
34 considered MVPA. The intervention is considered successful if the participants can
35 maintain their target heart rate (THR) for 15 minutes or above throughout the jogging
36 session. The Polar devices will also be used to calculate how long the participants are
37 within their THR (i.e. 50% or above the maximum HR). Meanwhile, jogging is
38 chosen because it is one of the most common exercises studied with regards to ASD⁵⁰
39 and can serve as endurance training, which is shown to be beneficial for sleep.²⁷
40 Participants will be positively reinforced verbally with compliments for their efforts
41 in jogging and their daily and weekly improvements will be visualized through graphs
42 and scales kept at home in the child's bedroom.³¹ After the intervention, the
43 participants will be required to follow their normal daily routine without participating
44 in any additional physical activity/exercise program (except the 60-minute weekly
45 physical education (PE) classes provided by school) throughout the follow-up period
46 (T2-T4).
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Control group: Participants in the control group will receive no physical intervention
4 (i.e. jogging program) and will be required to follow their daily routine without
5 participating in any additional physical activity/exercise program except the regular
6 PE classes throughout the whole study period (T1-T4).
7

8 9 **Study measures**

10 Before the initial assessment, participants' parents will be asked to provide
11 demographic data and a brief developmental history. Both participants and their
12 parents will undertake T1, T2, T3, and T4, where the following measurements will be
13 carried out.
14

15 16 *Primary outcome measures*

17 Sleep: Four sleep parameters including sleep onset latency (length of time taken to
18 fall asleep, expressed in minutes, SOL); sleep efficiency (actual sleep time divided by
19 time in bed, expressed as a percent, SE); wake after sleep onset (length of time they
20 were awake after sleep onset, expressed in minutes, WASO) and sleep duration (total
21 sleep in hours and minutes, SD) will be objectively measured by a GT3X
22 accelerometer.³⁰ Participants will be asked to wear the device on their non-dominant
23 wrist for seven consecutive days (Monday to Sunday). Non-wear time is defined as 60
24 min of consecutive zeros with a 2-min spike tolerance.³⁰ The night (2200–0700) will
25 be considered invalid if the wear time is less than 8 h and will be excluded from the
26 analysis. In addition, participants' sleep patterns will be logged by their parents using
27 Children's Sleep Habits Questionnaire (CSHQ), which is a validated 45-item
28 parent-administered questionnaire to examine sleep patterns of young children,⁵¹
29 including children with ASD.³⁰ Parents will be asked to recall specific sleep patterns
30 of their children over the assessment weeks (T1, T2, T3, and T4). Finally, parents will
31 also be asked to log the bedtime, sleep latency, sleep start, sleep end, wake-up time
32 and assumed sleep length in a sleep log for the whole assessment week. The sleep log
33 is used as part of the refined sleep algorithm (RSA) in Actigraph data analysis to
34 identify nocturnal sleep and to exclude non-wear time/wakefulness.⁵²
35

36 Melatonin level: All participants will be instructed to collect a 24-h urine sample.
37 6-sulfatoxymelatonin, a creatinine-adjusted morning urinary melatonin and
38 representative of melatonin level, will be measured from the sample.⁵³ The weekend
39 has been chosen to allow the participants to stay at home for sample collection. All
40 urine samples will be collected using 24-h urine bottles containing 0.1L of 0.5M
41 hydrochloric acid as a preservative. Upon completion, the research assistant will
42 immediately collect the urine sample from the participants' apartments and bring it to
43 the Sleep Assessment Unit of Sha Tin Hospital. The urine sample will be stored at
44 –80° before analysis.
45
46
47
48
49
50

51 52 *Secondary outcome measure*

53 Physical activity level: The physical activity level of the participants will also be
54 measured as secondary data to examine its relationship to sleep as suggested by
55 previous studies.^{5,30} It will be measured using the same accelerometer (i.e. GT3X).
56
57
58
59
60

1
2
3 The data used for analysis are the times spent in sedentary activity and MVPA based
4 on the default energy expenditure algorithm in the accelerometer device.⁵⁴ The day
5 (0700–2100) will be considered invalid if the wear time is less than 10 h and will then
6 be excluded from the analysis.
7

8 9 **Sample size**

10 A pilot study on improving sleeping quality among children with ASD³¹ showed that
11 physical activity had a notable effect (corresponding to a Cohen's *d* of about 1.0) on
12 improving sleeping quality. Given this effect size, a sample of 16 participants per
13 group is required to achieve a power of 80% and a level of significance of 5%.
14 Assuming 20% attrition, 20 participants will be recruited per group. They will be
15 recruited from 3 local special schools with existing research links to the PI and his
16 collaborators. More special schools will be invited to join the research project if there
17 is inadequate participant enrolment among the 3 participating schools.
18
19

20 21 **Randomization**

22 After screening, all the eligible participants will be randomly assigned to either the
23 intervention or control group. To ensure equal allocation ratios for the intervention
24 and control groups, block randomization⁵⁵ will be used. A block size of 8 will be used
25 in the proposed study (i.e. 4 in one group and 4 in the other group for every 8
26 consecutively entered participants). The block randomization process will be done by
27 a trained research assistant.
28
29

30 31 **Blinding**

32 The person responsible for analyzing the sleep parameters and melatonin level will be
33 blinded for the group assignment.
34
35

36 37 **Ethics and dissemination**

38 Prior to the study, information about the study will be provided to all participants and
39 their parents with the distribution of written consent form (see Appendix I). The
40 consent form will be collected from the participants and their parents by a physical
41 education teacher of each participating school. All participants and their parents will
42 be informed that withdrawal at any time will not result in any adverse consequences.
43 All sets of data will be encrypted with passwords. To prevent any leakage of sensitive
44 information, only the PI and his collaborators will have access to the datasets. Ethical
45 approval was obtained from the Human Research Ethics Committee, The Education
46 University of Hong Kong (reference number 2016-2017-0155). Results of the study
47 will be published in a peer-reviewed journal. Findings of the study will also be shared
48 with other university and non-governmental organizations in HK that specialize in
49 Autism by means of a formal dissemination seminar.
50
51

52 53 **Statistical methods**

54 All statistical analyses will be conducted using SPSS (version 23.0) for Windows
55 (SPSS Inc., Chicago, IL, USA) to assess sleep; multilevel regression or a generalized
56
57
58
59

1
2
3 estimating equation (GEE) will be used to assess the effects of the physical activity
4 intervention, time effect, and their interaction effect on sleep outcomes and melatonin
5 level outcomes. Two potential confounding variables (i.e., average time spent in daily
6 sedentary activity and average time spent in daily MVPA) will be used as covariates
7 because they may be closely related to sleep quality.⁵⁶ The effect size will be reported
8 as a Cohen's *d*. Bonferroni adjustment will be used to control for possible type I error
9 inflation caused by multiple comparisons. Intention to treat approach will be used to
10 handle any missing data.
11
12

