



Effect of Chronic Melatonin Supplementation on Fitness Profile Parameters in Sedentary Young Men of Kolkata, India

Sohini Basu

ORCID: 0000-0003-1582-1621

Sports and Exercise Physiology Laboratory, Department of Physiology,
University of Calcutta, University Colleges of Science and Technology,
92 APC Road, Kolkata: 700009, West Bengal, India

Anindita Mandal Majee

ORCID: 0000-0003-4973-5406

Department of Physiology, Raja Peary Mohan College, 1 Acharya Dhruba
Pal Road, P.O. Uttarpada, Dist. Hooghly, Pin Code: 712258, West Bengal,
India

Debasish Bandyopadhyay

ORCID: 0000-0001-7993-0777

Oxidative Stress and Free Radical Biology Laboratory, Department of
Physiology, University of Calcutta, University Colleges of Science and
Technology, 92 APC Road, Kolkata: 700009, West Bengal, India

Amit Bandyopadhyay

ORCID: 0000-0002-6811-4587

Sports and Exercise Physiology Laboratory, Department of Physiology,
University of Calcutta, University Colleges of Science and Technology,
92 APC Road, Kolkata: 700009, West Bengal, India

ABSTRACT

Purpose: Melatonin, a safe and pleiotropic biomolecule might have ergogenic benefits beyond its antioxidant and chronobiotic properties to combat deleterious health consequences of sedentary lifestyle. Present study was aimed to investigate the comparative fitness profile analysis following chronic melatonin supplementation in young, untrained sedentary male subjects in contrast to placebo. **Methods:** A single blinded randomized controlled trial was conducted with the melatonin group (MG) (n=14) and placebo group (PG) (n=14). Melatonin at a dose of 3mg/day, 30-60 mins before bed time was administered nocturnally to the MG for 4 weeks. All the measurements were taken weekly in the morning. Fitness profile broadly consisted of a battery of standardized physical and physiological tests (aerobic, anaerobic and motor fitness parameters). Statistical analyses comprised of repeated measures ANOVA with the level of significance set at $p < 0.05$. **Results:** In comparison to PG, a significant treatment effect was noted for vertical jump test (VJT) score and handgrip endurance, both higher in MG. Further, a significant time effect was observed with greater handgrip strengths (right and left hand), handgrip endurance, flexibility, VJT score, balance and lower blood pressure

(systolic and diastolic) in MG. **Conclusion:** Chronic nocturnal melatonin administration is capable of improving certain fitness performances.

Keywords: melatonin supplementation, fitness, balance, endurance, blood pressure, VJT

INTRODUCTION

Health is a pre-requisite to optimal wellbeing of an individual. In this context, exercise and physical activity serve as important factors that ensure both health and wellness thereby implying better quality of life [1]. However, in modern day many a times these get compromised due to sedentary lifestyle. By definition sedentary behaviour implies any waking behaviour such as leaning or sitting having an energy expenditure of 1.5 metabolic equivalent task (MET) or less [2]. The adverse health outcomes of sedentary behaviours have been well documented that are quite vast and range from increased all-cause mortality, cancer risk, cardiovascular disease mortality to risks of metabolic disorders [3]. Epidemiological data revealed engagement in insufficient physical activity by approximately 31% of the global population aged ≥ 15 years that has been further found to contribute to approximately 3.2 million deaths annually [4]. Therefore, in order to combat the situation where the prevalence of non-communicable diseases is going up due to sedentary lifestyles it is imperative to implement certain lifestyle modifications. These changes in lifestyle may be achieved either through incorporation of adequate exercise and physical activity or by means of nutrition in the form of supplement or diet. Under such circumstances, supplement in the form of melatonin may be a suitable choice that has garnered considerable attention in the recent past owing to its multifaceted potential in impacting the bodily system across several human centric studies.

Melatonin is a ubiquitous and phylogenetically conserved indoleamine that is produced by the pinealocytes of the pineal gland in humans during the hours of darkness at night and is then released into the bloodstream thereby entraining the biological rhythms in the mammals [5]. Besides the chronobiotic potential of melatonin, this biomolecule possessing a relatively large safety profile has also been reported to have plethora of potentials including free radical scavenging and anti-inflammatory activity, cytoprotective action and immunomodulatory property [6]. However, in the sports and exercise medicine sphere, studies conducted to deduce the specific interactions between melatonin and various physiological actions have generated numerous opinions and conflicting outcomes. Independent works have highlighted that based on the bouts of exercise, the melatonin concentrations increase, decrease or remain unaffected. The reason to these incongruent findings has been attributed to the day timing during which the participants exercised alongside the differences in lighting conditions [7]. Apart from these, both age as well as fitness status of an individual have been identified as crucial intervening factors for exercise-mediated alterations in the levels of melatonin. Moreover, administration of exogenous melatonin has also been reported to incur hypnotic and hypothermic effects that in turn has been linked to decline in short-term mental and physical performance [8, 9, 10]. Interestingly, the effects on physical performance have been found to be short-lived whereas the impacts are found to be still apparent 3-5 hours post administration as far as some of the cognitive tasks were concerned [11]. In fact, it has been hypothesized that melatonin might facilitate endurance exercise in case of humans, corroborating data retrieved from animal studies which suggested that for the exercising rats, liver and muscle glycogen is preserved with the help of melatonin [12]. Even then, studies involving interactions between melatonin, sleep and exercise concerning trained volunteers remain scanty.

The present study aimed to investigate the effect of chronic nocturnal melatonin ingestion on fitness profile variables measured in the following morning in healthy young sedentary men. We hypothesized that nocturnal melatonin ingestion could have potential ergogenic benefits. In order to examine this aim, specific tests were carried out for aerobic (VO_{2max}) and anaerobic (HIE score) fitness, VJT score, handgrip strengths and endurance as well as motor fitness (agility, flexibility, balance, reaction time).

METHODS

The entire work has been carried out employing the following standardized methods with the help of the requisite materials after seeking proper ethical clearance from the authority. Data pertaining to all the following non-invasive parameters have been procured on the same day in the morning following nocturnal ingestion of melatonin.

Calculation of Sample Size

Sample size was calculated by using PS Power and Sample Size Calculation version 2.1.30 software. It was deduced that a sample size of 11 subjects in each group would be required to fulfil the requirements of the study. However, considering a drop out of around 20% and also to be safe sided, we have recruited 14 subjects in each group for the study.

Selection of the Subjects

Sedentary, healthy male subjects were recruited from the postgraduate and higher levels in academics of University of Calcutta, who were not accustomed with regular physical activity and belonged to similar socio-economic status. Appropriate guidelines were followed to evaluate the sedentary behaviour of the subjects [13, 14]. The socio-economic status of each subject was noted through Modified Kuppaswamy socioeconomic scale [15]. Majority of the subjects belonged to lower middle socio-economic class. International Physical Activity Questionnaire was used to collect the data on health-related physical activity of each subject [16]. The subjects who participated in the study spent nearly 8-10 hours daily in sedentary activity and were not accustomed to undergo a minimum of 60 minutes of moderate to vigorous physical activity and thus were considered to be sedentary. A total of 28 subjects aged 21-26 years comprising of melatonin group (MG) (n=14) and placebo group (PG) (n=14) took part in the study after they were randomly assigned into the respective groups. Further, the subjects were not part of any physical conditioning or training programme. Individuals with hypertension, asthma, infectious diseases, diabetes, bronchitis, anaemia, cardiac problems, kidney or liver diseases and or any other major disease were excluded from the study. Further, non-smokers, non-alcoholic were selected for the study. A detailed history of daily physical activity, dietary intake, medication, vaccination status, incidence of any previous infectious disease etc. were collected through questionnaire method. They were asked to refrain from any physical exercise or any major activity during the days of the experimental trials. They were also asked to abstain from consuming caffeinated beverages for at least 24 hours prior to the experimental trials. A detailed history of any infectious disease such as COVID-19 and vaccination status of each volunteer were taken to assess their immune status. Each subject was informed about the entire experimental protocol before seeking written informed consent from them and after getting the due ethical approval from the Institutional Human Ethics Committee of the Department of Physiology, University of Calcutta. In order to allay the apprehensions of the subjects a familiarization trial was conducted seven days prior to the main trial for each of them.

