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Original Article

Does Melatonin Supplementation Affect Renal Function in Healthy Humans during Prolonged Exercise?

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ABSTRACT

Prolonged submaximal exercise induces reductions in vascular conductance to splanchnic and renal circulations. We aimed to evaluate in the present study the effect of melatonin on the renal response to submaximal exercise. Eight students aged between 20-23 years participated in the study. They ran at 60% of their maximal aerobic speed (MAS) for 45min after 50min of either melatonin-(6mg) or placebo ingestion, in a randomized order. The rectal temperature (Tre) and heart rate (HR) were measured. Felt arousal scale was measured at the beginning and at the end of the rest. Blood samples were taken before and after exercise, from which the hemoglobin (Hb), hematocrit (Hct), sodium (Na+), potassium (K+), bicarbonate (HCO_3^-), pH, urea, uric acid, creatinine, parathyroid hormone (PTH), calcium (Ca^{2+}), chloride (Cl^-), phosphor (P) and vitamin D concentrations were measured. Creatinine clearance was calculated based on the Cockcroft-Gault equation. Hb and Hct increased after exercise only in the placebo condition. Our results showed that T_{re} increased significantly at the end of exercise in both conditions ($P < 0.001$). HR was significantly attenuated in melatonin condition at the end of the exercise ($P < 0.01$). There was no statistical difference in creatinine clearance between conditions, but PTH was significantly higher in melatonin condition compared to placebo (68.6 and 51.9; $P = 0.01$) at the end of the exercise. Our finding revealed that melatonin increases the extent of the exercise-induced increase in PTH. We suggest that melatonin induce renal vasoconstriction during prolonged exercise owing to the alteration of renal blood flow.

Keywords: Exercise, kidney, melatonin, temperature, creatinine clearance, vasoconstriction

INTRODUCTION

Prolonged submaximal exercise is accompanied by an increase in body core temperature and elevation in cardiovascular strain (e.g. a rise in heart rate) and perceived exertion [1]. Therefore, the ability to maintain a given power output or running velocity progressively declines [2]. Typically, the human body's temperature is controlled by thermoregulatory reflexes (Vasoconstriction and vasodilatation) which allows for heat to be conserved or dissipated to the environment by way of conduction, convection, radiation, and evaporation [3]. Whole body pre-cooling strategies such as cold air, water immersion, water-perfused suits aim to reduce body temperature before exercise, thereby increasing the margin for metabolic heat

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production and increasing the time to exhaustion or the distance run or cycled [4]. These aforementioned strategies are worthwhile if the facilities are available. However, it's not always the case. In this context, nutritional strategies with antioxidants have been proposed to protect the body against oxidative stress and core temperature increase during exercise [5].

Melatonin (N-acetyl-5-methoxytryptamine), a hormone secreted by the pineal gland, acts as the major regulator of the circadian rhythm of core temperature in humans [6]. However, some unfavourable effects of this hormone such as increasing the blood pressure owing to down regulation of nitric oxide synthase (NOS) [7-9] and constrict the renal arteries in humans have been reported [10], which could induce a substantial drop in glomerular filtration rate and may cause renal failure [11,12]. Several studies have examined the hypothermic effect of melatonin, demonstrating a 0.25°C to 0.3°C decline in core temperature within several hours of consuming up to 5 mg dose [13,14]. Data available related to melatonin supplementation and exercise are limited and they are focused mainly on body temperature [15].

Few studies have examined the effects of exogenous melatonin supplementation on sports performance. Indeed, Atkinson et al (2005) examined the effects of melatonin supplementation on short term athletic performance, reporting no significant improvement in grip strength, 4 km cycling time or RPE despite the significant reductions in intra-aural temperature [16]. This study provides evidence that melatonin does not significantly affect absolute short term power output; however, there is little evidence available on its effect on continuous exercise performance. To our knowledge, all the studies investigated the effect of melatonin supplementation on continuous exercise performance found no effect on performance [16,17]. In the same context, it was shown that melatonin (5 mg) has no hypothermic effect during continuous exercise in hyperthermia [18].

