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**Effects of exercise on sleep, melatonin level and behavioral functioning in children with autism**

Andy C.Y. Tse, PhD1, Paul H. Lee, PhD2, Jihui Zhang, PhD3, Roy C.Y. Chan, BEd 1, Amy W.Y. Ho, PhD4, Elvis W.H. Lai, PhD5

1Department of Health and Physical Education, The Education University of Hong Kong, Hong Kong China; 2Department of Health Sciences, University of Leicester, United Kingdom; 3Department of Psychiatry, The Chinese University of Hong Kong, Hong Kong China; 4Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong China; 5Department of Psychiatry, The Hong Kong Castle Peak Hospital

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

Rm D4-2/F-13, Block D4,

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

Tel: (852) 2948 8074

Email: [andytcy@eduhk.hk](mailto:andytcy@ied.edu.hk)

**Abstract**

Poor sleep quality and low behavioral function are commonly reported in children with autism spectrum disorder (ASD). The objective of this study examined the impact of exercise on sleep on melatonin level and behavioral functioning in the population. Children with ASD (n = 55; age = 10.97±1.90) were randomly allocated to a 12-week morning jogging intervention group or a control group. Participants’ sleep was measured using actigraphy and sleep log assessments. 24-hour and first morning urinary 6-sulfatoxymelatonin (aMT6s) were used to determine if the exercise intervention could elicit changes in melatonin levels. Behavioral functioning of the participants was assessed by the repetitive subscale of the Gilliam Autism Rating Scale-3rd Edition. All assessments were carried out in baseline, post-intervention or regular treatment, and follow-up to elucidate the sustainability of the exercise effects.Positive changes were observed between baseline and post-intervention in actigraphy-assessed sleep efficiency and wake after sleep onset, as well as melatonin level and behavioral functioning within the intervention group (*ps* <.017). However, no significant changes were observed in all measurements between post-intervention and follow-up (*ps* >.05). The findings suggest that physical exercise is effective to improve sleep with an increase in melatonin level. It can also reduce repetitive behaviors in children with ASD.

**Introduction**

Children with autism spectrum disorder (ASD) are consistently shown to be at high risk for sleep and behavioral problems (Hirata et al., 2016; Malow et al., 2016; Maskey, Warnell, Parr, Le Couteur, & McConachie, 2013; Mazurek & Sohl, 2016; Souders et al., 2017). Children with ASD tend to experience significant difficulties in sleep onset and maintenance (Elrod & Hood, 2015; Malow et al., 2016) while having trouble to regulate their behaviors and attention (Keehn, Nair, Lincoln, Townsend, & Muller, 2016; Mayes, Calhoun, Mayes, & Molitoris, 2012). With these problems, their quality of life is further compromised (Dewald, Meijer, Oort, Kerkhof, & Bogels, 2010; Lord, 2019). Literatures demonstrated that poor sleep was closely associated with cognitive impairments (Dewald et al., 2010) and adaptive functioning (Taylor, Schreck, & Mulick, 2012) in children with ASD. Behavioral problems among the population further disturbed their social interactions, academic skills, self-worth, and independence (Huang, Yen, Tseng, Tung, Chen, & Chen, 2014; National Research Council, 2001). Apart from the children themselves, the sleep and behavioral problems in the population also adversely affected their caregivers, who were widely reported to have elevated parenting stress and social restrictions [See Martin, Papadopoulos, Chellew, Rinehart, & Sciberras (2019) and Yorke, White, Weston, Rafla, Charman, & Simonoff (2018) for reviews]. Therefore, a critical need exists for evidence-based effective and sustainable interventions that target their sleep and behavioral problems.