Nature of Study

The study was a longitudinal one whereby repeated observations of same variables were conducted over duration of four weeks. Moreover, a single blinded randomized control trial was performed in which only the investigator knew which treatment was assigned to which group of participants who were randomly assigned into two groups namely, the melatonin group (MG) and the placebo group (PG).

Duration of Supplement

The study was conducted for a timeframe of four weeks.

Time and Dose of Administration of the Supplement

The supplement or treatment compound of melatonin commercially available as Meloset (ARISTO Pharmaceuticals Pvt. Ltd.) having dosage 3 mg was administered to the experimental melatonin group (MG) participants. The minimal dose of 3 mg has been chosen for administration to the subjects out of the recommended dose range of 3-20 mg for use in humans [17]. Further, the placebo pill comprising of starch substance in gelatine capsule was offered to the placebo group (PG) participants. All the subjects were instructed to take the supplement, 30-60 minutes before bedtime at night. Nocturnal dosing of melatonin has been recommended to ensure greater safety in keeping with the natural circadian rhythm of endogenous melatonin in humans [18]. They were encouraged to ensure compliance by sending text messages to their respective registered mobile numbers everyday throughout the entire study period.

Frequency of Measurements

All the measurements were taken on a weekly basis with six days' interval between the consecutive sessions for five times in total including the baseline measurement.

Environmental Conditions Recording

The ambient temperature and relative humidity of the laboratory set up in which the subjects performed the tests was noted by the use of a psychrometer device comprising of wet bulb and dry bulb thermometers. The average dry bulb temperature, average wet bulb temperature and average relative humidity was recorded to be 24°C, 20°C and 70% respectively while the work was conducted between the months of August 2022 and April 2023.

Preparation of Subjects

Subjects were asked to refrain from heavy exercise during 72 hours before the tests. They were reminded to ensure compliance. Age of each subject was calculated in nearest year from the date of birth as obtained from their Photo ID issued by the Government of India. Subjects were instructed to have a light breakfast 2–3 hours before the trial. They came to the laboratory at 9 AM and took rest for half an hour. Measurements of body height and body mass were done with the subject standing barefoot and wearing minimum clothing on a weighing machine built-in with height measuring rod (Avery India Ltd, India) having an accuracy of ± 0.50 cm and ± 0.1 kg, respectively. Pre-exercise heart rate and blood pressure were also measured by standard methods [19].

Ethical Clearance of the Work

Ethical approval was given by the appropriate authority. All the participants who took part in the study gave their written informed consent at the beginning of the study. The study has been

performed in accordance with the principles as suggested in the Declaration of Helsinki by the World Medical Association [20].

Determination of maximum oxygen uptake or $VO_{2\max}$

Maximum oxygen uptake or $VO_{2\max}$ was estimated indirectly by employing the Queen's College Step Test (QCT) protocol [21].

Determination of High-Intensity Effort (HIE)

A 60-yard dash test which comprises of a shuttle run test of progressing distances was conducted to determine the High-Intensity Effort (HIE) for the subjects [22].

Determination of Handgrip Strength and Handgrip Endurance

The handgrip dynamometer instrument was used to measure both handgrip strength and endurance [23].

Measurement of Agility

A 30 ft shuttle run test was conducted to measure agility [22].

Determination of Flexibility

Flexibility was evaluated by employing the modified sit and reach test [22].

Determination of Vertical Jump Test (VJT) Score

VJT score was used to assess the explosive strength of the leg muscles of the subjects [19].

Determination of Reaction Time

The online reaction time program was used to test the reaction time of subjects [24].

Determination of Balance Score

The maximum duration that the subject was able to maintain balance while standing on their dominant leg with eyes open was monitored. The time was noted with the help of a stopwatch [25].

Statistical Analyses

All values are expressed as Mean $\pm$ SD (Standard deviation) in the Tables, while in case of the Figures, values are represented as Mean $\pm$ SE (Standard error). Statistical analyses have been performed by using SPSS (Statistical Package for Social Science) software (SPSS Inc., Chicago, IL), Version 21.0. The Shapiro-Wilk test was conducted to check whether data were normally distributed and the same was confirmed here. All the physical, physical fitness, motor fitness, body composition, lipid profile and fasting blood glucose parameters were analysed using a mixed model (Treatment $\times$ Time) repeated measures ANOVA for independent samples (i.e., Placebo and Melatonin treatment groups) with repeated measures for the time factor. This factor was "within-subjects" by definition and included five "levels" (Baseline/Week 1/Week 2/Week 3/Week4). When appropriate, comparison of significant difference of means was done through LSD (Least Significance Difference) post-hoc test. Effects of each factor in addition to combined factors (interactions) on each variable were analysed by using t-tests. Effect sizes were calculated as partial eta squared (η^2p). A probability value of $p < 0.05$ was selected as the criterion for statistical significance.

RESULTS

Mean $\pm$ SD values for the physical data of the participants measured across several timepoints are presented in Table 1.

Table 1: Physical data of the subjects

Variable	Time points	Placebo Group (n=14) Mean $\pm$ SD	Melatonin Group (n=14) Mean $\pm$ SD
Age (years)		22.57 $\pm$ 1.02	23.21 $\pm$ 1.37
Body height (cm)		167.51 $\pm$ 5.01	172.18 $\pm$ 5.19
Body weight (kg)	Baseline	62.25 $\pm$ 8.09	67.14 $\pm$ 8.53
	Week 1	62.04 $\pm$ 8.03	67.14 $\pm$ 8.85
	Week 2	62.43 $\pm$ 7.92	66.96 $\pm$ 9.13
	Week 3	62.25 $\pm$ 7.88	67.04 $\pm$ 9.08
	Week 4	61.89 $\pm$ 8.28	67.11 $\pm$ 8.56

Mean $\pm$ SD (Mean $\pm$ Standard deviation)

Body Height

Since body height did not vary in the studied population across various timepoints, hence no statistical analysis was performed.

Body Weight

Statistical analysis showed no significant Treatment effect, Time effect or interaction (Treatment $\times$ Time) effect on body weight (Table 3). Hence no post-hoc test was performed for noting where significant difference lied among the two groups. Mean $\pm$ SD values for the physiological fitness data of the participants investigated across numerous timepoints are depicted in Table 2.

Table 2: Physiological fitness data of the subjects

Variable	Time points	Placebo Group (n=14) Mean $\pm$ SD	Melatonin Group (n=14) Mean $\pm$ SD
Resting HR (beats/min)	Baseline	74.71 $\pm$ 7.53	76.29 $\pm$ 6.66
	Week 1	77.14 $\pm$ 5.60 *	78.07 $\pm$ 6.99
	Week 2	76.64 $\pm$ 4.73	78.50 $\pm$ 5.71 *
	Week 3	73.29 $\pm$ 4.55	79.57 $\pm$ 4.52
	Week 4	76.79 $\pm$ 4.42	74.86 $\pm$ 4.24 \$
SBP (mm of Hg)	Baseline	110.36 $\pm$ 7.70	115.14 $\pm$ 5.25
	Week 1	110.43 $\pm$ 8.16	114.57 $\pm$ 7.21
	Week 2	114.86 $\pm$ 8.10	112.43 $\pm$ 6.89
	Week 3	109.43 $\pm$ 5.52 \$	111.29 $\pm$ 5.00 *
	Week 4	107.71 $\pm$ 4.70 \$	110.86 $\pm$ 6.26 *#
DBP (mm of Hg)	Baseline	75.71 $\pm$ 3.31	77.29 $\pm$ 4.68
	Week 1	76.71 $\pm$ 4.61	76.71 $\pm$ 5.58
	Week 2	77.93 $\pm$ 5.64	75.00 $\pm$ 4.13
	Week 3	75.00 $\pm$ 2.32 \$	74.29 $\pm$ 3.67 *#
	Week 4	75.29 $\pm$ 2.43	74.14 $\pm$ 3.72 *#

Mean $\pm$ SD (Mean $\pm$ Standard deviation), HR = Heart rate, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, VO2max = maximum oxygen uptake

* = Significant with respect to Baseline in the same group at $p < 0.05$

= Significant with respect to Week 1 in the same group at $p < 0.05$

\$ = Significant with respect to Week 2 in the same group at $p < 0.05$

Resting Heart Rate

Statistical analysis depicted a significant interaction (Treatment $\times$ Time) effect on resting heart rate (Table 3). The post-hoc test elucidated that resting heart rate was significantly higher in Week 1 with respect to Baseline value in the PG. However, in the MG resting heart rate was significantly lowered in Week 4 compared to Week 2 but higher in Week 2 with respect to Baseline ($p < 0.05$ for all) (Table 2).

