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## Association of Endogenous Melatonin with Uric Acid and Traditional Cardiovascular Risk Factors in Healthy Young Male

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### Abstract

**Background.** Uric acid may act as an antioxidant; high serum uric acid levels are often associated with cardiovascular disease, such as coronary artery disease. Melatonin plays a role as a direct free radical scavenger and indirect antioxidant. No study has examined the relationships between endogenous melatonin and uric acid in order to predict the risk of future cardiovascular disease in male so far. To better characterize a possible relationship, we studied the association of endogenous melatonin with uric acid and traditional cardiovascular risk factors such as plasma lipids, and glucose in healthy young male.

**Material and Methods.** Twenty-one male participants [mean age: 22.6 ± 2.5 (18–26) year], non-smoking; their medication or alcohol consumption history were studied. Blood samples for measuring melatonin concentrations were collected in the supine position between 13:30–14:30 hours. After centrifugation, plasma samples were immediately frozen at –20°C until analysis.

**Results.** Although we found a significant negative correlation between the levels of endogenous melatonin and uric acid ( $p = 0.01$ ,  $r = -0.51$ ), we did not find any correlation among the melatonin and other anthropometric, hemodynamic and biochemical parameters in male subjects ( $p > 0.41$ ).

**Conclusions.** The present study demonstrated that a significant negative correlation between the levels of endogenous melatonin and uric acid in healthy young male (Adv Clin Exp Med 2015, 24, 2, 233–237).

**Key words:** melatonin, uric acid, traditional cardiovascular risk factors, healthy young male.

Uric acid, an end product of purine nucleotides degradation, is an anti-oxidant and is excreted in urine [1]. However, uric acid also has some pro-oxidant effects. As a result of these dual effects, high serum uric acid levels are often associated with cardiovascular disease such as coronary artery disease and hypertension [1–3]. Additionally, larger serum UA was found to be an independent risk factor for cardiovascular disease death in both sexes [3]. N-acetyl-5-methoxytryptamine, commonly known as melatonin, is a neurohormone that is mainly produced in the pineal gland which is a neuroendocrine organ of the brain. The release of melatonin from the pineal gland

is synchronized with the daylight cycle by a multisynaptic pathway. Melatonin secretion from pineal gland is inhibited by light [4, 5]. Melatonin affects various physiologic functions such as heart rate, arterial blood pressure, arterial dispensability, immune system and oxidation [4–6]. Studies demonstrated that it plays a role as direct free radical scavenger and indirect antioxidant [7, 8]. To our knowledge, UA reflects the systemic inflammation and low levels of UA may be explained by the anti-inflammatory effects of melatonin. No study has examined the relationships between endogenous melatonin and UA that would predict the risk of future cardiovascular disease in male so far.

To better characterize a possible relationship, we have studied the association of endogenous melatonin with UA and traditional cardiovascular risk factors such as plasma lipids, and glucose in healthy young males.

## Methods

### Study Population

Twenty-one male participants [mean age: 22.6  $\pm$  2.5 (18–26) year], non-smoking; their medication or alcohol consumption history were studied. All participants were healthy, as determined by their medical history and a routine examination. Written informed consent was obtained from all participants, and the study design was in accordance with the guidelines issued by the ethics committee. The investigation conformed to the principles outlined in the Declaration of Helsinki.

### Study Protocol

Participants had regular sleep-wake schedules and low amounts of caffeine consumption (less than 50 mg daily). All participants were requested to relax in a supine position, in a low noise, low light and constant temperature (20°C to 24°C) environment after consuming a light meal free of caffeine-containing beverages. The sleeping period was scheduled from 23:00 to 08:00. A full clinical evaluation, including measurements of vital signs and routine laboratory tests such as lipids and glucose levels, were performed one week before the start of the study. To measure melatonin, blood samples were taken with a two-way stopcock, heparinized, polyethylene cannula inserted into a vein in the forearm. The samples were centrifuged at 2000 rpm for 15 min and stored at –20°C until further analysis. While the subjects were in the supine position at a point in time, namely from 13:30–14:30, also taking plasma to measure the concentrations of melatonin.

Body mass index and waist to hip ratio measurements. Body mass index ( $\text{kg}/\text{m}^2$ ) was calculated by dividing the body weight in kilograms by the square of the body height in metres. Waist to hip ratios were calculated by dividing the circumference of the waist by the circumference of the hips.