One intervention that received considerable research interest is physical exercise. Previous studies provided empirical evidence that physical exercise was beneficial to sleep quality in children with ASD (Brand, Jossen, Holsboer-Trachsler, Pühse, & Gerber, 2015; Wachob and Lorenzi, 2015). Wachob and Lorenzi (2015)conducted a pilot study to explore the exercise-sleep relationship in 10 children with ASD. Accelerometers were utilized to measure participants’ 24-hour physical activity levels, sleep efficiency (SE), and wake after sleep onset (WASO). The actigraphy results showed that SE% was increased and WASO was reduced with increased participation in moderate-to-vigorous physical activity (MVPA). Similar sleep benefits following physical exercise were also evident in another investigation by Brand and colleagues (2015) in which the researchers showed that bicycle training significantly improved SE%, shortened sleep onset latency, and decreased WASO time in children with ASD (Brand, Jossen, Holsboer-Trachsler, Pühse, & Gerber, 2015). Most recently, it is also revealed that physical exercise significantly improved SE%, sleep onset latency, and sleep duration in the population (Tse, Lee, Chan, Edgar, Wilkinson-Smith, & Lai, 2019). Indeed, it appears that physical exercise can serve as an alternative way to ameliorate the symptoms of sleep disturbance and behavioral problems in children with ASD.

Whether the aforementioned benefits could be sustained, however remains largely undetermined. More importantly, the underlying mechanism of how physical exercise impacts sleep is unclear, particularly in children with ASD. Without knowing the mechanism, development of an optimal exercise intervention would be undermined. In neurotypical population, a melatonin-mediated model (Atkinson, Edwards, Reilly, & Waterhouse, 2007) was proposed. Melatonin is a natural hormone produced by pineal gland in the brain (Wirojanan et al., 2009). Its production is light dependent and generally increases with evening darkness to promote sleep onset and maintenance. Melatonin helps regulate circadian rhythm and synchronize sleep-wake cycle with day and night (Zisapel, 2018). Accumulating evidence shows that physical exercise can improve sleep by altering melatonin level (Buxton et al., 1997; Carlson, Pobocik, Lawrence, Brazeau, & Koch, 2019; Escames et al., 2012; Marrin et al., 2011). Pobocik and colleagues (2020) showed that aerobic exercise improved sleep quality in normal healthy adults with an increase in melatonin level (Pobocik, Rentzell, Leonard, Daye, & Evans, 2020). Given the melatonin level in children with ASD was generally lower than that in children with typical development (TD) (Babinska et al., 2019; Krakowiak, Goodlin-Jones, Hertz-Picciotto, Croen, & Hansen, 2008; Liu, Hubbard, Fabes, & Adam, 2006), it is hoped that physical exercise could also improve sleep in children with ASD by increasing their melatonin level. However, none of the previous studies have been conducted to examine this aspect. Therefore, one of the objectives of the present study is to fill this research gap.

Meanwhile, physical exercise was also demonstrated to be beneficial to behavioral disturbance in children with ASD (Bremer, Crozier, & Lloyd, 2016). For example, Bahrami and colleagues (2012) showed that Kata techniques (a technique of martial arts exercise) significantly reduced stereotypic behaviors (i.e., restrictive and repetitive behaviors) in children with ASD (Bahrami, Movahedi, Marandi, & Abedi, 2012). A similar finding was also shared in our previous study where ball-tapping exercise was beneficial in reducing hand-flapping stereotypic behaviors( Tse, Pang, & Lee, 2017). However, there were many inherent methodological limitations in the literature, such as very small sample sizes, no baseline measures of IQ, no objective diagnosis on ASD and behavioral functioning, and no longitudinal assessments of behavioral outcomes for sustainability of the exercise effect [see Bremer et al., (2016) for a review]. These inherent constraints have compromised the robustness and generalizability of evidence on the behavioral benefits of physical exercise in children with ASD. As a consequence, another objective of the present study is to investigate the impact of the physical exercise on behavioral functioning in children with ASD with a more methodologically robust study design.

**Methods**

*Trial design*

The study designed was referenced in a published study protocol (Tse, Lee, Zhang, & Lai, 2018) where a parallel, two-group randomized controlled trial (RCT) design was presented. A flow diagram of the study is presented in Fig. 1.