Blood Pressure

Statistical analysis revealed a significant Time effect and interaction (Treatment $\times$ Time) effect on SBP while only a significant Time effect was noted for DBP (Table 3). The post-hoc tests depicted that SBP was significantly lower in the MG for Week 3 and Week 4 with respect to Baseline values and between Week 1 and Week 4. However, in the PG, SBP values were significantly different between Week 2 and Week 3 as well as between Week 2 and Week 4. Moreover, significant differences in DBP values were represented within the groups for Week 3 and Week 4 with respect to Baseline values, between Week 2 and Week 3 and for Week 3 and Week 4 with respect to Week 1 value. ($p < 0.05$ for all) (Table 2).

Table 3: Statistical results for the physical and physiological fitness data of the subjects

Variable	ANOVA		
	Treatment	Time	Treatment $\times$ Time
Body weight (kg)	F = 2.369 $p > 0.05$ $\eta^2 p = 0.083$	F = 0.728 $p > 0.05$ $\eta^2 p = 0.027$	F = 1.906 $p > 0.05$ $\eta^2 p = 0.068$
Resting HR (beats/min)	F = 1.037 $p > 0.05$ $\eta^2 p = 0.038$	F = 1.938 $p > 0.05$ $\eta^2 p = 0.069$	F = 4.438 $p < 0.05$ $\eta^2 p = 0.146$
SBP (mm of Hg)	F = 1.267 $p > 0.05$ $\eta^2 p = 0.046$	F = 5.148 $p < 0.05$ $\eta^2 p = 0.165$	F = 3.214 $p < 0.05$ $\eta^2 p = 0.110$
DBP (mm of Hg)	F = 0.262 $p > 0.05$ $\eta^2 p = 0.010$	F = 3.843 $p < 0.05$ $\eta^2 p = 0.129$	F = 2.421 $p > 0.05$ $\eta^2 p = 0.085$

Mean $\pm$ SD values for the aerobic fitness and anaerobic fitness data of the participants investigated across numerous timepoints are depicted in Table 4.

Table 4: Aerobic fitness and Anaerobic fitness in the studied population

Variable	Time points	Placebo Group (n=14) Mean $\pm$ SD	Melatonin Group (n=14) Mean $\pm$ SD
VO _{2max} (ml/kg/min)	Baseline	57.81 $\pm$ 4.97	58.17 $\pm$ 8.40
	Week 1	58.29 $\pm$ 3.28	57.93 $\pm$ 3.56
	Week 2	56.37 $\pm$ 3.26	59.61 $\pm$ 3.31
	Week 3	56.37 $\pm$ 4.54	59.73 $\pm$ 5.57

	Week 4	56.25 ± 3.10	60.21 ± 7.73
HIE score (sec)	Baseline	12.59 ± 0.59	12.46 ± 1.46
	Week 1	12.44 ± 0.64	12.87 ± 0.93
	Week 2	12.65 ± 0.87	12.78 ± 0.91
	Week 3	12.77 ± 0.82	12.57 ± 0.97
	Week 4	12.33 ± 0.65	12.37 ± 1.03

Mean ± SD (Mean ± Standard deviation), VO₂max = maximum oxygen uptake, HIE = High intensity effort

Maximum Oxygen Uptake or VO₂max

Statistical analysis showed no significant Treatment effect, Time effect or interaction (Treatment × Time) effect on VO₂max (Table 5). Hence no post-hoc test was performed for noting where significant difference lied among the two groups (Placebo group or PG, Melatonin group or MG).

High-Intensity Effort (HIE)

Statistical analysis showed no significant Treatment effect, Time effect or interaction (Treatment × Time) effect on HIE score (Table 5). Hence no post-hoc test was performed for noting where significant difference lied among the two groups.

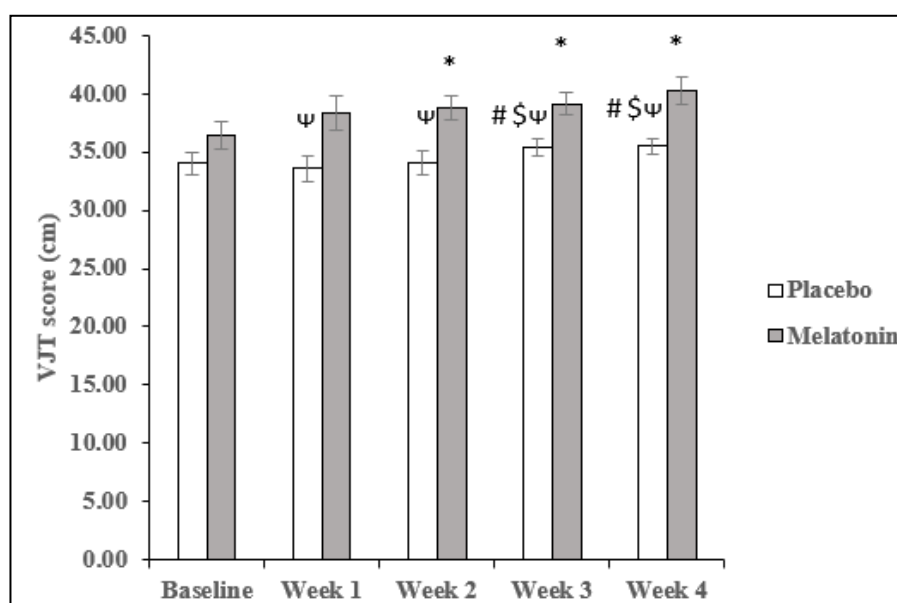


Fig 1: Vertical jump test (VJT) score (cm) in the studied population at various timepoints

* = Significant with respect to Baseline in the same group at p<0.05

= Significant with respect to Week 1 in the same group at p<0.05

\$ = Significant with respect to Week 2 in the same group at p<0.05

ψ = Significant with respect to Melatonin group in the same timepoint at p<0.05

Vertical Jump Test (VJT) Score

Statistical analysis demonstrated a significant Treatment and Time effects on VJT score (Table 5). Post-hoc test showed that within the groups, VJT score was significantly different for Week 2, Week 3, Week 4 with respect to baseline values, in Week 3 and Week 4 when compared to Week 1 as well as in Week 3 and Week 4 in comparison to Week 2. VJT score was significantly

higher in case of the MG in comparison to the PG for Week 1, Week 2, Week 3 and Week 4 ($p < 0.05$ for all) (Fig 1).

Table 5: Statistical results for the aerobic fitness, anaerobic fitness and VJT score data of the subjects

Variable	ANOVA		
	Treatment	Time	Treatment × Time
VO _{2max} (ml/kg/min)	F = 2.097 p > 0.05 $\eta^2 p = 0.075$	F = 0.020 p > 0.05 $\eta^2 p = 0.001$	F = 1.921 p > 0.05 $\eta^2 p = 0.069$
HIE score (sec)	F = 0.032 p > 0.05 $\eta^2 p = 0.001$	F = 2.572 p > 0.05 $\eta^2 p = 0.090$	F = 1.827 p > 0.05 $\eta^2 p = 0.066$
VJT score (cm)	F = 9.522 p < 0.05 $\eta^2 p = 0.268$	F = 7.194 p < 0.05 $\eta^2 p = 0.217$	F = 1.761 p > 0.05 $\eta^2 p = 0.063$

Mean ± SD values for the handgrip strengths data of the participants investigated across numerous timepoints are depicted in Table 6.