Previous research from our laboratory showed also that 6mg of melatonin has no hypothermic effect during continuous exercise in normothermia [19]. Evidence suggests that melatonin does not attenuate the increase of rectal temperature during continuous exercise. The biological parameters analyzed in our previous researches focused mainly on inflammatory biomarkers and biological parameters indicators of sweating. To the best of our knowledge, no studies have evaluated the effect of melatonin (>5mg) on the biological markers of the renal function. Since melatonin has been demonstrated to reduce renal blood flow in humans, we hypothesized that increases in melatonin would alter renal function at rest and during exercise.

METHODS

Study Design and Participants

Eight healthy, moderately trained male students [age: 21.8 ± 0.9 years; BMI: 21 ± 0.8] from the local higher institute of sport and physical education voluntarily participated in the study. After receiving a description of the protocol, risks of the study, each participant reviewed and signed a written informed consent form prior to participation. The study protocol complied with the Helsinki declaration for human experimentation and the protocol was fully approved by the local Ethics Committee on Human Research. All athletes reported being nonsmokers and not consuming alcohol and/or any antioxidant compounds, including neither vitamins nor medications (e.g. anti-inflammatory drugs or hypnotic agents).

The participants were screened by obtaining a medical history, including any history of severe physical or mental disorders, and performing physical examinations. During the first appointment, maximal aerobic speed (MAS) was assessed using the VAMEVAL test at 09:50 [20]. The second and third appointments were devoted to completing the two sessions of the protocol (melatonin or placebo) in a randomized order. All physical tests were performed at a temperature of 23 ± 0.1°C (maintained with a programmable temperature controller) and a relative humidity of 60% ± 3%. The participants were not specifically heat acclimated when they exercised for 45 min at 60% MAS.

Experimental Protocol

A rectal thermistor was inserted into the athletes (inserted 10 cm beyond the anal sphincter). At 09:00, the participants ingested either the melatonin or placebo capsule with water (500 ml) and then rested for 40 min in the dorsal position. During this period, the rectal temperature (T_{re}) and heart rate (HR) were recorded continuously with a HR monitor (Polar RS800, Finland) and a rectal probe (Universal YSI400, China). At 09:40, a blood sample was taken. At 09:50, the

participants started the exercise on a treadmill (Finnlo, Germany). They ran for 45 min at a submaximal intensity fixed at 60% of their MAS. Their HR and T_{re} were recorded continuously during all the period of rest and exercise (the data were selected at the beginning and the end of the exercise).

Blood samples were taken before and after exercise, from which the hemoglobin (Hb), hematocrit (Hct), sodium (Na+), potassium (K+), bicarbonate (HCO_3^-), pH, urea, uric acid, creatinine, parathyroid hormone PTH, calcium (Ca^{2+}), chloride (Cl^-), phosphor (P) and vitamin D concentrations were measured. Felt arousal scale (FAS) was measured at the beginning and at the end of the rest. The rating of perceived exertion (RPE) was measured using a modified Borg scale [21].

Biochemical Analyses

Biochemical assays were performed using standard techniques at the Laboratory of the Hospital of Children in Tunis, Tunisia, using the COBAS 6000. The total 25 OH vitamin D and PTH concentration was measured with electrochemiluminescence (ECLIA) on an automated Roche Cobas E 411 (Roche Diagnostics).

Statistical Analyses

Statistical Software Version 10.0 for Windows (StatSoft, Maisons-Alfort, France) was used for data analysis. The data are reported as the mean and standard deviation (± SD). Concerning the variables measured at the beginning and the end of the rest, the data were analyzed using repeated measures (condition × time) analysis of variance (ANOVA). The Bonferroni test was used to identify significant differences. Concerning the variables measured only at the end of the exercise, the data were analyzed using a paired Student's t-test. Effect sizes were calculated as partial eta-squared (η_p^2) to assess the practical significance of our findings. The level of significance was predetermined to be $P < 0.05$ for all statistical analyses.

RESULTS

Heart Rate and Rectal Temperature

The mean values of resting T_{re} and HR are displayed in Table 1. Post-hoc analysis revealed that HR was significantly higher at the end of exercise in placebo condition than melatonin condition (3.6%; $P < 0.01$) (Table 2).

Felt Arousal Scale and RPE

The mean values of the FAS are displayed in Tables 1. A significant interaction (condition × rest) was indicated for the FAS [$F = 43.75$, $P < 0.001$, $\eta_p^2 = 0.8$]. Post hoc analysis indicated that melatonin considerably decreased the level of arousal ($P < 0.001$) at rest. The T-test of student revealed a significant difference in RPE at the end of exercise between conditions. At the end of the exercise, the RPE was more elevated for the melatonin condition than for the placebo condition (13%; $P < 0.001$) (Table 2).

