

# Oral melatonin reduces blood coagulation activity: a placebo-controlled study in healthy young men

**Abstract:** Melatonin has previously been suggested to affect hemostatic function but studies on the issue are scant. We hypothesized that, in humans, oral administration of melatonin is associated with decreased plasma levels of procoagulant hemostatic measures compared with placebo medication and that plasma melatonin concentration shows an inverse association with procoagulant measures. Forty-six healthy men (mean age  $25 \pm 4$  yr) were randomized, single-blinded, to either 3 mg of oral melatonin ( $n = 25$ ) or placebo medication ( $n = 21$ ). One hour thereafter, levels of melatonin, fibrinogen, and D-dimer as well as activities of coagulation factor VII (FVII:C) and VIII (FVIII:C) were measured in plasma. Multivariate analysis of covariance and regression analysis controlled for age, body mass index, mean arterial blood pressure, heart rate, and norepinephrine plasma level. Subjects on melatonin had significantly lower mean levels of FVIII:C (81%, 95% CI 71–92 versus 103%, 95% CI 90–119;  $P = 0.018$ ) and of fibrinogen (1.92 g/L, 95% CI 1.76–2.08 versus 2.26 g/L, 95% CI 2.09–2.43;  $P = 0.007$ ) than those on placebo explaining 14 and 17% of the respective variance. In all subjects, increased plasma melatonin concentration independently predicted lower levels of FVIII:C ( $P = 0.037$ ) and fibrinogen ( $P = 0.022$ ) explaining 9 and 11% of the respective variance. Melatonin medication and plasma concentration were not significantly associated with FVII:C and D-dimer levels. A single dose of oral melatonin was associated with lower plasma levels of procoagulant factors 60 min later. There might be a dose–response relationship between the plasma concentration of melatonin and coagulation activity.

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## Introduction

Melatonin has pleiotropic action [1], and alterations in its 24-hr profile could relate to a variety of pathologic states and amongst them is cardiovascular disease. Oxidative stress, chronic systemic inflammation, and coagulation activation are cornerstone mechanisms in the initiation and progression of atherothrombotic disease such as coronary artery disease (CAD) complicated by acute coronary syndromes [2–4]. By virtue of exerting antioxidative, antiinflammatory, and antithrombotic activity, endogenous melatonin could favorably influence the course of CAD [5–7].

In support of this notion, nocturnal secretion of melatonin was found to be reduced in patients with CAD relative to healthy controls [8–10]. Patients with unstable angina showed lower melatonin excretion than those with stable angina suggesting that melatonin production was inversely associated with the risk of acute myocardial infarction (AMI) and cardiac death [11]. As compared with healthy controls, patients with AMI had lower elevation in serum melatonin at night than at day [12]. Melatonin has also been associated with biological risk factors for cardiovascular disease. For instance, circadian changes in

melatonin may partially be responsible for light dark variations in the production of the inflammatory marker C-reactive protein in patients with AMI [13]. In addition, patients with systemic hypertension who did not dip their blood pressure (BP) during the night showed lower nocturnal melatonin secretion than hypertensives who were night-time dippers [14].

Oral melatonin given in a typical pharmacological dose of several milligrams does not mimic the endogenous profile but leads to supraphysiological levels with potentially therapeutic properties in terms of mechanisms involved in atherothrombotic diseases [6]. Intake of melatonin for three nights before sleep decreased 24-hr ambulatory BP in patients with systemic hypertension [15]. Melatonin in a dosage similar to the concentration of the nocturnal surge reduced leukocyte rolling and adhesion in the microcirculation of rats during acute inflammation [16]. Aggregation of human platelets *in vitro* was inhibited by melatonin [17, 18] suggesting a relatively stronger inhibition with higher plasma melatonin concentration [17]. Melatonin also normalized shortened prothrombin time and elevated levels of fibrin degradation products in the blood of rats with thermal injury [19]. However, to the best of our knowledge, whether exogenously administered melatonin may influence

steady-state coagulation activity in humans has not been explored.