Table 6: Handgrip Strengths of the subjects

Variable	Time points	Placebo Group (n=14) Mean ± SD	Melatonin Group (n=14) Mean ± SD
Right handgrip strength (kg)	Baseline	15.01 ± 3.99	15.92 ± 7.01
	Week 1	13.66 ± 4.36	16.78 ± 6.40
	Week 2	13.83 ± 3.76	15.03 ± 6.97 *#
	Week 3	13.94 ± 3.51 *	15.22 ± 6.92
	Week 4	14.38 ± 3.71	15.86 ± 7.32 \$
Left handgrip strength (kg)	Baseline	12.84 ± 3.62	13.03 ± 6.60
	Week 1	13.32 ± 2.85	13.09 ± 5.46
	Week 2	14.12 ± 3.58	13.06 ± 6.17
	Week 3	12.77 ± 2.76	12.23 ± 5.72 #
	Week 4	14.11 ± 2.70 *£	13.20 ± 6.18 £

Mean ± SD (Mean ± Standard deviation)

* = Significant with respect to Baseline in the same group at $p < 0.05$

= Significant with respect to Week 1 in the same group at $p < 0.05$

\$ = Significant with respect to Week 2 in the same group at $p < 0.05$

£ = Significant with respect to Week 3 in the same group at $p < 0.05$

Handgrip Strength

Statistical analysis revealed a significant Time effect and interaction (Treatment × Time) effect on right handgrip strength while only a significant Time effect was noted for left handgrip strength (Table 7). Post-hoc tests demonstrated that in the MG right handgrip strength was significantly lower in Week 2 with respect to both Baseline as well as Week 1 although the value was significantly higher in Week 4 with respect to Week 2. However, the left handgrip strength within the groups was significantly different in Week 3 with respect to both Week 1 and Week 2, in Week 4 with respect to both Week 3 and Baseline values. In the PG, right handgrip strength was significantly less in Week 3 with respect to Baseline ($p < 0.05$ for all) (Table 6).

Handgrip Endurance

Statistical analysis revealed a significant Treatment effect and Time effect on handgrip endurance (Table 7). Post-hoc test elucidated that within the groups, handgrip endurance was significantly different for both Week 2, Week 3 and Week 4 with respect to Baseline as well as in Week 2 and Week 3 with respect to Week 1. Moreover, handgrip endurance was significantly higher in the MG in contrast to the PG for Week 3 ($p < 0.05$ for all) (Fig 2).

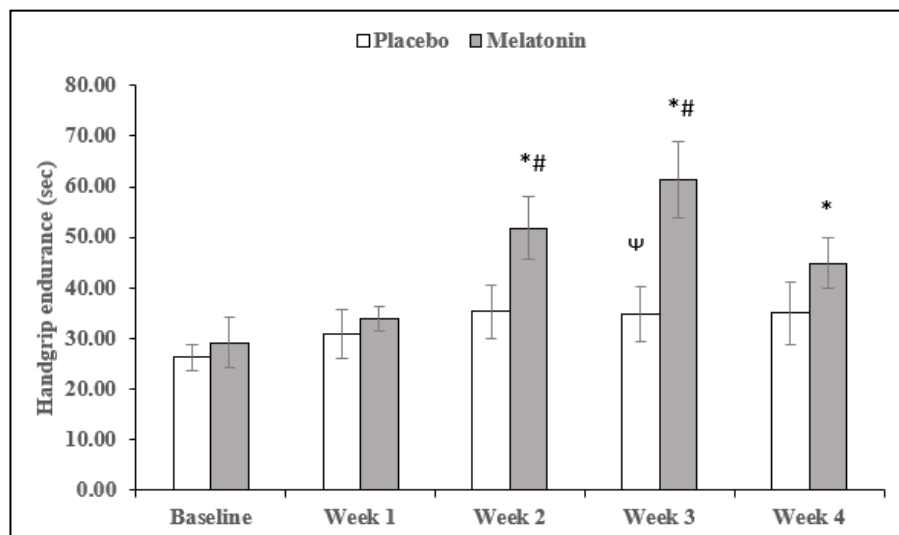


Fig 2: Handgrip endurance (sec) in the studied population at various timepoints

* = Significant with respect to Baseline in the same group at $p < 0.05$

= Significant with respect to Week 1 in the same group at $p < 0.05$

ψ = Significant with respect to Melatonin group in the same timepoint at $p < 0.05$

Table 7: Statistical results for the handgrip strengths and handgrip endurance data of the subjects

Variable	ANOVA		
	Treatment	Time	Treatment × Time
Right handgrip strength (kg)	F = 0.572 p > 0.05 η ² p = 0.022	F = 2.825 p < 0.05 η ² p = 0.098	F = 2.944 p < 0.05 η ² p = 0.102
Left handgrip strength (kg)	F = 0.083 p > 0.05 η ² p = 0.003	F = 4.661 p < 0.05 η ² p = 0.152	F = 1.625 p > 0.05 η ² p = 0.059
Handgrip endurance (sec)	F = 7.587 p < 0.05 η ² p = 0.226	F = 5.858 p < 0.05 η ² p = 0.184	F = 2.164 p > 0.05 η ² p = 0.077

Mean ± SD values for the motor fitness data of the participants assessed across various timepoints have been demonstrated in Table 8.

Table 8: Motor fitness data of the subjects

Variable	Time points	Placebo Group (n=14) Mean ± SD	Melatonin Group (n=14) Mean ± SD
Agility (sec)	Baseline	12.37 ± 1.25	12.63 ± 1.11

	Week 1	12.42 ± 1.15	12.29 ± 0.95
	Week 2	12.44 ± 1.08	12.21 ± 0.84
	Week 3	12.71 ± 1.05	12.16 ± 1.31
	Week 4	11.96 ± 1.11	11.63 ± 0.61
Flexibility (cm)	Baseline	13.40 ± 3.16	13.77 ± 3.67
	Week 1	13.47 ± 2.99	15.41 ± 3.28
	Week 2	14.67 ± 4.38	15.79 ± 3.37 *
	Week 3	14.62 ± 4.20 #	16.12 ± 4.34 *
	Week 4	14.37 ± 3.78	16.94 ± 4.53 *#
Balance score (sec)	Baseline	217.29 ± 138.51	173.76 ± 81.11
	Week 1	236.32 ± 158.11 *	185.97 ± 105.07
	Week 2	247.96 ± 156.86	192.93 ± 111.69
	Week 3	236.75 ± 156.30	227.78 ± 118.44 *#
	Week 4	237.60 ± 164.63	241.61 ± 120.19 *#
Reaction time (sec)	Baseline	0.6075 ± 0.09	0.5911 ± 0.06
	Week 1	0.6110 ± 0.08	0.6613 ± 0.04
	Week 2	0.6047 ± 0.08	0.6298 ± 0.07
	Week 3	0.6016 ± 0.05	0.6031 ± 0.07
	Week 4	0.6187 ± 0.07	0.6033 ± 0.04

Mean ± SD (Mean ± Standard deviation)

* = Significant with respect to Baseline in the same group at $p < 0.05$

= Significant with respect to Week 1 in the same group at $p < 0.05$

Agility

Statistical analysis showed no significant Treatment effect, Time effect or interaction (Treatment × Time) effect on agility score (Table 9). Hence no post-hoc test was performed for noting where significant difference lied among the two groups.

Flexibility

Statistical analysis showed a significant Time effect on flexibility score (Table 9). Post-hoc tests showed that flexibility score within the groups was significantly different in Week 2, Week 3 and Week 4 with respect to Baseline apart from Week 3 and Week 4 with respect to Week 1 ($p < 0.05$ for all) (Table 8).

Reaction Time

Statistical analysis showed no significant Treatment effect, Time effect or interaction (Treatment × Time) effect on reaction time (Table 9). Hence no post-hoc test was performed for noting where significant difference lied among the two groups.

Balance Score

Statistical analysis depicted a significant Time effect and interaction (Treatment × Time) effect on balance score (Table 9). The post-hoc test revealed that in the MG balance score was significantly higher in Week 3 and Week 4 with respect to Baseline while the values were also significantly greater in both Week 3 and Week 4 with respect to Week 1. In the PG, balance score was significantly higher only in Week 1 with respect to Baseline score ($p < 0.05$ for all) (Table 8).