13 14 **Discussion**

15 This study is the first randomized control trial examining the effectiveness of physical
16 activity intervention on sleep disorder among children with ASD. In addition, it is
17 also the first study investigating whether the melatonin-mediated mechanism would
18 be a possible underlying pathway by which physical activity impacts on sleep in
19 children with ASD. The results obtained in this study have two significant impacts.
20 First, if the intervention is effective, doctors can prescribe physical activity to children
21 with ASD who are not able to take drugs based on the concept of 'exercise is
22 medicine'. Second, the melatonin-mediated mechanism investigated in this study
23 could lead to further investigation of the interaction between physical activity and
24 melatonin in any population suffering from sleep disturbance. Such research could
25 include the comparison of the effectiveness of physical activity and supplemental
26 melatonin interventions on sleep quality in populations suffering from sleep disorders
27 and the manipulation of different physical activity intervention parameters (e.g.,
28 intensity, frequency, and time) on melatonin level and sleep quality.
29
30
31
32

33 34 **Acknowledgement**

35 This study is funded by the Early Career Scheme of Research Grants Council,
36 University Grants Committee of HKSAR Government (#28602517).
37

38 39 **Contributorship Statement**

40 AT and JZ conceived of the study and designed the study protocol, PL assisted in
41 defining the statistical analysis and provided input for the manuscript. EL provided
42 critical comment on implementation of participant's screening protocol. All authors
43 contributed to, read drafts of and approved the final manuscript.
44

45 46 **Competing interests**

47 The authors declare there is no competing interests with respect to the research,
48 authorship, and/or publication of this article.
49

50 51 **Funding**

52 This work is supported by the Early Career Scheme of Research Grant Council (Grant
53 number: 28602517).
54
55
56
57
58
59

References

References

1. World Health Organization. Questions and answers about autism spectrum disorders (ASD). 2016; <http://www.who.int/features/qa/85/en/>. Accessed 12th August, 2016.
2. Rapin I, Tuchman RF. Autism: definition, neurobiology, screening, diagnosis. *Pediatr Clin North Am*. 2008;55(5):1129-1146.
3. Child Assessment Service Department of Health, HKSAR. Child assessment service epidemiology and research bulletin. 2007. <https://www.dhcas.gov.hk/file/caser/CASER3.pdf>. Accessed 13th February, 2016.
4. Cortesi F, Giannotti F, Ivanenko A, Johnson K. Sleep in children with autistic spectrum disorder. *Sleep Med*. 2010;11(7):659-664.
5. Liu X, Hubbard JA, Fabes RA, Adam JB. Sleep disturbances and correlates of children with Autism Spectrum Disorders. *Child Psy Human Dev*. 2006;37(2):179-191.
6. Sivertsen B, Posserud M-B, Gillberg C, Lundervold AJ, Hysing M. Sleep problems in children with autism spectrum problems: a longitudinal population-based study. *Autism*. 2012;16(2):139-150.
7. Orsmond GI, Seltzer MM. Siblings of individuals with autism spectrum disorders across the life course. *Ment Retard Dev Disabil Res Rev*. 2007;13(4):313-320.
8. Goldman SE, Richdale AL, Clemons T, Malow BA. Parental sleep concerns in Autism Spectrum Disorders: Variations from childhood to adolescence. *J Autism and Dev Disord*. 2012;42(4):531-538.
9. Fricke-Oerkermann L, Pluck J, Schredl M, et al. Prevalence and course of sleep problems in childhood. *Sleep*. 2007;30(10):1371-1377.
10. Calhoun SL, Fernandez-Mendoza J, Vgontzas AN, Liao D, Bixler EO. Prevalence of insomnia symptoms in a general population sample of young children and preadolescents: gender effects. *Sleep Med*. 2014;15(1):91-95.
11. Souders MC, Mason TB, Valladares O, et al. Sleep behaviors and sleep quality in children with autism spectrum disorders. *Sleep*. 2009;32(12):1566-1578.
12. Richdale AL, Schreck KA. Sleep problems in autism spectrum disorders: prevalence, nature, & possible biopsychosocial aetiologies. *Sleep Med Rev*. 2009;13(6):403-411.
13. Dewald JF, Meijer AM, Oort FJ, Kerkhof GA, Bögels SM. The influence of sleep quality, sleep duration and sleepiness on school performance in children and adolescents: A meta-analytic review. *Sleep Med Rev*. 2010;14(3):179-189.
14. Curcio G, Ferrara M, De Gennaro L. Sleep loss, learning capacity and academic performance. *Sleep Med Rev*. 2006;10(5):323-337.
15. Mazurek MO, Sohl K. Sleep and behavioral problems in children with Autism Spectrum Disorder. *J Autism Dev Disord*. 2016;46(6):1906-1915.
16. Schreck KA, Mulick JA, Smith AF. Sleep problems as possible predictors of intensified symptoms of autism. *Res Dev Disabil*. 2004;25(1):57-66.

17. Doo S, Wing YK. Sleep problems of children with pervasive developmental disorders: correlation with parental stress. *Dev Med & Child Neuro.* 2006;48(8):650-655.
18. Yu XT, Lam HS, Au CT, Chan SHY, Chan DFY, Li AM. Extended parent-based behavioural education improves sleep in children with Autism Spectrum Disorder. *Hong Kong J Ped.* 2015;20(4):219-225.
19. Richdale AL. Sleep problems in autism: prevalence, cause, and intervention. *Dev Child Neuro.* 1999;41(01):60-66.
20. Malow BA, Byars K, Johnson K, et al. A practice pathway for the identification, evaluation, and management of insomnia in children and adolescents with Autism Spectrum Disorders. *Pediatrics.* 2012;130(2):106-124.
21. Bramble D. Consumer opinion concerning the treatment of a common sleep problem. *Child Care Health Dev.* 1996;22(6):355-366.
22. Vriend JL, Corkum PV, Moon EC, Smith IM. Behavioral interventions for sleep problems in children with Autism Spectrum Disorders: Current findings and future directions. *J Ped Psycho.* 2011;36(9):1017-1029.
23. Weiskop S, Richdale A, Matthews J. Behavioural treatment to reduce sleep problems in children with autism or fragile X syndrome. *Dev Med Child Neuro.* 2005;47(2):94-104.
24. Lancioni GE, O'Reilly MF, Basili G. Review of strategies for treating sleep problems in persons with severe or profound mental retardation or multiple handicaps. *Am J Mental Retard.* 1999;104(2):170-186.
25. Driver HS, Taylor SR. Exercise and sleep. *Sleep Med Rev.* 2000;4(4):387-402.
26. Kredlow MA, Capozzoli MC, Hearon BA, Calkins AW, Otto MW. The effects of physical activity on sleep: a meta-analytic review. *J Behav Med.* 2015;38(3):427-449.
27. Youngstedt SD, O'Connor PJ, Dishman RK. The effects of acute exercise on sleep: a quantitative synthesis. *Sleep.* 1997;20(3):203-214.
28. Lundahl A, Nelson TD, Van Dyk TR, West T. Psychosocial Stressors and Health Behaviors: Examining Sleep, Sedentary Behaviors, and Physical Activity in a Low-Income Pediatric Sample. *Clinical Pediatrics.* 2013;52(8):721-729.
29. Stone MR, Stevens D, Faulkner GEJ. Maintaining recommended sleep throughout the week is associated with increased physical activity in children. *Prev Med.* 2013;56 (2):112-117.
30. Wachob D, Lorenzi DG. Brief Report: Influence of Physical Activity on Sleep Quality in Children with Autism. *J Autism Dev Disord.* 2015;45(8):2641-2646.
31. Brand S, Jossen S, Holsboer-Trachsler E, Pühse U, Gerber M. Impact of aerobic exercise on sleep and motor skills in children with autism spectrum disorders – a pilot study. *Neuropsychiatric Disease and Treatment.* 2015;11:1911-1920.
32. McGinty D, Szymusiak R. Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. *Trends Neurosci.* 1990;13(12):480-487.
33. Adam K, Oswald I. Protein synthesis, bodily renewal and the sleep-wake cycle. *Clinical Science (London, England : 1979).* 1983;65(6):561-567.