We investigated the effect of a single oral dose of 3 mg melatonin on plasma levels of coagulation factor VII activity (FVII:C), FVIII:C, fibrinogen, and D-dimer, all of which have previously been associated with an increased risk of coronary thrombotic events [20–23]. In brief, FVII together with tissue factor initiates the extrinsic pathway of blood coagulation when endothelial cells are damaged and prothrombotic material becomes exposed to the circulation [24]. Factor VIII is activated by thrombin further downstream in the coagulation cascade [25], where fibrinogen is ultimately converted to fibrin, the main component of a thrombus [26]. Dissolution of fibrin by the fibrinolytic system results in production of the fibrin degradation product D-dimer that is a coagulation activation marker reflecting the amount of fibrin formed during coagulation and subsequent fibrinolysis [27].

We hypothesized that healthy young men allocated to melatonin would show lower plasma levels of FVII:C, FVIII:C, fibrinogen, and D-dimer relative to subjects allocated to placebo medication 60 min after administration of 3 mg of melatonin or placebo. We further hypothesized that plasma melatonin concentration would show an inverse relationship with levels of coagulation factors. We postulated that relationships between melatonin and coagulation activity would be independent of common demographic (i.e., age), metabolic [i.e., body mass index (BMI), mean arterial blood pressure (MAP), heart rate (HR)], and neuroendocrine (i.e., plasma norepinephrine) correlates of hemostatic function.

## Materials and methods

### Participants and study design

Our study sample consisted of 46 medication-free healthy men between 20 and 34 yr of age from whom we obtained complete data (Table 1). Subjects with any acute or chronic somatic or psychiatric disorders were excluded. Additional exclusion criteria, as obtained by subjects' self-report, included regular strenuous exercise, smoking, alcohol and illicit drug abuse, heart disease, elevated blood sugar and diabetes, elevated cholesterol, liver and renal diseases, chronic obstructive pulmonary disease, allergies and atopic diathesis, rheumatic diseases, and current infectious diseases. If the personal history was not conclusive, the subjects' primary care physician was contacted for clarification.

We applied a randomized, placebo-controlled, single-blinded design (i.e., participants were unaware of whether they received verum or placebo medication). All subjects reported to the laboratory on two consecutive days. Participants abstained from food and drink (other than water) for 2 hr before reporting to the laboratory, and also from physical exercise, alcohol, and caffeinated beverages starting the evening before day 1. On day 1, systolic and diastolic BP, as well as HR, were measured once after the subjects had been sitting quietly for 20 min, and BMI was also assessed. On day 2, sessions commenced between 14:00 and 16:00 hours and lasted for approximately 90 min. Twenty-five minutes after insertion of a venous catheter, melatonin or placebo was administered. Following the previous observations on melatonin kinetics after oral intake of 1–5 mg of melatonin [28], we took blood samples under resting conditions 60 min after medication intake for assessment of coagulation activity, norepinephrine, and melatonin levels. The study protocol was formally approved by Switzerland's regulatory agency for therapeutic products (Swissmedic) and the ethics committee of the State of Zurich, Switzerland. The study was carried out in accordance with the Declaration of Helsinki principles. All participants provided written informed consent.

### Study medication

Verum medication was 3 mg of melatonin purchased from Physiologics ('Melatonin Standard', order No. 7915; Physiologics, Northglenn, CO, USA). Placebo pills contained lactose and amyllum (lactosi cum amylo) and were purchased from Häseler AG (order No. 13-3400-2; Herisau, Switzerland).

### Metabolic factors

Systolic BP, diastolic BP and HR were measured using the fully automated Omron device (Omron 773; Omron Medizintechnik Handelsgesellschaft mbH, Mannheim, Germany). The MAP was computed by the formula  $(2/3 \times \text{systolic BP} + 1/3 \times \text{diastolic BP})$  and used for statistical analysis. The BMI was calculated by dividing the weight in kilograms by the square of height in meters ( $\text{kg/m}^2$ ).