<Fig. 1 is inserted here>

*Changes to trial design*

The present study has one important change of trial design from the registered study protocol (Tse, Lee, Zhang, & Lai, 2018). That is, the present study did not incorporate the last assessment time point (i.e. T4: 12 weeks after the intervention) due to insufficient research funding.

*Participants*

A total of seventy-two participants were recruited from five local special schools for mild intellectual disabilities using the following inclusion criteria: (1) 8–12 years of age; (2) pre-puberty or early puberty, as indicated by Tanner stage I or II ; (3) diagnosed with ASD by an interdisciplinary team, including physicians and psychologists, based on a standardized diagnostic tool – the Autism Diagnostic Observation Schedule – 2nd Edition (Lord et al., 2012) (4) non-verbal IQ over 50 as assessed by the Wechsler Intelligence Scale for Children (Chinese revised) (C-WISC) [see Gong & Cai (1993) for more information]; (5) able to follow instructions; (6) physically able to participate in the intervention (recommended by school physical education teachers); (7) no additional regular participation in physical exercise other than school physical education classes (mean duration: 63.61 mins/week) for at least three months prior to the study reported by parents or guardians and (8) no concurrent medication for at least three months before the study or any prior melatonin treatment. The exclusion criteria were: (1) one or more co-morbid psychiatric disorders; (2) other medical conditions that limit physical activity capacity (e.g., asthma, seizure, cardiac disease); and (3) a complex neurological disorder (e.g., epilepsy, phenylketonuria, fragile X syndrome, tuberous sclerosis).

*Randomization*

The participants were randomly assigned to one of two groups: intervention (n = 36) and control (n = 36). To ensure equal allocation ratios for both groups, block randomization was utilized. A block size of eight was used in the study. The block randomization process was performed by a trained research assistant. Of the 72 participants, 17 were excluded (13 from the intervention group and 4 from the control group) due to incomplete intervention participation or incomplete urine collection, resulting in 23 participants in the intervention group (21 boys and 2 girls; mean age = 10.86 ± 1.99) and 32 participants in the control group (26 boys and 6 girls; mean age = 11.17± 1.93). Severity of participants’ autism symptoms was collected by the parental reported Social Responsiveness Scale (Constantino & Gruber, 2012). Information about each participant’s ongoing treatments (e.g., medications, speech therapy, occupational therapy) was gathered from the participants’ parents. Written informed consent was obtained from participants’ parents/guardians. The study was approved by the university ethics committee. Demographic data for the two groups is shown in Table 1.

<Table 1 is inserted here>

*Study settings*

Each participant was asked to attend three 1-week assessments. Their habitual sleep and behavioral patterns were assessed before the baseline, post-intervention, and follow-up (6 weeks after the post-intervention.

*Intervention*

Intervention group: The intervention was a 12-week morning jogging program consisting of 24 sessions (2 sessions per week, 30 min per session) in and outdoor environment of each participating school. Morning session and outdoor environment were chosen to control for sunlight exposure for all participants. The total of 24 sessions was selected based on previous studies involving jogging in this population (Kern, Koegel, Dyer, Blew, & Fenton, 1982; Petrus et al., 2008). The intervention period was from April to July with increasing length of daylight. Each intervention session was conducted by the corresponding physical education (PE) teacher of the participants or a trained research assistant aided by a group of student helpers. The staff-to-participant ratio for both groups was 1:2 to 1:1, depending on attendance. The overall attendance rate was 96.92%. Each intervention session was performed in an identical format, comprising three activities: warm-up (5 min), jogging (20 min), and cool-down (5 min). Jogging was selected because it is the most commonly used exercise for studies in children with ASD [see Lang et al., (2010) for review]. Participants were required to run at a moderate intensity level, as determined by exhibiting an increased breathing rate and mildly flushed face [see Rosenthal-Malek & Mitchell (1997) for more information]. Verbal reinforcement and verbal cueing were employed to encourage the participants to jog continuously. Participants were required to follow their normal daily routine without engaging in any additional physical activity/exercise program throughout the follow-up period (i.e. 6 weeks).