Table 9: Statistical results for the motor fitness data of the subjects

Variable	ANOVA		
	Treatment	Time	Treatment × Time
Agility (sec)	F = 0.480 p > 0.05 $\eta^2 p = 0.018$	F = 3.192 p > 0.05 $\eta^2 p = 0.109$	F = 0.902 p > 0.05 $\eta^2 p = 0.034$
Flexibility (cm)	F = 1.313 p > 0.05 $\eta^2 p = 0.048$	F = 6.635 p < 0.05 $\eta^2 p = 0.203$	F = 1.606 p > 0.05 $\eta^2 p = 0.058$
Balance score (sec)	F = 0.396 p > 0.05 $\eta^2 p = 0.015$	F = 5.736 p < 0.05 $\eta^2 p = 0.181$	F = 3.334 p < 0.05 $\eta^2 p = 0.114$
Reaction time (sec)	F = 0.207 p > 0.05 $\eta^2 p = 0.008$	F = 2.474 p > 0.05 $\eta^2 p = 0.087$	F = 2.351 p > 0.05 $\eta^2 p = 0.083$

DISCUSSION

Results of our study depicted that there was no significant effect of melatonin (3mg) on body weight in the health sedentary young men. A systematic review and meta-analysis of controlled trials carried out among human subjects was performed to deduce the role of melatonin on body weight. Results of this review suggested that melatonin exerted its effects on body weight when used in adjunction to a medication that had either increased or reduced body weight of the patient. The study findings highlighted that while the standard treatment led to a decrease in body weight, melatonin was capable of diminishing this impact. Contrarily melatonin exerted buffering effect in situations where standard treatment led to an increase in body weight. Thus, it became apparent that melatonin when used in adjunction with first choice of medication aided in reducing the side effects on body weight and these effects were pronounced in children and adolescents. Hence, all these evidences indicated towards melatonin's buffering role with respect to fluctuations in body weight [26]. Further, in another study, reduction in body weight was noted in case of female subjects supplemented with melatonin (3mg) who were early chronotypes exhibiting high circadian misalignment devoid of any alterations in physical activity levels or calorie intake [27]. Similar findings of reduction in body weight were achieved in melatonin supplemented groups compared to placebo groups particularly better in studies that utilized ≤ 8 mg melatonin daily doses as depicted in systematic review and meta-analysis [28]. However, in another randomised-controlled trial, post-menopausal women when supplemented with 1or 3mg of daily doses of melatonin at nighttime for 1 year showed no significant change in body weight between the two groups [29]. Our study deviated from all the previous findings in manner that the dose (3mg) of melatonin utilized was not enough to induce weight loss or weight gain particularly when administered alone without any other medication or supplement.

Our study showed no significant change in VO_{2max} among or between the two groups due to melatonin or placebo ingestion. In a study by Atkinson et al. [30] administration of 5mg of melatonin in a double-blind manner among physically active male participants showed that melatonin had no influence on time trial performance and our findings were consistent with their results. The dose of 3mg of melatonin administered nocturnally for 4 weeks was not sufficient to elicit any ergogenic benefit in healthy sedentary young men. However, acute

supplementation of oral melatonin (6mg) among healthy men were found to enhance the aerobic tolerance at maximal aerobic capacity intensity (iMAC) in 19% at the cyclo-ergometer [31]. This was contradictory to our finding and the reason may be due to the variation in dose of melatonin administered in the present study.

Data pertaining to the effect of melatonin administration on resting heart rate in humans are limited. Nevertheless, Souissi et al. [32] in their randomised controlled study on young healthy and moderately trained male participants acutely administered melatonin (6mg) 50 min prior to exercise where they ran at 60% of their maximal aerobic speed for 45 min. Heart rate was measured for both the melatonin and placebo groups. It was observed that at the end of exercise, heart rate was significantly lower in melatonin group in contrast to their placebo counterparts. Our findings were consistent with these results whereby an interaction effect was prominent with respect to resting heart rate and in MG at the end of the study duration in Week 4 the values were lowered compared to Week 2. Further in recent research by Eo et al. [33] it has been demonstrated that circadian rhythmicity of melatonin like endocrine hormone is correlated with cardiovascular rhythmicity such as RR intervals. In this context, heart rate has been deemed as a suitable alternative to melatonin as a reliable circadian biomarker for personal healthcare that do not have the problem of assessment and monitoring over 24hour duration.

The potential impact of melatonin supplementation on the blood pressure (BP) of people with metabolic disorders has been evaluated in a systematic review and meta-analysis of randomised controlled trials (RCTs). The pooled analysis of the study findings confirmed that melatonin is capable of significantly reducing both the systolic blood pressure (SBP) and diastolic blood pressure (DBP) in patients with metabolic disorders. It has been suggested that the BP lowering effect of melatonin is achieved through stabilizing the functioning of the biological clock as well as indirectly the hypotensive effect is incurred through improvement of the metabolic status of the person involved [34]. These were in congruity with our findings where we found that in MG there was a declining trend in both SBP and DBP over time and values were consistently lowered at the end of the study. In yet another systematic review and meta-analysis of RCTs, the favourable impact of melatonin in regulating blood pressure has been revealed thereby making it a potent and novel candidate for treating essential hypertension [35]. Research has further shown that temporal rise in nocturnal melatonin concentration has the ability to reduce high peripheral vascular resistance, thereby lowering blood pressure and heart rate and enhancing the cardiac output. Additionally, supplementation of nocturnal melatonin for three weeks regulated the circadian rhythm of blood pressure, thus lowering the risk of ventricular hypertrophy [36]. Probing deeper, it has been found that the regulation of the suprachiasmatic nucleus and vasopressin release dictate the impact of melatonin in the cardiovascular context. The regulatory mechanism behind the control of blood pressure by melatonin involves membrane G-protein coupled receptors viz., MT1 and MT2 receptors located in the tunica intima and media layers of the vascular wall alongside lowered levels of catecholamines [37]. Further, nitric oxide (NO) also plays a vital role in blood pressure regulation and exogenous melatonin intake through modulating the activity of endothelial nitric oxide synthase (eNOS) enzyme and significantly decreasing the oxidative stress and levels of NF- κ B [38].

High intensity effort (HIE) score is indicative of the anaerobic power of an individual and refers to the capacity to overcome heavy resistance with high speed (Basu et al., 2019)[39]. In our study there was no significant change in HIE score among the participants. Contradictory to our findings, Paryab et al. [40] in their study depicted that ingestion of 6mg of melatonin prior to 30 mins of exercise among collegiate student athletes improved the anaerobic power following both 4 hr of sleep deprivation as well as 24 hr of sleep deprivation. Faria et al. [41] in their systematic review studied the influence of acute melatonin administration on human physical performance and found no significant change in power due to melatonin while a reduction in power performance was noted in specific tests following melatonin ingestion. In yet another study it was found that there was no significant change in anaerobic power in young trained males who were supplemented with melatonin (10mg) following total night sleep deprivation [42]. Similar findings have been stated in our study and thus anaerobic power remained unchanged due to nocturnal melatonin (3mg) ingestion in sedentary young men.

Handgrip strength is reflective of the upper body muscular strength [43]. In male individuals, testosterone hormone plays a vital role in muscle enlargement [44, 45]. Moreover, males possess greater number of fast types II fibres having enhanced glycolytic enzyme activity under the influence of male hormone testosterone [44, 46]. In a double-blind randomized study design melatonin (5mg) was administered in the morning 30 mins before conducting the test battery on male soccer players to study the impact of morning melatonin ingestion on physical and cognitive tasks measured during afternoon. Handgrip performances among the study participants were evaluated at three test periods at 08:00 h, 12:00 h and 16:00 h. In case of the melatonin supplemented group, handgrip performances were significantly greater at 16:00 h than 12:00 h and 08:00 h. Additionally, when compared with the placebo group, melatonin group depicted significantly lowered handgrip performance in the morning [47]. In line with the beneficial impact of melatonin on handgrip strength performance our study showed that both right handgrip strength and left handgrip strength showed marked improvement in the MG with an optimal performance being noted for Week 4, i.e., at the end of the study.

Handgrip endurance is another vital indicator of the functional status of an individual particularly concerning the functional integrity of the upper extremity [48]. Several factors like that of age, sex, muscle strength, built, nutritional status and arm span impact handgrip endurance. Moreover, BMI, handgrip strength and handgrip endurance have been reported to have a correlation [49]. Dhananjaya et al. [49] in their work further established that in case of the normal weight individuals, there exists a statistically insignificant positive correlation between BMI and handgrip endurance. However, to the best of our knowledge no studies until now have explored the impact of chronic melatonin intake on handgrip endurance and thus our finding will add value to this under-explored parameter. Congruent to the results reported in previous works, our study confirmed that as compared to the PG, the MG showed a significantly improved handgrip performance with time and optimal handgrip endurance performance was noted for Week 3 probably due to the ergogenic impact of melatonin supplementation.