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
 - 17
 - 18
 - 19
 - 20
 - 21
 - 22
 - 23
 - 24
 - 25
 - 26
 - 27
 - 28
 - 29
 - 30
 - 31
 - 32
 - 33
 - 34
 - 35
 - 36
 - 37
 - 38
 - 39
 - 40
 - 41
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
34. Atkinson G, Edwards B, Reilly T, Waterhouse J. Exercise as a synchroniser of human circadian rhythms: an update and discussion of the methodological problems. *Eur J Appl Physiol*. 2007;99(4):331-341.
35. Lee H, Kim S, Kim D. Effects of exercise with or without light exposure on sleep quality and hormone responses. *J Exerc Nutr & Biochem*. 2014;18(3):293-299.
36. Wirojanan J, Jacquemont S, Diaz R, et al. The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X syndrome. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine*. 2009;5(2):145-150.
37. Rossignol DA, Frye RE. Melatonin in autism spectrum disorders: a systematic review and meta-analysis. *Dev Med & Child Neuro*. 2011;53(9):783-792.
38. Krakowiak P, Goodlin-Jones B, Hertz-Picciotto I, Croen LA, Hansen RL. Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study. *J Sleep Res*. 2008;17(2):197-206.
39. Veatch OJ, Goldman SE, Adkins KW, Malow BA. Melatonin in Children with Autism Spectrum Disorders: How Does the Evidence Fit Together? *J Nat and Sci*. 2015;1(7):125.
40. Goldman SE, Adkins KW, Calcutt MW, et al. Melatonin in children with autism spectrum disorders: endogenous and pharmacokinetic profiles in relation to sleep. *J Autism Dev Disord*. 2014;44(10):2525-2535.
41. Arendt J. Importance and relevance of melatonin to human biological rhythms. *J Neuroendocrinol*. 2003;15(4):427-431.
42. Marrin K, Drust B, Gregson W, Morris CJ, Chester N, Atkinson G. Diurnal variation in the salivary melatonin responses to exercise: relation to exercise-mediated tachycardia. *Eur J of Appl Physiol*. 2011;111(11):2707-2714.
43. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders: DSM-5* Washington, DC: American Psychiatric Association; 2016.
44. Giannotti F, Cortesi F, Cerquiglini A, Bernabei P. An Open-Label Study of Controlled-Release Melatonin in Treatment of Sleep Disorders in Children with Autism. *J Autism and Dev Disord*. 2006;36(6):741-752.
45. Petrus C, Adamson SR, Block L, Einarson SJ, Sharifnejad M, Harris SR. Effects of exercise interventions on stereotypic behaviours in children with autism spectrum disorder. *Physiothera Can* 2008;60(2):134-145.
46. Kern L, Koegel RL, Dyer K, Blew PA, Fenton LR. The effects of physical exercise on self-stimulation and appropriate responding in autistic children. *J Autism and Dev Disord*. 1982;12(4):399-419.
47. Centers of Disease Control and Prevention. Measuring physical activity intensity: Target heart rate and estimated maximum heart rate; <http://www.cdc.gov/physicalactivity/basics/measuring/hearttrate.htm>. Accessed 26th March, 2017.
48. Londeree BR, Moeschberger ML. Effect of Age and Other Factors on Maximal Heart Rate. *Res Q Exerc Sport*. 1982;53(4):297-304.

- 1
- 2
- 3 49. Pan CY, Chu CH, Tsai CL, Sung MC, Huang CY, Ma WY. The impacts of
- 4 physical activity intervention on physical and cognitive outcomes in children with
- 5 autism spectrum disorder. *Autism*. 2016.
- 6
- 7 50. Lang R, Koegel LK, Ashbaugh K, Regester A, Ence W, Smith W. Physical
- 8 exercise and individuals with autism spectrum disorders: A systematic review.
- 9 *Res Autism Spectr Disord*. 2010;4(4):565-576.
- 10
- 11 51. Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire
- 12 (CSHQ): psychometric properties of a survey instrument for school-aged children.
- 13 *Sleep*. 2000;23(8):1043-1051.
- 14
- 15 52. Barreira TV, Schuna JM, Jr., Mire EF, et al. Identifying children's nocturnal sleep
- 16 using 24-h waist accelerometry. *Med Sci Sports Exerc*. 2015;47(5):937-943.
- 17
- 18 53. ActiGraph. What is MVPA and how I can view in ActiLife. 2016;
- 19 <https://help.theactigraph.com/entries/22148365-What-is-MVPA-and-how-can-I-view-it-in-ActiLife->. Accessed 2nd September,2017.
- 20
- 21 54. Schernhammer ES, Kroenke CH, Dowsett M, Folkerd E, Hankinson SE. Urinary
- 22 6-sulfatoxymelatonin levels and their correlations with lifestyle factors and
- 23 steroid hormone levels. *J Pineal Res*. 2006;40(2):116-124.
- 24
- 25 55. Efrid J. Blocked Randomization with Randomly Selected Block Sizes.
- 26 *International J Enviro Res Public Health*. 2011;8(1):15-20.
- 27
- 28 56. Kline CE, Crowley EP, Ewing GB, et al. The effect of exercise training on
- 29 obstructive sleep apnea and sleep quality: a randomized controlled trial. *Sleep*.
- 30 2011;34(12):1631-1640.
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
- 39
- 40
- 41
- 42
- 43
- 44
- 45
- 46
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 1. Flow chart of the proposed study