Hb, Hct, [La], Urea, Uric Acid, Bicarbonate, Ph, Creatinine and Creatinine Clearance

The mean [La] values for both conditions were less than 2.5 mmol/L. Post hoc analysis indicated that the Hb and Hct increased after exercise only in placebo condition (respectively, with $P = 0.01$ and $P = 0.011$) (Table 3). The mean values of urea,

Table 1: The physiological and psychological variable results at rest for both conditions

Variable		Placebo		Melatonin		Global effect
		0_{min}	40_{min}	0_{min}	40_{min}	
HR (beats/min)	*Mean*	66.7	58.3	69	57.2	Condition: NS
	SD	8.7	7.6 *	8.3	6.9 *	Rest: *** Interaction: *
T_{re} (°C)	*Mean*	37.22	37.01	37.43	37.02	Condition: NS
	SD	0.19	0.18 *	0.15	0.16 *	Rest: *** Interaction: **
FAS	*Mean*	5	4.75	5.12	2.3	Condition: **
	SD	1.06	1.28	0.83	0.51 *§	Rest: *** Interaction: ***

*: Significant difference from the pre-exercise value ($p < 0.05$)
§: Significant difference from the placebo condition ($p < 0.05$)
NS: Non significant ($p > 0.05$)

Table 2: The pre- and post-exercise results of the physiological and psychological variables for both conditions

Variable		Placebo		Melatonin		Global effect
		0_{min}	45_{min}	0_{min}	45_{min}	
HR (beats/min)	*Mean*	68.3	165.5	66.2	159	Condition: *
	SD	6.2	5.5 *	7.5	3.7 *§	Exercise: *** Interaction: **
T_{re} (°C)	*Mean*	37.04	38.42	36.97	38.69	Condition: NS
	SD	0.17	0.22 *	0.20	0.37 *	Exercise: *** Interaction: **
RPE	*Mean*		13.12		14.87	Condition: ***
	SD		2.23		1.95 §	

*: Significant difference from the pre-exercise value ($p < 0.05$)
§: Significant difference from the placebo condition ($p < 0.05$)
NS: Non significant ($p > 0.05$)

uric acid, bicarbonate, pH, creatinine and estimated creatinine clearance are displayed in Table 3. The creatinine clearance was estimated by Cockcroft-Gault equation [22].

CreatClear = ((140 - Age)/(SerumCreat)) * (Weight/72)

No significant (condition × exercise) interaction was obtained for urea [F = 4.06, P = 0.08, η_p^2 = 0.36]. Post-hoc analysis revealed that urea increased only in melatonin condition after exercise (P= 0.004). A significant (condition x exercise) interaction was obtained for uric acid [F = 8, P = 0.025, η_p^2 = 0.53]. Post-hoc analysis revealed that uric acid was significantly higher under melatonin than placebo condition at rest (P < 0.001) and at the end of the exercise (P < 0.0001). No significant (condition × exercise) interaction was obtained for creatinine [F = 0.71, P = 0.42, η_p^2 = 0.09]. No significant (condition × exercise) interaction was obtained for creatinine clearance [F = 0, P = 1, η_p^2 = 0]. No significant (condition × exercise) interaction was obtained for bicarbonate [F = 0.003, P = 0.95, η_p^2 < 0.001]. No significant (condition × exercise) interaction was obtained for pH [F = 1.3, P = 0.28, η_p^2 = 0.16].

Table 3: The pre- and post-exercise biochemical parameter results for the two conditions