### Blood Pressure Measurements

Arterial blood pressure was measured by the same observer in each subject and in the supine position after at least 20 min of rest. Clinic blood pressure was measured, using a mercury

sphygmomanometer with a cuff appropriate to the arm circumference (Korotkoff phase I for systolic blood pressure and V for diastolic blood pressure). In each subject 2 blood pressure measurement were performed, and their mean was considered for analysis. Mean blood pressure = [systolic blood pressure + 2 X diastolic blood pressure]/3 pulse pressure = systolic blood pressure – diastolic blood pressure.

### Assays

Assessment of plasma melatonin levels was performed within one month from the blood sampling. Melatonin levels were measured with a commercially available radioimmunoassay kit (RE29301, IBL, Germany). Samples of each participant's plasma were processed in the same assay, thus eliminating interassay variability. The assay has a sensitivity of less than 3.5 pg/mL and an intra-assay coefficient of variation of less than 8%.

### Statistical Analysis

Statistics were obtained using the ready-to-use software SPSS (version 8.0, SPSS Inc, USA). All the values are expressed as means  $\pm$  SD. Relations between melatonin and antropometric, hemodynamic and biochemical variables were calculated using Pearson correlation tests.  $P < 0.05$  was considered significant.

## Results

Antropometric, hemodynamic and biochemical values in males are presented in Table 1. Although we found a significant negative correlation between the levels of endogeneous melatonin and uric acid ( $p = 0.01$ ,  $r = -0.51$ ) (Fig. 1), we did not find any correlation among the melatonin and age, weight, height, body mass index, waist circumference, hip circumference, waist/hip ratio, systolic blood pressure, diastolic blood pressure, mean blood pressure, pulse pressure, heart rate, calcium, glucose, cholesterol, high density lipoprotein, low density lipoprotein, and triglyceride levels in male subjects ( $p > 0.05$ ).

## Discussion

To our knowledge, this is the first article focusing on a direct relation between endogenous melatonin level and blood biomarkers associated with cardiovascular disease in healthy males. We found a negative correlation between the levels of

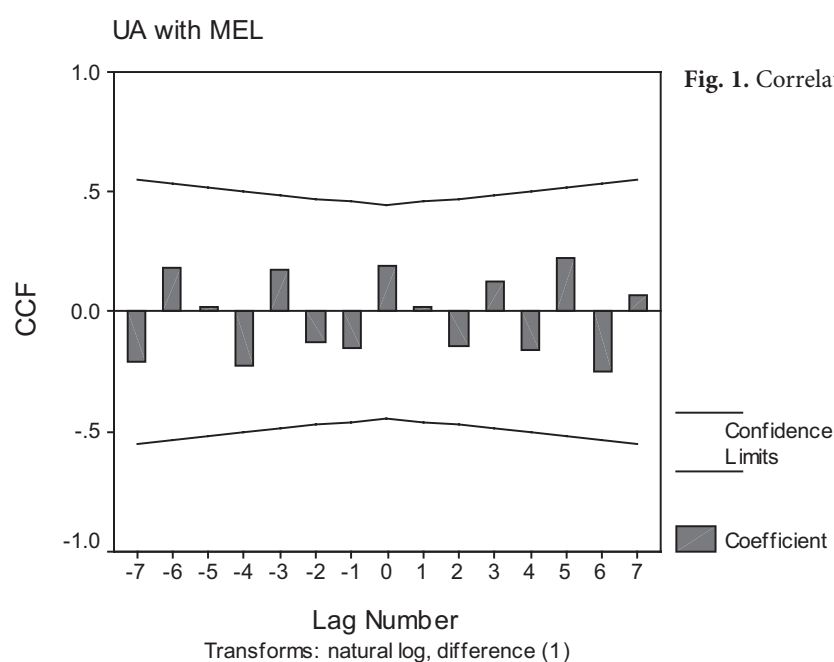
**Table 1.** Antropometric, hemodynamic and biochemical values in male

	Male
Age (years)	22.6 ± 2.5
Weight (kg)	74.42 ± 9.41
Height (m)	177.28 ± 4.86
Body mass index (kg/m <sup>2</sup> )	23.61 ± 2.27
Waist circumference (cm)	83.42 ± 9.01
Hip circumference (cm)	96.28 ± 6.25
Waist/hip ratio	0.86 ± 0.01
Systolic blood pressure (mm Hg)	111.90 ± 7.49
Diastolic blood pressure (mm Hg)	65.23 ± 8.13
Mean blood pressure (mm Hg)	80.76 ± 6.23
Pulse pressure (mm Hg)	46.66 ± 10.40
Heart rate (beat/min)	73.47 ± 8.99
Melatonin (pg/mL)	6.84 ± 7.79
Uric acid (g/dL)	5.45 ± 1.14
Calcium (g/dL)	8.86 ± 0.70
Glucose (mg/dL)	72.71 ± 9.90
Cholesterol (mg/dL)	147.47 ± 21.41
HDL (mg/dL)	37.33 ± 7.30
LDL (mg/dL)	83.47 ± 22.65
Triglyceride (mg/dL)	134.14 ± 51.51