For peer review only

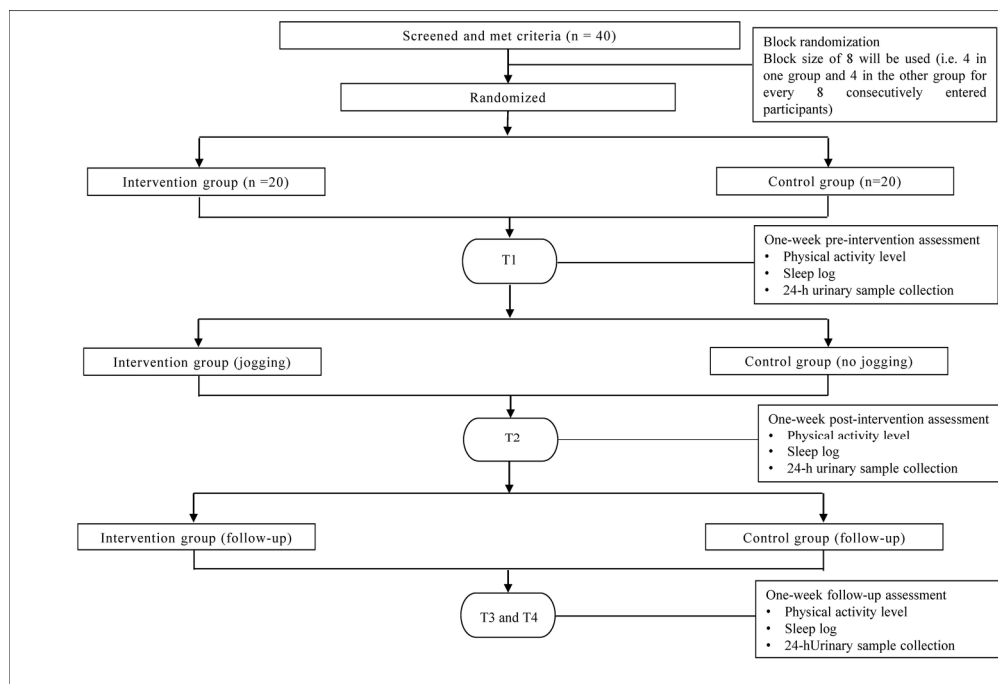


Figure 1. Flow chart of the proposed study

190x142mm (300 x 300 DPI)



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			

1 2 3 4 5 6 7 8 9 10	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
11 12 13 14 15 16		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
17 18 19 20 21 22 23 24	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
25 26 27 28 29	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
30 31 32		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P.9-10
33 34 35 36 37 38		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
39	Methods: Monitoring			
40 41 42 43 44 45 46 47 48	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
49 50 51 52 53 54 55 56 57 58 59 60		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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" 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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020944.R3
Article Type:	Protocol
Date Submitted by the Author:	10-Mar-2018
Complete List of Authors:	Tse, Choi Yeung Andy; Department of Health and Physical Education Lee, Paul; Hong Kong Polytechnic University, School of Nursing Zhang, Jihui; The Chinese University of Hong Kong, Department of Psychiatry Lai, Elvis, W.H.; The Hong Kong Castle Peak Hospital, Department of Psychiatry
Primary Subject Heading:	Research methods
Secondary Subject Heading:	Sports and exercise medicine
Keywords:	Physical activity, Sleep, Children with autism spectrum disorders, Melatonin

SCHOLARONE™
Manuscripts

Only

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**

Andy C.Y. Tse¹, Paul H. Lee², J. Zhang³, Elvis W.H. Lai⁴

¹Department of Health and Physical Education, The Education University of Hong Kong, Hong Kong
China

²School of Nursing, The Hong Kong Polytechnic University, Hong Kong China

³Department of Psychiatry, The Chinese University of Hong Kong, Hong Kong China

⁴Department of Psychiatry, The Hong Kong Castle Peak Hospital

Dr. Andy C.Y. Tse [Corresponding Author]

Rm D4-2/F-03, Block D4,
10 Lo Ping Road, Tai Po, N.T., Hong Kong.

Tel: (852) 29488074

Email: andytcy@eduhk.hk

Dr. J. Zhang

G/F Multicentre, Tai Po Hospital, Tai Po, N.T.,
Hong Kong

Tel: (852) 26475321

Email: jihui.zhang@cuhk.edu.hk

Dr. Paul H. Lee

School of Nursing, GH527
Hong Kong Polytechnic University, Hung Hom,
Kowloon, Hong Kong

Tel: (852) 3400 8275

Email: paul.h.lee@polyu.edu.hk

Dr. Elvis W.H. Lai

15, Ching Chung Koon Road,
Tuen Mun, N.T., Hong Kong

Tel: (852)24567111

Email: lwh041@ha.org.hk

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.

For peer review only

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

1
2
3 Kredlow et al.[26] examined the effects of acute and regular exercise on sleep. Their
4 findings indicated that both acute and regular exercise could increase total sleep time,
5 improve sleep onset latency and sleep efficiency, reduce rapid eye movement, and
6 promote slow wave sleep.
7

8 Although physical activity has been shown to be beneficial to sleep quality, most
9 studies have been conducted on healthy adults and good sleepers[25,27] and only a
10 small number of studies have been conducted in the child population.[28,29] Recently,
11 two small-scale studies were conducted to explore the impact of physical activity on
12 sleep in children with ASD.[30,31] In one study, Wachob et al.[30] used
13 accelerometers to objectively measure subjects' physical activity level and sleeping
14 quality (i.e., sleep efficiency [SE] and wake after sleep onset [WASO]) in 10 children
15 with ASD. The results revealed a negative relationship between the average time
16 spent in moderate-to-vigorous physical activity (MVPA) and average WASO time,
17 and a positive relationship between average sedentary time and average SE
18 percentages. In another study Brand et al.[31] found a similar association between PA
19 and sleep quality. In that study, researchers asked 10 children with ASD to participate
20 in thrice-weekly 30-min sessions of bicycle workout followed by 30 min of
21 coordination and balance training. A sleep-encephalography (sleep-EEG) device was
22 used to objectively measure several sleep parameters (e.g., total sleep time, sleeping
23 period, and SE). The results revealed that physical exercise was associated with
24 increased SE, shortened sleep onset latency, and decreased WASO time.[31]
25
26
27
28