Agility or coordinative abilities is considered a vital component of motor fitness and is usually referred to as the generalized and relatively stabilized pattern of motor control and regulation process [39]. No significant difference in agility were observed between MG and PG thereby highlighting no impact of chronic 3mg melatonin supplementation in altering agility score among healthy sedentary young men. The effect of morning melatonin (5mg) intake on physical

performance assessed in the afternoon among soccer players was evaluated [47]. A significant difference in terms of agility was noted among the melatonin group when measured at 08:00 h, 12:00 h and 16:00 h with performances being higher at 16:00 h than 08:00 h and 12:00 h. However, with respect to any daytime no significant difference was noted between melatonin and placebo group. A randomized study design was adopted to determine if melatonin (6mg) intake was capable of improving recovery in the female athletes following an intermittent training session. It was shown that melatonin did not have any significant effect of Condition, Exercise and interaction Condition x Exercise for performance variable measured through modified agility test [50]. A similar finding was reported by Ghattassi et al. [51] where they reported no significant difference between placebo and nocturnal 5mg melatonin intake in agility test among professional soccer players. Even our findings were consistent with the outcomes as reported in previous studies.

Flexibility is yet another crucial component of motor fitness and is reflective of the strength of both the lower back muscle and hamstring muscle [52]. In a study conducted by Farjallah et al. [53] effect of daily melatonin administration (5mg) during an intensive training schedule on physical performance was investigated in case of soccer players. It was concluded that melatonin ingestion had beneficial impact as it caused alleviation of deterioration in physical performance as observed through countermovement jump, five-jump test and others when compared with placebo. Another study focused on assessing the safety and efficacy of nocturnal melatonin (6mg) ingestion with respect to functional mobility and risk of falling in case of adults having multiple sclerosis disease. It was reflected that melatonin probably by means of improvement of sleep quality and cognitive function led to enhanced functional mobility due to acute melatonin intake [54]. All these findings were also congruent to our observations that depicted a significant progressive rise in flexibility score in the MG with the optimal enhanced performance observed at the end of the study period i.e., in Week 4.

The explosive strength of the leg muscles is usually evaluated by means of the VJT score [39]. A study conducted among trained young men assessed the impacts of melatonin (10mg) supplementation on physiological and physical performance following total night sleep deprivation. It was presented that total night sleep deprivation led to impaired jump strength performance although no significant change in jump strength was noted due to melatonin administration in the studied group as well as in condition of normal habitual sleep [42]. Research has exposed that inside vascular smooth muscles, melatonin acts as a mediator between two opposing responses in a concentration-dependent manner. At lower concentrations, melatonin increases contractions, while in higher concentrations it exerts a negative impact [55]. Mero et al. [56] in their randomized controlled study design carried out among healthy male resistance trained volunteers depicted no significant acute effect on maximal jumping ability when supplemented with melatonin (10mg) orally prior to resistance exercise session. Interestingly, daytime oral ingestion of melatonin (5mg) among soccer players showed a significant daytime effect on jump test when performances were greater at 16:00 h than 08:00 h in the melatonin group, although no significant difference was noted between placebo and melatonin group for any daytime [47]. Interestingly in our study we found that chronic nocturnal melatonin (3mg) supplementation significantly differed between the PG and MG with the MG exhibiting greater VJT score at the end of the study period in Week 4 with progressive better score from Week 1 onwards. The observations were taken diurnally on a weekly basis in the next morning following nocturnal ingestion. The improved VJT score may

be accorded to the chronobiotic role of melatonin that favoured good sleep health and consequently providing better strength to perform the next morning [54]. However, in yet another study that administered a single dose of oral melatonin (10mg) following strenuous late-evening exercise among healthy male adolescent athletes, it was seen that while there was no significant difference in horizontal and vertical jumps, contrarily an improved performance was recorded in 5-jump test upon ingesting melatonin [57]. Similar findings have been reported in the study by Farjallah et al. [50] where acute supplementation with oral melatonin (6mg) on elite female handball players did not show any significant difference in short-term physical performance for parameters such as squat jump, counter-movement jump in both before and after intermittent training session.

Reaction time is considered as one of the most vital components of motor fitness and appears as a reliable measure for the central nervous system's rate of processing of sensory stimuli as well as its execution as motor response [39]. It is useful for denoting the general alertness of a subject [58]. The outcomes of our study did not reveal any significant effect of melatonin on reaction time when administered nocturnally in a daily dose of 3mg for 4 weeks among healthy sedentary young men. Ghattassi et al. [59] also investigated the effects of morning ingestion of melatonin (5mg) in male soccer players by performing simple reaction time test. Reaction time performances were measured at three times of the day such as 08:00 h, 12:00 h and 16:00 h, while the performance was lower at 08:00 h in the melatonin group compared to their placebo counterparts; performances were higher at 12:00 h and 16:00 h regardless of the treatment given. Interestingly data retrieved from a systematic review and meta-analysis of RCTs exposed that in case of the healthy subjects, daytime supplementation of melatonin did not impair reaction time. Moreover, subgroup analysis of data also highlighted that supplementation with a 5mg dose of melatonin during daytime did not negatively correlate with the reaction time score, although doses with 10mg or more adversely correlated with the reaction time score [60]. In our study the selected dose of 3mg of melatonin was given to the subjects at nighttime and 30-60 mins before bedtime and therefore this dose might have been insufficient to alter the reaction time when measured the following morning every week.

An integral component of neuromuscular fitness is assigned to balance that in turn helps to successfully perform a motor activity. Fundamental movement skills are performed, learned and controlled through the power of balance. Investigation carried out with respect to acute supplementation with melatonin on physical performance in humans showed that melatonin improved balance performance at least in non-athletes [41]. Further, a pilot study conducted on adults with multiple sclerosis depicted that acute nocturnal administration of melatonin (6mg) was safe for improving postural balance when evaluated the following morning possibly through improvement in quality of sleep as well as cognitive functions [54]. However, it has been reported that diurnal melatonin ingestion in healthy young men might cause loss of balance [61]. Our study refutes this particular finding as it was evident from our observation that in the MG there was a gradual improvement in balance with the optimal attained at the end of study duration in Week 4. Our findings were also congruent with the findings by Khaleghi-Mamaghani et al. [42] who upon conducting a study on trained young males revealed that melatonin (10mg) supplementation following total night sleep deprivation led to significant improvement in both static and dynamic balance.

CONCLUSION

The present study highlights that chronic nocturnal melatonin supplementation for four weeks is effective in improving sedentary behaviour in healthy, young men through betterment of certain fitness variables. Melatonin significantly bettered both leg explosive strength evident through increased VJT score over time and handgrip endurance compared to the placebo. In addition to these, a temporal improvement in flexibility, balance, handgrip strengths (right and left hand) and blood pressure (both systolic and diastolic) was observed in the group treated with melatonin thereby indicating its positive impact on health and fitness.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Sugata Sen Roy for his invaluable guidance in performing the statistical analyses and to Mr. Debajyoti Mukherjee for his unconditional support during the execution of the study. We are also indebted to all the participants for their whole-hearted participation and cooperation in conducting the work.

DECLARATIONS

Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

Funding

The authors did not receive support from any organization for the submitted work.

Ethics approval

Approval was obtained from the Institutional Human Ethics Committee of the Department of Physiology, University of Calcutta (Ref No. IHEC/AB/P84/2019). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Informed Consent

All the participants gave their written informed prior to taking part in the study.