Control group: Participants in the control group did not receive any intervention, and were asked to follow their daily routine without taking part in any additional physical exercise program throughout the entire study period.

*Outcomes*

Sleep: Three sleep parameters, including sleep efficiency (actual sleep time divided by time in bed, expressed as a percentage); wake after sleep onset (length of time awake after sleep onset, expressed in minutes), and sleep duration (total sleep duration in hours and minutes) were measured using an actigraphy Gfollow-upX accelerometer and a sleep log (Tse A. C. Y. Tse et al., 2019). Similar to our previous study (A. C. Y. Tse et al., 2019), participants were asked to wear the device on the non-dominant wrist for seven full consecutive days (i.e., Monday to Sunday, days and nights), and were instructed to remove it only when they were taking a bath. Non-wear time was defined as 60 min of consecutive zero scores with a 2-min spike tolerance (Wachob & Lorenzi, 2015) The night (22:00–07:00) was considered invalid if the wear time was less than 8 h and was excluded from data analysis. The Sadeh algorithm (Sadeh, Sharkey, & Carskadon, 1994) was implemented to identify sleep onset and sleep offset. For the sleep log, participants’ parents were asked to recall sleep information (e.g., bedtime, sleep start, sleep end, wake-up time, and sleep length) for the whole assessment week. Parents were told to record the sleep information accurately with the assistance of clock or watch.

Melatonin level: All participants were required to collect a 24-h urine and a first morning void urine sample. 6-sulfatoxymelatonin (aMT6s), a creatinine-adjusted morning urinary melatonin being considered as representative of melatonin level, was measured from the sample using Enzyme-Linked Immunosorbent Assay (ELISA) (IBL, Hamburg, Germany) (Middleton, 2013). Both 24-h urine and the first morning void urine sample were used so as to increase the reliability of the measurements. For the convenience of data collection, parents were asked to collect the 24-h urinary samples of the participants from Saturday evening to Sunday evening, as well as to collect the first morning urinary samples of the participants on Monday prior to going to school. The parents were instructed to store the urine sample in the fridge temporarily before Monday. The Monday urinary samples were collected in order to maximize the data collection compliance rate as participants needed to go to school on Monday. Upon completion, the urine samples were immediately stored at −80° in a chemical pathology laboratory before analysis.

Behavioral functioning: Similar to our previous study (Tse et al., 2017), Gilliam Autism Rating Scale- 3rd Edition (Gilliam, 2014) was used for behavioral assessment. The GARS-3 is a survey consisting of 42 items divided into three subscales to measure stereotypic behavior, communication, and social interaction. Behavior of a children in 6-hour time periods was scored as follows: 0 – never observed, 1 – seldom observed (1-2 times/ 6

hours), 2 – sometimes (3-4 times), 3 - frequently (at least 5-6 times) (Robinson, 2013). For the purpose of this study, only the repetitive behavior subscale was used to obtain the scaled score. Higher scaled scores indicated greater severity of stereotypic behavior. There were several changes to trial outcomes. First, we included an additional assessment of first morning urinary sample in order to provide a more comprehensive measurement of aMT6s level. Second, we included the behavioral functioning assessment in order to examine the impact of physical exercise on behavioral functioning in children with ASD. Third, we were not able to measure the physical activity level of the participants in the present study due to the reluctance of participants to wear the heart rate monitor (Polar H10).

*Sample size*

A pilot study on improving sleeping quality among children with ASD revealed that PA had a notable effect (corresponding to a Cohen’s d of approximately 1.0) on improving sleeping quality (Brand et al., 2015). Given this effect size, a minimum sample of 16 participants per group is required to achieve a power of 80% and a level of significance of 5%. In the present study, a total of 72 participants (36 participants in each group) were recruited. 55 participants (23 in the intervention group and 32 in the control group) had successfully completed the study.