29 Given the benefits of physical activity on sleep, it has been suggested that
30 physical activity may provide an alternative treatment for sleep disturbances in
31 children with ASD.[30,31] However, it remains unclear whether the effect of
32 exercise-based interventions can be sustained. Also, precautions should be taken when
33 interpreting the results because of the small sample sizes and lack of control
34 conditions.[30,31] More importantly, the mechanism of how physical activity impacts
35 on sleep remains unclear, particularly in children with ASD. Indeed, it is important to
36 understand such mechanisms to design an effective physical activity intervention for
37 sleep disturbances among children with ASD. In the normal population, several
38 mechanism models (e.g., the thermoregulatory hypothesis,[32] body restoration
39 theory[33] and melatonin-mediated mechanism[34]) have been proposed. Of
40 particular interest to the current study is the melatonin-mediated mechanism model,
41 which suggests that physical activity affects the circadian rhythm by altering
42 melatonin levels.[35]
43
44
45

46 Melatonin, a natural hormone produced by the pineal gland, functions as a key
47 regulator of the circadian rhythm and promotes sleep onset and sleep maintenance.[36]
48 Secretion of melatonin normally increases shortly after darkness, peaks in the middle
49 of the night and falls slowly during the early morning hours.[37] This hormonal
50 response allows for maintaining a normal circadian rhythm and sleeping through the
51 night. Compared with TD children, melatonin levels have been reported to be
52 lower[5,38] in some children with ASD, although no such difference was shown in
53 two other studies.[39,40] To counter this melatonin deficit, supplemental melatonin is
54 commonly used to treat insomnia in children with ASD.[41] Recently, researchers
55
56
57
58
59
60

1
2
3 suggested that melatonin levels could also be altered by physical activity.[42] In one
4 experiment, Marrin et al.[42] asked seven healthy participants to complete a moderate
5 intensity morning cycling exercise while measuring their salivary melatonin
6 concentration at different time points: baseline, during exercise, after exercise and
7 recovery. The results revealed that participants' melatonin levels significantly
8 increased during and after exercise compared with those at baseline and recovery.
9 However, to the best of our knowledge, no previous studies have examined the
10 relationship between physical activity, melatonin, and sleep, particularly in children
11 with ASD. Therefore, the mechanisms by which physical activity impacts on sleep in
12 children with ASD remains unclear. To examine this question, here we propose a new
13 study protocol, which began enrolment in February 2018.
14
15
16

17 **Objectives**

18 This study protocol has two objectives: (1) to examine the associations between
19 physical activity, melatonin, and sleep quality in children with ASD, to ultimately
20 elucidate how physical activity impacts on sleep in children with ASD, from the
21 perspective of the melatonin-mediated mechanism model; and (2) to examine the
22 possible sustained effect of physical activity on improved melatonin secretion and
23 sleep quality in children with ASD. We hypothesize that physical activity can improve
24 sleep quality in children with ASD by increasing their endogenous melatonin levels,
25 and these beneficial effects can be sustained.
26
27
28
29

30 **Methods/design**

31 **Study design**

32 The proposed study will have a parallel, two-group randomized controlled trial (RCT)
33 design, with equal allocation of participants to the intervention and control groups
34 (1:1). A flow diagram of the study is shown in Fig. 1.
35
36

37 <Fig. 1 is inserted here>
38
39

40 **Data collection**

41 Each participant will attend four 1-week assessments, where we will assess their
42 habitual sleep patterns: before the intervention (T1), immediately after the
43 intervention (T2), 6 weeks after and 12 weeks after the intervention (T3 and T4,
44 respectively). T1 and T2 represent the pre-and post-intervention, respectively. T3
45 serves as the 6-week follow-up and T4 serves as the 12-week follow-up.
46
47

48 **Participants**

49 Children will be screened using the following inclusion criteria.

50 The inclusion criteria are: (1) 9–12 years of age; (2) pre-puberty or early puberty as
51 indicated by Tanner stage I or II ; (3) ASD diagnosis from a physician based on the
52 Diagnostic and Statistical Manual of Mental Disorders, 5th edition, (DSM-V);[43] (4)
53 non-verbal IQ over 40; (5) able to follow instructions; (6) physically able to
54 participate in the intervention; (7) no additional regular participation in physical
55
56
57
58
59
60

1
2
3 exercise other than school physical education classes for at least 6 months prior to the
4 study; (8) no concurrent medication for at least 6 months before the study or any prior
5 melatonin treatment; and (9) sleep difficulties, including sleep onset insomnia and
6 frequent and prolonged nightwaking and/or early morning awakening (see Giannotti
7 et al.[44] for definitions) reported by parents.

8
9 The exclusion criteria are: (1) one or more co-morbid psychiatric disorders, as
10 established by a structured interview based on DSM-V; (2) other medical conditions
11 that limit physical activity capacity (e.g., asthma, seizure, cardiac disease); and (3) a
12 complex neurological disorder (e.g., epilepsy, phenylketonuria, fragile X syndrome,
13 tuberous sclerosis). All screening will be carried out by a psychologist and a
14 physician.
15
16

17 **Intervention**

18
19 Intervention group: The intervention is a 12-week jogging program consisting of 24
20 sessions (two sessions per week, 30 min per session) in a hall/gymnasium of each
21 participating school. The total of 24 sessions was selected based on previous studies
22 involving jogging in this population.[45,46] Each intervention session will be
23 conducted in the morning by a trained research assistant and student helpers. The
24 staff-to-participant ratio for both groups is 1:2 to 1:1 depending on the attendance.
25 The research assistant and student helpers must be majoring in physical education or
26 adapted physical education and must have experience with children with ASD. Each
27 intervention session will be conducted in an identical format, comprising three
28 activities: warm-up (5 min), jogging (20 min), and cool-down (5 min). In the jogging
29 activity group, participants will be asked to jog around an activity circuit (57 m × 50
30 m) marked with four red cones together with the research staff. The activity circuit
31 will be set up in an outdoor sports ground or indoor gymnasium depending on the
32 weather and the arrangement of the participating schools. Participants are required to
33 run at a moderate intensity level. The intensity level of jogging will be objectively
34 measured by asking participants to wear a heart rate monitor (Polar H1) during each
35 jogging session. According to the Center for Disease Control and Prevention,[47]
36 MVPA is achieved with a target heart rate above 60% of the maximum heart rate
37 (subtracting the participant's age from 220)[48]. Considering the low physical activity
38 level of children with ASD,[49] physical activity with a heart rate (HR) above 50% of
39 the maximum heart rate should be considered MVPA. The intervention is considered
40 successful if the participants can maintain their target heart rate (THR) for 15 minutes
41 or above throughout the jogging session. A Polar device will also be used to calculate
42 how long the participants are within their THR (i.e., 50% or above the maximum HR).
43 Meanwhile, jogging is chosen because it is one of the most common exercises studied
44 with regard to ASD[50] and can serve as endurance training, which is shown to be
45 beneficial for sleep.[27] Participants will be positively reinforced verbally with
46 compliments for their efforts in jogging and their daily and weekly improvements will
47 be visualized through graphs and scales kept at home in the child's bedroom.[31]
48 After the intervention, the participants will be required to follow their normal daily
49 routine without participating in any additional physical activity/exercise program
50
51
52
53
54
55
56
57
58
59
60