References

- [1] Penedo FJ, Dahn JR. Exercise and well-being: a review of mental and physical health benefits associated with physical activity. *Curr Opin Psychiatry*. 2005;18(2):189-93. doi: 10.1097/00001504-200503000-00013.
- [2] Jochem C, Wallmann-Sperlich B, Leitzmann MF. The Influence of Sedentary Behavior on Cancer Risk: Epidemiologic Evidence and Potential Molecular Mechanisms. *Curr Nutr Rep*. 2019;8(3):167-174. doi: 10.1007/s13668-019-0263-4.
- [3] Park JH, Moon JH, Kim HJ, Kong MH, Oh YH. Sedentary Lifestyle: Overview of Updated Evidence of Potential Health Risks. *Korean J Fam Med*. 2020;41(6):365-373. doi: 10.4082/kjfm.20.0165.
- [4] Moxley E, Webber-Ritchey KJ, Hayman LL. Global impact of physical inactivity and implications for public health nursing. *Public Health Nursing*. 2022;39(1):180-8. doi: <https://doi.org/10.1111/phn.12958>.
- [5] Hemati K, Pourhanifeh MH, Dehdashtian E, Fatemi I, Mehrzadi S, Reiter RJ, Hosseinzadeh A. Melatonin and morphine: potential beneficial effects of co-use. *Fundam Clin Pharmacol*. 2021;35(1):25-39. doi: 10.1111/fcp.12566.
- [6] Cardinali DP. Are melatonin doses employed clinically adequate for melatonin-induced cytoprotection? *Melatonin Res*. 2019; 2(2):106-32. doi: <https://doi.org/10.32794/mr11250025>

- [7] Drust B, Cable NT, Reilly T. Investigation of the effects of the pre-cooling on the physiological responses to soccer-specific intermittent exercise. *Eur J Appl Physiol*. 2000;81(1-2):11-7. doi: 10.1007/PL00013782.
- [8] Dollins AB, Lynch HJ, Wurtman RJ, Deng MH, Kischka KU, Gleason RE, Lieberman HR. Effect of pharmacological daytime doses of melatonin on human mood and performance. *Psychopharmacology (Berl)*. 1993;112(4):490-6. doi: 10.1007/BF02244899.
- [9] Slotten HA, Krekling S. Does melatonin have an effect on cognitive performance? *Psychoneuroendocrinology*. 1996;21(8):673-80. doi: 10.1016/s0306-4530(96)00027-3.
- [10] Cagnacci A, Kräuchi K, Wirz-Justice A, Volpe A. Homeostatic versus circadian effects of melatonin on core body temperature in humans. *J Biol Rhythms*. 1997;12(6):509-17. doi: 10.1177/074873049701200604.
- [11] Rogers NL, Phan O, Kennaway DJ, Dawson D. Effect of daytime oral melatonin administration on neurobehavioral performance in humans. *J Pineal Res*. 1998;25(1):47-53. doi: 10.1111/j.1600-079x.1998.tb00385.x.
- [12] Mazepa RC, Cuevas MJ, Collado PS, González-Gallego J. Melatonin increases muscle and liver glycogen content in nonexercised and exercised rats. *Life Sci*. 2000;66(2):153-60. doi: 10.1016/s0024-3205(99)00573-1.
- [13] Tremblay MS, Colley RC, Saunders TJ, Healy GN, Owen N. Physiological and health implications of a sedentary lifestyle. *Appl Physiol Nutr Metab*. 2010;35(6):725-40. doi: 10.1139/H10-079.
- [14] Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, Carty C, Chaput JP, Chastin S, Chou R, Dempsey PC, DiPietro L, Ekelund U, Firth J, Friedenreich CM, Garcia L, Gichu M, Jago R, Katzmarzyk PT, Lambert E, Leitzmann M, Milton K, Ortega FB, Ranasinghe C, Stamatakis E, Tiedemann A, Troiano RP, van der Ploeg HP, Wari V, Willumsen JF. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med*. 2020;54(24):1451-1462. doi: 10.1136/bjsports-2020-102955.
- [15] Saleem SM, Jan SS. Modified Kuppuswamy socioeconomic scale updated for the year 2019. *Indian J Forensic Community Med*. 2019;6(1):1-3. doi: <https://doi.org/10.18231/2394-6776.2019.0001>
- [16] Craig C, Marshall A, Sjostrom M, Bauman A, Lee P, Macfarlane D, Lam T, Stewart S. International physical activity questionnaire-short form. *J Am Coll Health*. 2017;65(7):492-501.
- [17] Maldonado MD, Manfredi M, Ribas-Serna J, Garcia-Moreno H, Calvo JR. Melatonin administered immediately before an intense exercise reverses oxidative stress, improves immunological defenses and lipid metabolism in football players. *Physiol Behav*. 2012;105(5):1099-103. doi: 10.1016/j.physbeh.2011.12.015.
- [18] Foley HM, Steel AE. Adverse events associated with oral administration of melatonin: A critical systematic review of clinical evidence. *Complementary therapies in medicine*. 2019; 42:65-81. doi: 10.1016/j.ctim.2018.11.003.
- [19] Dalui R, Bandyopadhyay A. Fitness Profile of Indian Male Karate Players. *Journal of Combat Sports & Martial Arts*. 2016;7(1). doi: 10.5604/20815735.1223401.
- [20] World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-4. doi: 10.1001/jama.2013.281053.
- [21] Nabi T, Rafiq N, Qayoom O. Assessment of cardiovascular fitness [VO2 max] among medical students by Queens College step test. *Int j Biomed adv res*. 2015;6(5):418-21. doi: 10.7439/ijbar
- [22] Roy AS, Bandyopadhyay A. Effect of Ramadan intermittent fasting on selective fitness profile parameters in young untrained Muslim men. *BMJ Open Sport Exerc Med*. 2015;1(1): e000020. doi: 10.1136/bmjsem-2015-000020.
- [23] Dalui R, Roy AS, Bandyopadhyay A. Fitness profile of male swimmers and water polo players of kolkata, India-A comparative study. *Indian J Physiol Pharmacol*. 2018;62(4):397-406.
- [24] Pollack AE. Exploring the Complexities of Experimental Design: Using an On-line Reaction Time Program as a Teaching Tool for Diverse Student Populations. *J Undergrad Neurosci Educ*. 2010;9(1): A47-50.

- [25] Balogun JA, Ajayi LO, Alawale F. Determinants of single limb stance balance performance. *Afr J Med Med Sci.* 1997;26(3-4):153-7.
- [26] Mostafavi SA, Akhondzadeh S, Mohammadi MR, Keshtkar AA, Hosseini S, Eshraghian MR, Motlagh TA, Alipour R, Keshavarz SA. Role of Melatonin in Body Weight: A Systematic Review and Meta-Analysis. *Curr Pharm Des.* 2017;23(23):3445-3452. doi: 10.2174/1381612822666161129145618.
- [27] Marqueze EC, Nogueira LFR, Vetter C, Skene DJ, Cipolla-Neto J, Moreno CRC. Exogenous melatonin decreases circadian misalignment and body weight among early types. *J Pineal Res.* 2021;71(2):e12750. doi: 10.1111/jpi.12750.
- [28] Delpino FM, Figueiredo LM. Melatonin supplementation and anthropometric indicators of obesity: A systematic review and meta-analysis. *Nutrition.* 2021;91-92:111399. doi: 10.1016/j.nut.2021.111399.
- [29] Amstrup AK, Sikjaer T, Pedersen SB, Heickendorff L, Mosekilde L, Rejnmark L. Reduced fat mass and increased lean mass in response to 1 year of melatonin treatment in postmenopausal women: A randomized placebo-controlled trial. *Clin Endocrinol (Oxf).* 2016;84(3):342-7. doi: 10.1111/cen.12942.
- [30] Atkinson G, Jones H, Edwards BJ, Waterhouse JM. Effects of daytime ingestion of melatonin on short-term athletic performance. *Ergonomics.* 2005;48(11-14):1512-22. doi: 10.1080/00140130500100967.
- [31] Beck WR, Messias LH, Silva FC, Manchado-Gobatto FB, Gobatto CA. Acute melatonin administration enhances aerobic tolerance: an analysis of biochemical and hematological parameters. *Motriz: Revista de Educação Física.* 2018;24. doi: <https://doi.org/10.1590/S1980-6574201800010014>.
- [32] Souissi A, Yousfi N, Dabboubi R, Aloui G, Haddad M, Souissi N. Effect of acute melatonin administration on physiological response to prolonged exercise. *Biological Rhythm Research.* 2020;51(6):980-7. doi: 10.1080/09291016.2019.1573462.
- [33] Eo YJ, Park J, Kim S, Lee KN, Lee SM, Kim DH, Kim C, Do YR. Estimation of melatonin level and core body temperature: heart rate and heart rate variability as circadian rhythm markers. *Biological Rhythm Research.* 2022;53(11):1735-52. doi: 10.1080/09291016.2021.2024408.
- [34] Akbari M, Ostadmohammadi V, Mirhosseini N, Lankarani KB, Tabrizi R, Keshtkaran Z, Reiter RJ, Asemi Z. The effects of melatonin supplementation on blood pressure in patients with metabolic disorders: a systematic review and meta-analysis of randomized controlled trials. *J Hum Hypertens.* 2019;33(3):202-209. doi: 10.1038/s41371-019-0166-2.
- [35] Hadi A, Ghaedi E, Moradi S, Pourmasoumi M, Ghavami A, Kafeshani M. Effects of Melatonin Supplementation on Blood Pressure: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Horm Metab Res.* 2019;51(3):157-164. doi: 10.1055/a-0841-6638.
- [36] Draper B, Yee WL, Pedrana A, Kyi KP, Qureshi H, Htay H, Naing W, Thompson AJ, Hellard M, Howell J. Reducing liver disease-related deaths in the Asia-Pacific: the important role of decentralised and non-specialist led hepatitis C treatment for cirrhotic patients. *Lancet Reg Health West Pac.* 2022; 20:100359. doi: 10.1016/j.lanwpc.2021.100359.
- [37] Macchi MM, Bruce JN. Human pineal physiology and functional significance of melatonin. *Frontiers in neuroendocrinology.* 2004;25(3-4):177-95. doi: 10.1016/j.yfrne.2004.08.001.
- [38] Pandi-Perumal SR, BaHammam AS, Ojike NI, Akinseye OA, Kendzerska T, Buttoo K, Dhandapany PS, Brown GM, Cardinali DP. Melatonin and Human Cardiovascular Disease. *J Cardiovasc Pharmacol Ther.* 2017;22(2):122-132. doi: 10.1177/1074248416660622.
- [39] Basu S, Roy AS, Dalui R, Bandyopadhyay A, Basu S, Roy S. Physical fitness of basketball and volleyball players of Kolkata, India. *International Journal of Clinical and Experimental Physiology.* 2019;6(4):111-7. doi: <https://doi.org/10.5530/ijcep.2019.6.4.31>.
- [40] Paryab N, Taheri M, H'Mida C, Irandoust K, Mirmoezzi M, Trabelsi K, Ammar A, Chtourou H. Melatonin supplementation improves psychomotor and physical performance in collegiate student-athletes following a sleep deprivation night. *Chronobiol Int.* 2021;38(5):753-761. doi: 10.1080/07420528.2021.1889578.