*Blinding*

The persons responsible for screening the eligibility of participants and for analyzing the data were blinded to the group assignment*.*

**Statistical Methods**

All statistical analyses were conducted using SPSS (version 23.0) for Windows (SPSS Inc., Chicago, IL, U.S.A.). Multilevel regression was utilized to assess the effect of the physical exercise intervention, the effect of time (i.e. assessment time points), and their interaction effect on sleep, melatonin level, and behavioral functioning outcomes. Two potential confounding variables (i.e., average time spent in daily sedentary activity and average time spent in daily MVPA) were included as covariates because they may be closely related to sleep quality (Kline et al., 2011). A minute was classified as sedentary or MVPA if the accelerometer count per minute at the frequency of 80Hz was <232 or >4514, respectively (Lee & Tse, 2019), and the average time spent in daily sedentary activity and MVPA were calculated by the sum of the total time spent on the corresponding type of activity divided by the number of valid accelerometer wearing days. Bonferroni-adjusted alpha cut-off of p<.017 (0.05/3 = 0.017) was used to identify a significant time effect to control for possible type I error inflation resultant from multiple comparisons.

**Community involvement**

There is no community involved in this study.

**Results**

Changes of all dependent variables across times are depicted in Table 2.

<Table 2 is inserted here>

*Sleep parameters*

At baseline, all the sleep parameters measured by actigraphy assessment and sleep logs were comparable between groups (all *p* values> .05).

Actigraphy assessments

Sleep efficiency: A significant time effect was identified in the intervention group (*p* <.017). It was shown that the sleep efficiency of the intervention group increased significantly from baseline (mean = 89.1%; SD = .89) to post-intervention (mean = 94.8%, SD = .37) (*p* *<.*017) but it has later decreased from post-intervention to follow-up (mean = 88.7%; SD = 2.28) (*p* <.017). The sleep efficiency did not change significantly within the control group from baseline to follow-up (*ps >* .05).

Wake after sleep onset: There was a significant time effect in the intervention group (*p* < .017). Pairwise comparisons revealed that the WASO of the intervention group was significantly reduced form baseline (mean = 16.57 min; SD = 9.44) to post-intervention (mean = 11.80 min; SD = 8.27) (*p* <.01).

Sleep duration: A significant between-group difference was shown at post-intervention (*p* < .05). Participants in the intervention group (mean = 377.56 min; SD = 91.67) slept significantly longer than those in the control group (mean = 328.84 min; SD = 81.90) at post-intervention (*p* < .05). Within-group comparisons showed no differences in each group throughout the whole study (all *p* values >.05).

Sleep log

Sleep efficiency: No significant changes were observed on both within- and between-group comparisons (*ps* > .05). The sleep efficiency of both groups was above 85% throughout the study.

Sleep duration: Similar to sleep efficiency, no significant changes were found on both within- and between-group comparisons (*ps* > .05).

*Melatonin level*

24-h aMT6s

There was a significant group difference at post-intervention (*p* <.05). Specifically, the melatonin level of the intervention group (31.25 nmol/24 h) was statistically higher than that of the control group (20.86 nmol/24 h) (*p* = .04). No significant between-group difference was found during the follow-up period (*p* > .05). Meanwhile, the 24-h aMT6s level increased significantly within the intervention group from baseline to post-intervention (*p <* .017).

First morning urinary aMT6s

Significant between-group difference was observed at post-intervention (*p* *<.* 05). The melatonin level of the intervention group (23.15 nmol/mmol Cr) was significantly higher than that of the control group (15.79 nmol/mmol Cr). Similar to 24-h aMT6s, the first morning urinary aMT6s level increased significantly within the intervention group from baseline to post-intervention (*p* < .017).

*Behavioral functioning*

As shown in Table 2, no significant between-group differences were present in the scaled scores of the GAR-3’s repetitive behaviour subscale at all timeslots (*ps* >.05), indicating that both groups exhibited similar behavioral patterns throughout the whole study. However, a significant within-group differences were found in the intervention group from baseline to post-intervention (*p* = .01) and from post-intervention to follow-up (*p* =.01). These findings suggested that the exercise intervention could be able to reduce repetitive behaviors but this effect could not be sustained over times.