(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

1
2
3 Physical activity level: The physical activity level of the participants will also be
4 measured as secondary data to examine its relationship to sleep, as suggested by
5 previous studies.[5,30] This measurement will be conducted using the same
6 accelerometer (i.e., GT3X). The data used for analysis are the times spent in sedentary
7 activity and MVPA based on the default energy expenditure algorithm in the
8 accelerometer device.[54] The day (0700–2100) will be considered invalid if the wear
9 time is less than 10 h and will then be excluded from the analysis.
10
11

12 **Sample size**

13
14 A pilot study on improving sleeping quality among children with ASD[31] revealed
15 that physical activity had a notable effect (corresponding to a Cohen's *d* of
16 approximately 1.0) on improving sleeping quality. Given this effect size, a sample of
17 16 participants per group is required to achieve a power of 80% and a level of
18 significance of 5%. Assuming a 20% attrition rate, 20 participants will be recruited
19 per group. They will be recruited from three local special schools with existing
20 research links to the principal investigator (PI) and collaborators. More special
21 schools will be invited to join the research project if there is inadequate participant
22 enrolment among the three participating schools.
23
24
25

26 **Randomization**

27 After screening, all eligible participants will be randomly assigned to either the
28 intervention or control group. To ensure equal allocation ratios for the intervention
29 and control groups, block randomization[55] will be used. A block size of eight will
30 be used in the proposed study (i.e., four in one group and four in the other group for
31 every eight consecutively entered participants). The block randomization process will
32 be performed by a trained research assistant.
33
34
35

36 **Patient and public involvement**

37 Patients and public will not be involved in the study.
38
39

40 **Blinding**

41 The person responsible for analyzing the sleep parameters and melatonin level will be
42 blinded to the group assignment.
43
44

45 **Ethics and dissemination**

46 Prior to the study, information about the study will be provided to all participants and
47 their parents with the distribution of written consent forms (see Appendix I). The
48 consent forms will be collected from the participants and their parents by a PE teacher
49 at each participating school. All participants and their parents will be informed that
50 withdrawal at any time will not result in any adverse consequences. All sets of data
51 will be encrypted with passwords. To prevent any leakage of sensitive information,
52 only the PI and collaborators will have access to the datasets. Ethical approval was
53 obtained from the Human Research Ethics Committee, The Education University of
54 Hong Kong (reference number 2016-2017-0155). The results of the study will be
55
56
57
58
59

1
2
3 published in a peer-reviewed journal. Findings of the study will also be shared with
4 other university and non-governmental organizations in Hong Kong that specialize in
5 autism by means of a formal dissemination seminar.
6
7

8 **Statistical methods**

9 All statistical analyses will be conducted using SPSS (version 23.0) for Windows
10 (SPSS Inc., Chicago, IL, USA) to assess sleep; multilevel regression or a generalized
11 estimating equation (GEE) will be used to assess the effect of the physical activity
12 intervention, the effect of time, and their interaction effect on sleep outcomes and
13 melatonin level outcomes. Two potential confounding variables (i.e., average time
14 spent in daily sedentary activity and average time spent in daily MVPA) will be used
15 as covariates because they may be closely related to sleep quality.[56] The effect size
16 will be reported as a Cohen's *d*. Bonferroni adjustment will be used to control for
17 possible type I error inflation caused by multiple comparisons. The intention to treat
18 approach will be used to handle any missing data.
19
20
21

22 **Discussion**

23 This study is the first randomized control trial designed to examine the effectiveness
24 of physical activity intervention on sleep disorder among children with ASD. In
25 addition, it is the first study designed to investigate whether the melatonin-mediated
26 mechanism is a potential underlying pathway by which physical activity impacts on
27 sleep in children with ASD. The results obtained in this study will potentially have
28 two significant implications. First, if the intervention is effective, doctors can
29 prescribe physical activity to children with ASD who are not able to take drugs based
30 on the notion of "exercise as medicine". Second, the melatonin-mediated mechanisms
31 investigated in this study could lead to further investigation of the interaction between
32 physical activity and melatonin in any population suffering from sleep disturbance.
33 Such research could include a comparison of the effectiveness of physical activity and
34 supplemental melatonin interventions on sleep quality in populations suffering from
35 sleep disorders and the manipulation of different physical activity intervention
36 parameters (e.g., intensity, frequency, and time) on melatonin level and sleep quality.
37
38
39
40
41

42 **Acknowledgements**

43 This study was funded by the Early Career Scheme of Research Grants Council,
44 University Grants Committee of HKSAR Government (#28602517). We thank
45 Benjamin Knight, MSc., from Edanz Group (www.edanzediting.com/ac) for editing a
46 draft of this manuscript.
47
48

49 **Contributorship statement**

50 ACYT and JZ conceived of the study and designed the study protocol, PHL assisted
51 in defining the statistical analysis and provided input for the manuscript. EWHL
52 provided critical comment on implementation of the participant screening protocol.
53 All authors contributed to, read drafts of and approved the final manuscript.
54
55
56
57
58
59
60

Competing interests

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

Funding

This work was supported by the Early Career Scheme of Research Grant Council (Grant number: 28602517).