Variable		Placebo		Melatonin		Global effect
		0_{min}	45_{min}	0_{min}	45_{min}	
Hb (g/dl)	*Mean* *SD*	14.3 0.5	15 0.7 *	14.2 0.6	14.4 0.5 §	Condition: NS Exercise: *** Interaction: NS
Hct (%)	*Mean* *SD*	43 1.5	45.5 2.1 *	42.3 2	43.3 1.4 §	Condition: * Exercise: *** Interaction: NS
Na+ (mmol/l)	*Mean* *SD*	139 1.3	140 1.2	139 1.2	140 1.7	Condition: NS Exercise: NS Interaction: NS
K+ (mmol/l)	*Mean* *SD*	4.4 0.2	4.4 0.1	4.2 0.1	4.4 0.1	Condition: NS Exercise: NS Interaction: NS
Urea (µmol/l)	*Mean* *SD*	5.25 1.3	5.37 1.24	5.67 1.23	5.92 1.2	Condition: NS Exercise: ** Interaction: NS
Uric acid (µmol/l)	*Mean* *SD*	295 58	304 58	304 55	318 52	Condition: NS Exercise: ** Interaction: *
Creatinine (µmol/l)	*Mean* *SD*	74.2 4.6	79.8 2.9	74.6 5.6	79.1 4.4	Condition: NS Exercise: ** Interaction: NS
CreatClear	*Mean* *SD*	130 14	120 8.5	131 11.8	121 10.8	Condition: NS Exercise: * Interaction: NS
HCO_3^-(mmol/l)	*Mean* *SD*	27.9 2.7	27.3 3.1	27.9 2.8	27.3 2.6	Condition: NS Exercise: NS Interaction: NS
pH	*Mean* *SD*	7.44 0.03	7.43 0.04	7.44 0.03	7.45 0.01	Condition: NS Exercise: NS Interaction: NS
[La] (mmol/l)	*Mean* *SD*	1.8 0.3	2 0.5	1.7 0.4	1.8 0.4	Condition: NS Exercise: NS Interaction: NS

*: Significant different from corresponding pre-exercise value (p<0.05)
§: Significant different from placebo condition (p<0.05)
NS: Non-significant (p>0.05)

Parathyroid Hormone, Calcium, Chloride, Phosphor and Vitamin D

The mean values of plasmatic PTH, calcium, chloride, phosphor and vitamin D are displayed in Table 4. A significant (condition x exercise) interaction was obtained for PTH [F = 20.55, P < 0.01, η_p^2 = 0.7]. Post-hoc analysis revealed that PTH was significantly higher in melatonin condition compared to placebo condition at the end of the exercise (32%; P = 0.01). A significant (condition x exercise) interaction was obtained for calcium [F = 35.32, P < 0.001, η_p^2 = 0.8]. A significant (condition x exercise) interaction was obtained for chloride [F = 5.9, P < 0.046, η_p^2 = 0.45]. A significant (condition x exercise) interaction was obtained for vitamin D [F = 6.26, P = 0.04, η_p^2 = 0.47]. No significant (condition × exercise) interaction was obtained for phosphor [F = 1.1, P = 0.32, η_p^2 = 0.13].

Table 4: Pre and post exercise result for biochemical parameter and inflammatory biomarkers

Variable		Placebo		Melatonin		Global effect
		0_{min}	45_{min}	0_{min}	45_{min}	
PTH (pg/mL)	*Mean* *SD*	27.8 7.5	51.9 18.3 *	29.6 6	68.6 20.6 *§	Condition: *** Exercise: *** Interaction: **
Ca^{2+} (mmol/l)	*Mean* *SD*	2.41 0.06	2.52 0.08 *	2.40 0.05	2.42 0.06	Condition: * Exercise: *** Interaction: ***
Cl^- (mmol/l)	*Mean* *SD*	99.7 1.4	100.5 0.8	99.4 1.1	100.7 1.3	Condition: NS Exercise: * Interaction: *
P (mmol/l)	*Mean* *SD*	1.06 0.07	1.35 0.13	1.05 0.14	1.27 0.11	Condition: NS Exercise: ** Interaction: NS
Vit.D (ng/ml)	*Mean* *SD*	21.5 9	*24.2 9.4	22.5 8.7	*21.9 7.8	Condition: NS Exercise: NS Interaction: *

*: Significant difference from the pre-exercise value (p < 0.05)
§: Significant difference from the placebo condition (p < 0.05)
NS: Non significant (p > 0.05)

DISCUSSION

The purpose of this study was to investigate for the first time the effects of a single dose (6 mg) of melatonin on renal response to continuous, submaximal exercise. Our study is the first study investigating the effect of a single dose of melatonin (>5mg) on renal response to continuous exercise. The main finding of our study is that a single 6 mg dose of melatonin increased PTH but did not affect glomerular filtration at the end of the exercise.