HDL – high-density lipoprotein, LDL – low-density lipoprotein.

endogeneous melatonin and UA. UA, the most abundant antioxidant, may also act as a prooxidant under conditions of oxidative stress [1]. It is a weak organic acid which is a product of purine nucleotides metabolism that leads to the oxidation of hypoxanthine to xanthine and its further oxidation to UA [9]. During the production of UA, catalyzed by xanthine-oxidase, reactive oxygen species are generated as a by-product, which has a significant role in the increased vascular oxidative stress considered to be one of the main reasons of cells function impairment [9]. Reactive oxygen species and elevated serum concentration of UA are associated with an increased risk for cardiovascular disease [1, 10]. The mechanism by which UA may cause cardiovascular disease has been explored in some studies [2, 11–13]. These studies have linked elevated UA with endothelial dysfunction and a reduction in nitric oxide levels is a primary determinant of blood vessel tone and thrombogenicity [11–13]. Khosla et al. [11] demonstrated that UA impairs nitric oxide generation in cultured endothelial cells, inhibits both basal and vascular endothelial growth factor (VEGF)-induced nitric oxide production in bovine aortic endothelial cells, and reduce circulating nitrites in male Sprague-Dawley rats. Gersch et al. [12] showed that UA reacts directly with nitric oxide in a rapid irreversible reaction resulting in the formation of 6-aminouracil and depletion of nitric oxide. In pulmonary arterial endothelial cells uric acid-induced arginase activation reduces nitric oxide production [13].

Melatonin, or N-acetyl-5-methoxytryptamine, is an indole mainly produced in the pineal gland

**Fig. 1.** Correlation between melatonin and uric acid

during the night. The amount of melatonin and its main urinary metabolite, 6-sulphatoxymelatonin decreases with the advancing age [14, 15]. In humans, melatonin production not only diminishes with age, but it is also significantly lower in many age-related diseases such as cardiovascular disease [16, 17]. Therefore, we studied healthy people of similar age. Studies demonstrated that the rhythmicity of melatonin has an important role in a variety of cardiovascular pathophysiology including anti-inflammatory and antioxidant functions [7]. Melatonin stimulates both the gene expression for antioxidative enzymes, such as superoxide dismutase, glutathione peroxidase as well as the levels of glutathione and to increase their activity [7]. Furthermore, melatonin and its metabolites scavenger free radicals such as hydroxyl radicals, superoxide radicals, and hydrogen peroxide which are continuously produced in cells by oxidative phosphorylation in mitochondria and by fatty acid oxidation in peroxisomes and thus terminate the initiation and propagation of lipid peroxidation [18]. High affinity G protein-coupled membrane receptors known as MT1 and MT2 and nuclear receptors called RZR/ROR are responsible for melatonin's effects on cells [19–21]. The relationship between endogenous melatonin and UA levels may be responsible for the pathogenesis of future coronary artery disease. Some studies demonstrated elevated UA and a reduced nitric oxide levels [11–13]. Also, elevated UA and reduced melatonin levels may be a reflection as in our study. Masue et al. [22] studied the association between the endogenous melatonin level and various established

blood biomarkers of risk of cardiovascular disease such as plasma lipids, homocysteine, UA, and high-sensitivity C-reactive protein in 181 Japanese women. They found the urinary 6-sulphatoxymelatonin level was inversely associated with established independent risk factors for cardiovascular disease, including UA, as in our study, and high-sensitivity C-reactive protein. If our evidence was extrapolated into clinical situations, the use of exogenous melatonin as a prophylactic agent for cardiovascular disease may offer benefits to decreasing the blood level of UA in men.

In conclusion, the current study demonstrated that a significant negative correlation between the levels of endogenous melatonin and UA in healthy young males. Our results may encourage studies investigating the role of melatonin in the development of CVD.

## Study Limitations

Studies on physiologic melatonin plasma levels throughout the menstrual cycle have resulted in conflicting reports [23–25]. The melatonin levels during the luteal phase may increase, may decrease (compared with the follicular phase), or may not change between the menstrual phases [23–25]. Though, we did not include female subjects in this study. Also, we cannot give a clear explanation of our results. Further, large group studies are needed to best understand the possible interactions of melatonin and UA. Anthropometric, hemodynamic and biochemical values in male.

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