- [41] Faria VS, Messias LHD, Pejon TMM, Beck WR. Influence of Acute Melatonin Administration on Human Physical Performance: A Systematic Review. *Sports Health*. 2024;16(1):70-78. doi: 10.1177/19417381231155142.
- [42] Khaleghi-Mamaghani E, Rahmani-Nia F, Arazi H. Evaluation of the effects of melatonin supplementation on the physical and physiological performance following total night sleep deprivation in trained young males. *Journal of Turkish Sleep Medicine*. 2021;8(2):151. doi: 10.4274/jtsm.galenos.2021.63626.
- [43] Guidetti L, Musulin A, Baldari C. Physiological factors in middleweight boxing performance. *J Sports Med Phys Fitness*. 2002;42(3):309-14.
- [44] Astrand, PO, Rodahl K. Neuromuscular junction. In: *Textbook of Work Physiology*, International Student edition, Tokyo: McGraw-Hill Kogakusha Ltd.; 1970. pp. 35-100.
- [45] Manjuanth H, Venkatesh D, Rajkumar S, Taklikar RH. Gender difference in hand grip strength and electromyogram (EMG) changes in upper limb. *Res. J. Pharm. Biol. Chem. Sci*. 2015;6(4):1889-93.
- [46] Morehouse LE, Miller AT. Strength. In: *Physiology of Exercise*. 5th ed. London: The CV Mosby Company; 1967. pp.50-60.
- [47] Ghattassi K, Hammouda O, Graja A, Boudhina N, Chtourou H, Hadhri S, Driss T, Souissi N. Morning melatonin ingestion and diurnal variation of short-term maximal performances in soccer players. *Physiol Int*. 2016;103(1):94-104. doi: 10.1556/036.103.2016.1.9.
- [48] Gurav P, Saini S, Palekar TJ. Correlation between body composition and hand grip strength amongst young physical therapists. *Journal of Emerging Technologies and Innovative Research (JETIR)*. 2019;6(6):279-84.
- [49] Dhananjaya JR, Veena HC, Mamatha BS, Sudarshan CR. Comparative study of body mass index, hand grip strength, and handgrip endurance in healthy individuals. *National Journal of Physiology, Pharmacy and Pharmacology*. 2017;7(6):594. doi: 10.5455/njppp.2017.7.1030007022017.
- [50] Farjallah MA, Hammouda O, Zouch M, Ghattassi K, Graja A, Driss T, Chamari K, Souissi N. Effect of melatonin ingestion on physical performance, metabolic responses, and recovery after an intermittent training session. *Physiol Int*. 2018;105(4):358-370. doi: 10.1556/2060.105.2018.4.24.
- [51] Ghattassi K, Graja A, Hammouda O, Chtourou H, Boudhina N, Chaouachi A, Souissi N. Effect of nocturnal melatonin ingestion on short-term anaerobic performance in soccer players. *Biological Rhythm Research*. 2014;45(6):885-93. doi: 10.1080/09291016.2014.929853.
- [52] Basu S, Roy AS, Bandyopadhyay A. Fitness Profile in Male Boxers of Kolkata, India. *Sports Medicine Journal/Medicina Sportivâ*. 2016;12(2),1-8.
- [53] Farjallah MA, Ghattassi K, Kamoun A, Graja A, Ben Mahmoud L, Driss T, Jamoussi K, Sahnoun Z, Souissi N, Zmijewski P, Hammouda O. Melatonin supplementation alleviates cellular damage and physical performance decline induced by an intensive training period in professional soccer players. *PLoS One*. 2022;17(9): e0273719. doi: 10.1371/journal.pone.0273719.
- [54] Jallouli S, Ghroubi S, Dhia IB, Yahia A, Elleuch MH, Sakka S, Mhiri C, Hammouda O. Effect of melatonin intake on postural balance, functional mobility and fall risk in persons with multiple sclerosis: a pilot study. *Int J Neurosci*. 2022; 12:1-11. doi: 10.1080/00207454.2022.2090353.
- [55] Baker J, Kimpinski K. Role of melatonin in blood pressure regulation: An adjunct anti-hypertensive agent. *Clin Exp Pharmacol Physiol*. 2018;45(8):755-766. doi: 10.1111/1440-1681.12942.
- [56] Mero AA, Vähälummukka M, Hulmi JJ, Kallio P, von Wright A. Effects of resistance exercise session after oral ingestion of melatonin on physiological and performance responses of adult men. *Eur J Appl Physiol*. 2006 Apr;96(6):729-39. doi: 10.1007/s00421-005-0119-z.
- [57] Cheikh M, Hammouda O, Gaamouri N, Driss T, Chamari K, Cheikh RB, Dogui M, Souissi N. Melatonin ingestion after exhaustive late-evening exercise improves sleep quality and quantity, and short-term performances in teenage athletes. *Chronobiol Int*. 2018;35(9):1281-1293. doi: 10.1080/07420528.2018.1474891.

-
- [58] Bandyopadhyay A, Basu S. Motor Fitness in Children: A Brief Update. *Res. Inves. Sports. Med.* 2022; 8(5): 772–778. doi:10.31031/RISM.2022.08.000699.
- [59] Ghattassi K, Hammouda O, Graja A, Boudhina N, Chtourou H, Hadhri S, Driss T, Souissi N. Morning melatonin ingestion and diurnal variation of short-term maximal performances in soccer players. *Physiol Int.* 2016 Mar;103(1):94-104. doi: 10.1556/036.103.2016.1.9.
- [60] Sumsuzzman DM, Choi J, Jin Y, Hong Y. Neurocognitive effects of melatonin treatment in healthy adults and individuals with Alzheimer's disease and insomnia: A systematic review and meta-analysis of randomized controlled trials. *Neurosci Biobehav Rev.* 2021; 127:459-473. doi: 10.1016/j.neubiorev.2021.04.034.
- [61] Souissi A, Dergaa I. An overview of the potential effects of melatonin supplementation on athletic performance. *Int. J. Sport. Stud. Health.* 2021;4(1): 1-4. doi: 10.5812/intjssh.121714.