Our finding indicated that melatonin expand the exercise-induced increasing in PTH, is known to be a vasodilator and to exert a hypotensive action to induce a reduction in BP [23]., Brickman et al. (1991) recently demonstrated that there was a positive relation between PTH and BP in nonhypertensive subjects [24]. Moreover, since the renal vasoconstriction, occurs during prolonged exercise might, increases renal blood pressure (BP) and parathyroid hormone (PTH). A positive relation between PTH and BP has been demonstrated in nonhypertensive subjects [24] PTH is known to be a vasodilator and to exert a hypotensive action to induce a reduction in BP [23]. Authors could speculate that the important increase of PTH in melatonin condition is due to an important elevation in BP. In this context, the results of the present study support the results of Cook et al.(2011) indicating that melatonin constrict the renal arteries and increase BP. Otherwise, authors suggest that PTH seems to counteract elevation of BP induced by renal vasoconstriction during prolonged exercise and increase glomerular filtration.

On the other hand, our finding revealed that melatonin decreased significantly FAS at rest. Previous research demonstrated that melatonin decreases regional cerebral blood flow in the rat [25], but not in humans [10,26]. Thus, we speculate that melatonin does not have an acute regulatory effect on cerebral blood flow in humans. Further studies were needed to investigate the effect of melatonin on cerebral blood flow in athletes after exercise. High dose of melatonin induces renal vasoconstriction and increase BP, but the mechanisms of its hypertensive actions are not well understood. Melatonin increases renal blood pressure by increasing the renal sympathetic outflow [10] and/or by its NO-scavenging action [7,8,27]. Nitric oxide synthase (NOS) activity was inhibited in a dose-dependent manner by physiological concentrations of melatonin [28,29]. The NO-scavenging action of melatonin induces vasoconstrictor activity [30,31] and may affect inflammatory response to prolonged continuous exercise [20]. The effect of exogenous melatonin on blood pressure appears to be dose related. Melatonin functions differently within the body depending on the ingested dose [10,32-34]. Research in animals confirms a dose response to melatonin concentration in relation to vascular changes [35,36], adrenal nerve activity [37], and hormonal secretion responses [38].

In fact, exercise increases the HR, which will almost certainly enhance the mechanical forces of blood flow, such as shear stress, pressure, and cyclic strain, on the vascular wall [39]. In conductance arteries, shear stress has been shown to increase endothelial superoxide generation [40]. Prolonged sustained high levels of shear stress, as encountered during exercise as a result of increased cardiac output, have been shown to stimulate NO production, hydrogen peroxide and vascular superoxide production [41]. NO, when produced in large amounts, binds O_2 to form peroxynitrite (ONOO−), an unstable compound that decomposes into toxic hydroxyl radicals [42]. High production of superoxide and NO affect the antioxidant defense. Therefore, because of the low antioxidant defense, prolonged periods of exercise (or intensive exercise) induce an increase in vascular oxidative stress, which is considered a pathogenic factor that induces cardiac fatigue [39] and renal inflammation [43].

We suggest that the administration of low dose of melatonin just before exercise could improve cardiovascular and renal function during exercise by reducing vascular oxidative stress and the production of ONOO− in the endothelium, which increases NO bioavailability, reduces inflammation, favors vasorelaxation and improves cardiac force and renal function. Furthermore, patients of older age have significantly higher NO and peroxynitrite levels [44] and melatonin could be helpful for elderly people and for patients who suffer from chronic diseases.

A limitation of the present study is that we used maximal aerobic speed rather than maximal oxygen uptake (VO2max). Moreover, the study was conducted only on male athletes. Therefore, different finding might be among female. The sweet loss was more important in the placebo condition than melatonin condition. Therefore, the results of the present study may underestimate the true perturbation of the renal

function that melatonin could induce during exercise. Finally, melatonin (NO-scavenger) competitively inhibits NO-production but it will be interesting to confirm that with analyze of circulating NO. It is difficult to analyze NO due to its volatile nature.

CONCLUSION

The data of this study showed that a single dose (6mg) of melatonin supplementation increases the extent of exercise-induced increase in PTH. Thus, we suggest that the administration of high dose (≥6mg) of melatonin before exercise might disturb the renal function because of its vasoconstrictor effect. The present study sends a warning signal to the acute use of very high dose of melatonin as a dietary supplement before exercise.

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Declaration of interests

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