For peer review only

References

- 1 World Health Organization. Questions and answers about autism spectrum disorders (ASD). 2016 <http://www.who.int/features/qa/85/en/> (accessed 12 Aug 2016).
- 2 Rapin I, Tuchman RF. Autism: definition, neurobiology, screening, diagnosis. *Pediatr Clin North Am* 2008;55(5):1129-1146.
- 3 Yip L. Update on autism. *Public Health Epidemiol Bull* 2012;21(1):11-6.
- 4 Cortesi F, Giannotti F, Ivanenko A, et al. Sleep in children with autistic spectrum disorder. *Sleep Med* 2010;11(7):659-664.
- 5 Liu X, Hubbard JA, Fabes RA, et al. Sleep disturbances and correlates of children with Autism Spectrum Disorders. *Child Psy Human Dev* 2006;37(2):179-191.
- 6 Sivertsen B, Posserud M-B, Gillberg C, et al. Sleep problems in children with autism spectrum problems: a longitudinal population-based study. *Autism* 2012;16(2):139-150.
- 7 Orsmond GI, Seltzer MM. Siblings of individuals with autism spectrum disorders across the life course. *Ment Retard Dev Disabil Res Rev* 2007;13(4):313-320.
- 8 Goldman SE, Richdale AL, Clemons T, Malow BA. Parental sleep concerns in Autism Spectrum Disorders: Variations from childhood to adolescence. *J Autism Dev Disord* 2012;42(4):531-538.
- 9 Fricke-Oerkermann L, Pluck J, Schredl M, et al. Prevalence and course of sleep problems in childhood. *Sleep* 2007;30(10):1371-1377.
- 10 Calhoun SL, Fernandez-Mendoza J, Vgontzas AN, et al. Prevalence of insomnia symptoms in a general population sample of young children and preadolescents: gender effects. *Sleep Med* 2014;15(1):91-95.
- 11 Souders MC, Mason TB, Valladares O, et al. Sleep behaviors and sleep quality in children with autism spectrum disorders. *Sleep* 2009;32(12):1566-1578.
- 12 Richdale AL, Schreck KA. Sleep problems in autism spectrum disorders: prevalence, nature, & possible biopsychosocial aetiologies. *Sleep Med Rev* 2009;13(6):403-411.
- 13 Dewald JF, Meijer AM, Oort FJ, et al. The influence of sleep quality, sleep duration and sleepiness on school performance in children and adolescents: a meta-analytic review. *Sleep Med Rev* 2010;14(3):179-189.
- 14 Curcio G, Ferrara M, De Gennaro L. Sleep loss, learning capacity and academic performance. *Sleep Med Rev* 2006;10(5):323-337.
- 15 Mazurek MO, Sohl K. Sleep and behavioral problems in children with Autism Spectrum Disorder. *J Autism Dev Disord* 2016;46(6):1906-1915.
- 16 Schreck KA, Mulick JA, Smith AF. Sleep problems as possible predictors of intensified symptoms of autism. *Res Dev Disabil* 2004;25(1):57-66.
- 17 Doo S, Wing YK. Sleep problems of children with pervasive developmental disorders: correlation with parental stress. *Dev Med Child Neuro* 2006;48(8):650-655.
- 18 Yu XT, Lam HS, Au CT, et al. Extended parent-based behavioural education improves sleep in children with Autism Spectrum Disorder. *Hong Kong J Ped* 2015;20(4):219-225.

19. Richdale AL. Sleep problems in autism: prevalence, cause, and intervention. *Dev Child Neuro* 1999;41(01):60-66.
20. Malow BA, Byars K, Johnson K, et al. A practice pathway for the identification, evaluation, and management of insomnia in children and adolescents with Autism Spectrum Disorders. *Pediatrics* 2012;130(2):106-124.
21. Bramble D. Consumer opinion concerning the treatment of a common sleep problem. *Child Care Health Dev* 1996;22(6):355-366.
22. Vriend JL, Corkum PV, Moon EC, et al. Behavioral interventions for sleep problems in children with Autism Spectrum Disorders: current findings and future directions. *J Ped Psycho* 2011;36(9):1017-1029.
23. Weiskop S, Richdale A, Matthews J. Behavioural treatment to reduce sleep problems in children with autism or fragile X syndrome. *Dev Med Child Neuro* 2005;47(2):94-104.
24. Lancioni GE, O'Reilly MF, Basili G. Review of strategies for treating sleep problems in persons with severe or profound mental retardation or multiple handicaps. *Am J Mental Retard* 1999;104(2):170-186.
25. Driver HS, Taylor SR. Exercise and sleep. *Sleep Med Rev* 2000;4(4):387-402.
26. Kredlow MA, Capozzoli MC, Hearon BA, et al. The effects of physical activity on sleep: a meta-analytic review. *J Behav Med* 2015;38(3):427-449.
27. Youngstedt SD, O'Connor PJ, Dishman RK. The effects of acute exercise on sleep: a quantitative synthesis. *Sleep* 1997;20(3):203-214.
28. Lundahl A, Nelson TD, Van Dyk TR, et al. Psychosocial stressors and health behaviors: examining sleep, sedentary behaviors, and physical activity in a low-income pediatric sample. *Clinical Pediatrics* 2013;52(8):721-729.
29. Stone MR, Stevens D, Faulkner GEJ. Maintaining recommended sleep throughout the week is associated with increased physical activity in children. *Prev Med* 2013;56 (2):112-117.
30. Wachob D, Lorenzi DG. Brief report: influence of physical activity on sleep quality in children with autism. *J Autism Dev Disord* 2015;45(8):2641-2646.
31. Brand S, Jossen S, Holsboer-Trachsler E, et al. Impact of aerobic exercise on sleep and motor skills in children with autism spectrum disorders – a pilot study. *Neuropsychiatric Dis Treat* 2015;11:1911-1920.
32. McGinty D, Szymusiak R. Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. *Trends Neurosci* 1990;13(12):480-487.
33. Adam K, Oswald I. Protein synthesis, bodily renewal and the sleep-wake cycle. *Clin Sci* 1983;65(6):561-567.
34. Atkinson G, Edwards B, Reilly T, et al. Exercise as a synchroniser of human circadian rhythms: an update and discussion of the methodological problems. *Eur J Appl Physiol* 2007;99(4):331-341.
35. Lee H, Kim S, Kim D. Effects of exercise with or without light exposure on sleep quality and hormone responses. *J Exerc Nutr Biochem* 2014;18(3):293-299.
36. Wirojanan J, Jacquemont S, Diaz R, et al. The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X syndrome. *J Clin Sleep Med* 2009;5(2):145-150.