**Discussion**

To the best of our knowledge, this study constitutes the first randomized control trial (RCT) designed to examine the impact of physical exercise and its sustainability on sleep and behavioral functioning in children with autism spectrum disorder (ASD). In addition, this study also extends the current literature by investigating the melatonin-mediated mechanism on the exercise-sleep relationship in children with ASD. In line with previous findings (Brand et al., 2015; Tse et al., 2019), the present study showed significant improvements in actigraphy-assessed sleep efficiency and wake after sleep onset in the exercise intervention group. More remarkably, there were significant increases in melatonin level in the exercise intervention group as indicated by both 24-h aMT6s and first morning urinary aMT6s levels between pre- and post-exercise (i.e. baseline and post-intervention).

These findings not only supported the notion on the ability of physical exercise to alter melatonin levels (Carlson et al.; Escames et al., 2012; H. Lee, Kim, & Kim, 2014; Marrin et al., 2011), but also showed that same mechanism may also be applied in children with ASD. These findings have two significant implications. First, it reconfirms the effectiveness of physical exercise on improving sleep in children with ASD. Second, the melatonin-related findings suggest that exercise may be an alternative way to counter for melatonin deficits in children with ASD. Indeed, melatonin supplement - a nutritional supplement with sleep-promoting and sleep phase shifting properties, has gained widespread public acceptance as an alternative to medications due to its favourable side-effect profile and relatively low cost (Abdelgadir, Gordon, & Akobeng, 2018). Many studies have also shown that melatonin supplement positively impacts sleep in children with ASD, including reduced sleep onset latency, increased total sleep duration and improved sleep efficiency (Rossignol & Frye, 2011). The melatonin-related findings of exercise shown in this study suggests that exercise may be a non-prescription pharmaceutical approach for children with ASD to improve their sleep quality. Nevertheless, endogenous melatonin level is shown sensitive to multiple factors such as light exposure (Gooley et al., 2011), dietary intake (Peuhkuri, Sihvola, & Korpela, 2012) and emotion (Liu et al., 2017), therefore it is unclear whether the increased melatonin levels of the participants in the present study are purely due to the exercise intervention or any other factors. Therefore, further studies examining the exercise-melatonin relations are warranted.

Comparing with actigraphy data, it is worthy to note that physical exercise did not show any significant impact on all sleep log assessed parameters. This discrepancy between actigraphy and sleep log assessments may due to reduced accuracy and potential parental bias of the sleep log assessment in the present study. Previous studies (Dayyat, Spruyt, Molfese, & Gozal, 2011; Iwasaki et al., 2010; Sadeh, 1994) showed that parental observation became less accurate with increasing assessment period. For example, Sadeh (1994) observed that the discrepancy between actigraphy and sleep log assessments increased over a period of weeks when parents grew tired or less motivated with time. In the present study, we noticed that some parents becoming less motivated to record the required information towards the end of each assessment period. For example, they did not record the information in details and they tended to rely on their memory to record the information rather than punctually recorded them in the sleep log. These may affect the accuracy of the data, which in turns masked the power of physical exercise. Also, similar to many other studies (Dayyat et al., 2011; Iwasaki et al., 2010; Short, Gradisar, Lack, Wright, & Chatburn, 2013), our findings also revealed a typical overestimate of sleep duration in sleep log assessment. As shown in Table 2, parental estimates of sleep indicated longer sleep duration when compared with actual sleep duration as derived from actigraphy assessment, with an overestimate of more than 60 minutes in all records. This typical overestimate of sleep log assessment may also hinder the accuracy of the sleep log assessment.

The current study also sought to examine the impact of physical exercise on behavioral functioning in children with ASD. The results indicate that exercise can be an effective behavioral intervention for children with ASD with a significant decrease of GAR-3’s score on repetitive behavior subscale. This finding has reconfirmed our previous findings that exercise can help reduce the stereotypic behaviors ( Tse, 2020; Tse et al., 2017).