- 1
- 2
- 3 37. Rossignol DA, Frye RE. Melatonin in autism spectrum disorders: a systematic
- 4 review and meta-analysis. *Dev Med Child Neuro* 2011;53(9):783-792.
- 5
- 6 38. Krakowiak P, Goodlin-Jones B, Hertz-Picciotto I, et al. Sleep problems in
- 7 children with autism spectrum disorders, developmental delays, and typical
- 8 development: a population-based study. *J Sleep Res* 2008;17(2):197-206.
- 9
- 10 39. Veatch OJ, Goldman SE, Adkins KW, Malow BA. Melatonin in children with
- 11 autism spectrum disorders: how does the evidence fit together? *J Nat and Sci*
- 12 2015;1(7):125.
- 13
- 14 40. Goldman SE, Adkins KW, Calcutt MW, et al. Melatonin in children with autism
- 15 spectrum disorders: endogenous and pharmacokinetic profiles in relation to sleep.
- 16 *J Autism Dev Disord* 2014;44(10):2525-2535.
- 17
- 18 41. Arendt J. Importance and relevance of melatonin to human biological rhythms. *J*
- 19 *Neuroendocrinol* 2003;15(4):427-431.
- 20
- 21 42. Marrin K, Drust B, Gregson W, et al. Diurnal variation in the salivary melatonin
- 22 responses to exercise: relation to exercise-mediated tachycardia. *Eur J Appl*
- 23 *Physiol* 2011;111(11):2707-2714.
- 24
- 25 43. American Psychiatric Association. Diagnostic and Statistical Manual of Mental
- 26 Disorders: DSM-5. Washington DC: American Psychiatric Association 2016.
- 27
- 28 44. Giannotti F, Cortesi F, Cerquiglioni A, et al. An open-label study of
- 29 controlled-release melatonin in treatment of sleep disorders in children with
- 30 autism. *J Autism and Dev Disord* 2006;36(6):741-752.
- 31
- 32 45. Petrus C, Adamson SR, Block L, et al. Effects of exercise interventions on
- 33 stereotypic behaviours in children with autism spectrum disorder. *Physiothera*
- 34 *Can* 2008;60(2):134-145.
- 35
- 36 46. Kern L, Koegel RL, Dyer K, et al. The effects of physical exercise on
- 37 self-stimulation and appropriate responding in autistic children. *J Autism Dev*
- 38 *Disord* 1982;12(4):399-419.
- 39
- 40 47. Centers of Disease Control and Prevention. Measuring physical activity intensity:
- 41 target heart rate and estimated maximum heart rate;
- 42 <http://www.cdc.gov/physicalactivity/basics/measuring/hearttrate.htm> (accessed 26
- 43 Mar 2017).
- 44
- 45 48. Londeree BR, Moeschberger ML. Effect of age and other factors on maximal
- 46 heart rate. *Res Q Exerc Sport* 1982;53(4):297-304.
- 47
- 48 49. Pan CY, Chu CH, Tsai CL, et al. The impacts of physical activity intervention on
- 49 physical and cognitive outcomes in children with autism spectrum disorder.
- 50 *Autism* 2016;21(2):190-202.
- 51
- 52 50. Lang R, Koegel LK, Ashbaugh K, et al. Physical exercise and individuals with
- 53 autism spectrum disorders: a systematic review. *Res Autism Spectr Disord*
- 54 2010;4(4):565-576.
- 55
- 56 51. Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire
- 57 (CSHQ): psychometric properties of a survey instrument for school-aged children.
- 58 *Sleep* 2000;23(8):1043-1051.
- 59
- 60 52. Barreira TV, Schuna JM, Jr., Mire EF, et al. Identifying children's nocturnal sleep
- using 24-h waist accelerometry. *Med Sci Sports Exerc* 2015;47(5):937-943.

- 1
- 2
- 3 53. ActiGraph. What is MVPA and how I can view in ActiLife. 2016;
- 4 [https://help.theactigraph.com/entries/22148365-What-is-MVPA-and-how-can-I-v](https://help.theactigraph.com/entries/22148365-What-is-MVPA-and-how-can-I-view-it-in-ActiLife-)
- 5 [iew-it-in-ActiLife-](https://help.theactigraph.com/entries/22148365-What-is-MVPA-and-how-can-I-view-it-in-ActiLife-) (accessed 2 Sep 2017).
- 6
- 7 54. Schernhammer ES, Kroenke CH, Dowsett M, et al. Urinary 6-sulfatoxymelatonin
- 8 levels and their correlations with lifestyle factors and steroid hormone levels. *J*
- 9 *Pineal Res* 2006;40(2):116-124.
- 10 55. Efird J. Blocked randomization with randomly selected block sizes. *Int J Enviro*
- 11 *Res Public Health* 2011;8(1):15-20.
- 12
- 13 56. Kline CE, Crowley EP, Ewing GB, et al. The effect of exercise training on
- 14 obstructive sleep apnea and sleep quality: a randomized controlled trial. *Sleep*
- 15 2011;34(12):1631-1640.
- 16
- 17
- 18
- 19
- 20
- 21
- 22
- 23
- 24
- 25
- 26
- 27
- 28
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
- 39
- 40
- 41
- 42
- 43
- 44
- 45
- 46
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

1
2
3 Figure 1. Flow chart of the proposed study
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

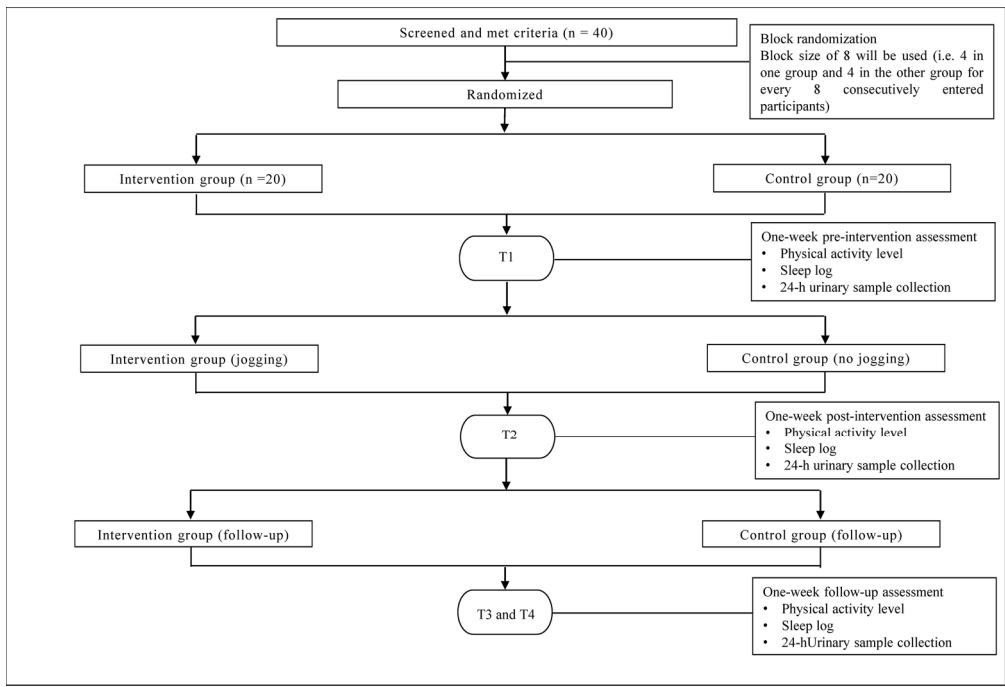


Figure 1. Flow chart of the proposed study

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

1 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
2 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
3 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
4 處。在此多謝你的參與。
5
6
7
8
9
10
11

12 家長回條

13
14 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
15 容，並同意參加這項研究。
16
17

18
19 此外，本人亦 ** 同意 / 不同意 本人之兒女 _____ (學童名稱) _____ (學童班
20 別) _____ (學童學號) 參與是項研究。[** 請刪去不適用者]
21
22
23
24
25
26
27

28 _____
29 家長聯絡電話

_____ 日期

30
31
32
33
34
35 _____
36 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			

1 2 3 4 5 6 7 8 9 10	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
11 12 13 14 15 16		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
17 18 19 20 21 22 23 24	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
25 26 27 28 29	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
30 31 32		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P.9-10
33 34 35 36 37 38		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
39	Methods: Monitoring			
40 41 42 43 44 45 46 47 48	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
49 50 51 52 53 54 55 56 57 58 59 60		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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.