Finally, the present study also conducted follow-up assessments to determine whether sleep and behavioral benefits of exercise could be sustained. Results showed that all the sleep and behavioral outcomes were back to their baseline levels six week after the exercise intervention, suggesting sleep and behaviour may only have short-term changes following exercise. As thus, routine physical exercise habit could be beneficial to maintain sleep and behavioral benefits in children with ASD.

Several limitations exist in the present study that require attention in future investigations. First, we did not exclude individuals with sleep disorders that are not behavioral in nature (e.g., obstructive sleep apnea, restless leg syndrome, periodic limb movement disorder) and this may confound our findings. It is recommended that future similar studies should exclude individuals with these sleep disorders. Second, there is a lack of objective measurement of exercise intensity (e.g., heart rate monitor). Without such measurement, it is difficult to control for exercise intensity, which may confound the study. We recommend that future intervention studies utilize heart rate monitors to control for exercise intensity. Third, light exposure should be measured and controlled for examining melatonin level in order to detect any effect of light exposure on melatonin secretion in children with ASD. Finally, this study is further constrained by the relatively few intervention sessions (i.e., only two times per week). Future similar studies may consider conducting more intervention sessions (e.g., three times per week or above).

**Conclusion**

This RCT study examined the immediate and sustainable impact of physical exercise intervention on sleep through the melatonin-mediated mechanism model, as well as behavioral functioning, in children with ASD. Results indicated that exercise improved objectively-measured sleep and reduced stereotypic behaviors in the population. Significant changes of melatonin level were detected in the exercise intervention group. We concluded that the exercise of jogging twice a week seems to be beneficial for sleep and behavioral outcomes but the effect could not be sustained over time. Particular attention should continue to be paid to the impact of physical exercise on melatonin secretion of children with ASD. Future studies should examine this aspect by controlling factor of light exposure to confirm if the increase of endogenous melatonin level was due to exercise or light exposure or possibly other factors in children with ASD. Finally, it is beneficial for further researchers to include screening for sleep quality and objective measures of exercise intensity in order to elucidate the treatment effect of exercise on sleep disturbance in children with ASD.

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**Conflicts of Interest**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Diagram

Description automatically generatedFigure 1: Flow Diagram of the Study

Table 1. Demographic statistics of participants of each group

|  |  |  |  |
| --- | --- | --- | --- |
|  | Intervention group  (n =23) | Control group  (n =32) | *p* |
| Gender | 21 boys and 2 girls | 26 boys and 6 girls |  |
| Age (years) | 10.86±1.99 | 11.17±1.93 | 0.20 |
| Weight (kg) | 42.75±9.45 | 39.67±7.78 | 0.18 |
| Height (m) | 1.47±0.97 | 1.44±0.11 | 0.36 |
| BMI (kg/m2) | 19.61±2.91 | 18.90±1.90 | 0.27 |
| Non-verbal IQ | 58.32±6.78 | 57.59±4.99 | 0.64 |
| SRS-2 T-scores | 88.48±11.38 | 86.68±11.13 | 0.55 |

Table 2. Changes of sleep parameters, melatonin level and behavioral functioning across times

|  |  |  |  |
| --- | --- | --- | --- |
| Dependent variables | Intervention group, n (SD) | Control group, n (SD) | p-value (between group) |
| Actigraphy assessment |  |  |  |
| Sleep efficiency (%) |  |  |  |
| T1 | 89.1 (0.89) | 91.5 (1.07) | 0.36 |
| T2 | 94.8 (0.37) | 89.6 (1.13) | 0.08 |
| T3 | 88.7 (2.28) | 89.6 (1.68) | 0.82 |
| p-value (within group) |  |  |  |
| p-value (T1 vs T2) | 0.02\* | 0.49 |  |
| p-value (T1 vs T3) | 0.73 | 0.83 |  |
| p-value (T2 vs T3) | 0.01 | 0.91 |  |
| Wake after sleep onset (min) |  |  |  |
| T1 | 16.57 (9.44) | 12.93 (8.69) | 0.15 |
| T2 | 11.80 (8.27) | 10.96 (6.70) | 0.43 |
| T3 | 13.55 (11.20) | 10.69 (7.09) | 0.15 |
| p-value (within group) |  |  |  |
| p-value (T1 vs T2) | 0.01\* | 0.11 |  |
| p-value (T1 vs T3) | 0.10 | 0.08 |  |
| p-value (T2 vs T3) | 0.22 | 0.66 |  |
| Sleep duration (min) |  |  |  |
| T1 | 346.06 (102.70) | 321.91 (110.70) | 0.41 |
| T2 | 377.56 (91.67) | 328.84 (81.90) | 0.04\* |
| T3 | 335.84 (92.11) | 324.67 (103.12) | 0.72 |
| p-value (within group) |  |  |  |
| p-value (T1 vs T2) | 0.15 | 0.73 |  |
| p-value (T1 vs T3) | 0.66 | 0.88 |  |
| p-value (T2 vs T3) | 0.08 | 0.89 |  |
| Sleep log |  |  |  |
| Sleep efficiency (%) |  |  |  |
| T1 | 88.43 (8.47) | 88.05 (11.46) | 0.88 |
| T2 | 89.82 (7.03) | 85.52 (12.43) | 0.09 |
| T3 | 85.90 (14.39) | 86.89 (11.68) | 0.62 |
| p-value (within group) |  |  |  |
| p-value (T1 vs T2) | 0.55 | 0.11 |  |
| p-value (T1 vs T3) | 0.16 | 0.39 |  |
| p-value (T2 vs T3) | 0.22 | 0.58 |  |
| Sleep duration (hours) |  |  |  |
| T1 | 445.75 (49.96) | 466.71 (44.30) | 0.09 |
| T2 | 458.13 (51.19) | 453.60 (93.14) | 0.81 |
| T3 | 472.00 (68.30) | 455.63 (91.20) | 0.42 |
| p-value (within group) |  |  |  |
| p-value (T1 vs T2) | 0.33 | 0.46 |  |
| p-value (T1 vs T3) | 0.09 | 0.52 |  |
| p-value (T2 vs T3) | 0.36 | 0.64 |  |
| Melatonin level |  |  |  |
| 24-hour aMT6s (nmol/24hr) |  |  |  |
| T1 | 21.51 (15.45) | 19.80 (15.42) | 0.68 |
| T2 | 31.25 (19.91) | 20.86 (17.29) | 0.04\* |
| T3 | 29.30 (12.94) | 25.10 (20.16) | 0.52 |
| p-value (within group) |  |  |  |
| p-value (T1 vs T2) | 0.04\* | 0.82 |  |
| p-value (T1 vs T3) | 0.09 | 0.18 |  |
| p-value (T2 vs T3) | 0.37 | 0.25 |  |
| First morning urinary aMT6s (nmol/mmol Cr) |  |  |  |
| T1 | 16.55 (7.61) | 18.37 (16.50) | 0.58 |
| T2 | 23.15 (12.62) | 15.79 (8.27) | 0.02\* |
| T3 | 15.11 (7.47) | 16.09 (9.39) | 0.67 |
| p-value (within group) |  |  |  |
| p-value (T1 vs T2) | 0.01\* | 0.40 |  |
| p-value (T1 vs T3) | 0.18 | 0.36 |  |
| p-value (T2 vs T3) | 0.001 | 0.86 |  |
| Behavioral functioning (GAR-3 Scaled score) | | | |
| T1 | 18.80 (6.60) | 18.97 (8.98) | 0.93 |
| T2 | 15.33 (8.73) | 18.24 (8.10) | 0.20 |
| T3 | 18.08 (7.41) | 19.79 (7.60) | 0.38 |
| p-value (within group) |  |  |  |
| p-value (T1 vs T2) | 0.04\* | 0.58 |  |
| p-value (T1 vs T3) | 0.56 | 0.59 |  |
| p-value (T2 vs T3) | 0.03\* | 0.31 |  |

T1: Baseline; T2: Post-intervention; T3: Follow-up; SD: Standard deviation

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