### Biochemical analyses

For the determination of FVII:C, FVIII:C, fibrinogen, and D-dimer, 4.5 mL of blood was added to 0.5 mL of citrate, 0.106 mol/L. All samples were immediately centrifuged for

Variable	Melatonin (n = 25)	Placebo (n = 21)	P-value
Melatonin (pg/mL)	365.6 ± 103.2	2.25 ± 0.5	<0.001
Age (yr)	24.6 ± 0.7	25.1 ± 0.8	0.679
Body mass index ( $\text{kg/m}^2$ )	23.0 ± 0.6	22.7 ± 0.4	0.688
Mean arterial pressure (mmHg)	94.1 ± 1.6	94.7 ± 2.2	0.812
Heart rate (bpm)	71.5 ± 2.3	65.6 ± 2.5	0.087
Norepinephrine (pg/mL)	321.6 ± 23.9	359.3 ± 31.1	0.334

Values are given as mean values ± S.E.M.  
Group comparison was by Student's *t*-test.

Table 1. Characteristics of the 46 men studied

20 min at 3000 g at room temperature. Plasma aliquots were frozen in polypropylene test tubes at  $-80^{\circ}\text{C}$  until further analyses. Fibrinogen levels were quantified in g/L as per a modified Clauss method [29]. FVII:C and FVIII:C were determined by standard coagulometric methods using factor-deficient standard human plasma and reagents (Dade Behring, Liederbach, Germany) and are expressed as percent relative to normal plasma. D-dimer was determined in ng/mL using a commercially available enzyme-linked immunosorbent assay (Asserachrom Stago, Asnières, France). All hemostatic measures were determined in duplicates, and all inter and intra-assay coefficients of variation were  $< 10\%$ .

For plasma norepinephrine and melatonin measurements, blood was drawn into ethylenediaminetetraacetic acid (EDTA)-coated monovettes (Sarstedt, Numbrecht, Germany), and immediately centrifuged for 10 min at 2000 g; plasma was stored at  $-80^{\circ}\text{C}$  until analysis. Plasma norepinephrine and melatonin levels were determined by a commercial lab (Labor für Stressmonitoring, Göttingen, Germany). The norepinephrine levels were determined by means of high-performance liquid chromatography (HPLC) and electrochemical detection after liquid-liquid extraction [30]. Melatonin was extracted with methylene chloride and analyzed with a reversed phase HPLC with fluorescence detection [31]. Detection limits were 10 pg/mL for norepinephrine and 2.5 pg/mL for melatonin. Samples with a melatonin concentration below the detection limit were set at half the limit of detection (i.e., 1.25 pg/mL) as was previously recommended for a skewed data set [32, 33]. Inter- and intra-assay coefficients of variation were  $< 10\%$  for all analyses.

### Statistical analysis

All data were analyzed using SPSS version 13.0 for Windows (Statistical Package for the Social Sciences, SPSS, Chicago, IL, USA). Significance level was set at  $P < 0.05$  and all tests were two-tailed. Normal distribution of data was tested by the Kolmogorov-Smirnov test. As values of FVIII:C, D-dimer, and plasma melatonin were skewed, we logarithmically transformed these data. Data are given as means  $\pm$  S.E.M. or as geometric means with 95% confidence interval. Differences in subjects' characteristics between the melatonin and placebo group were calculated by the Student's *t*-test. Pearson correlation analysis was used to estimate the bivariate correlation coefficient between two variables.

We applied multivariate analysis of variance (MANOVA; with medication as the between-groups factor), and

multivariate analysis of covariance (MANCOVA; with age, BMI, MAP, HR, and norepinephrine levels as covariates) to test for a difference in coagulation measures between subjects with melatonin and those with placebo. The number and type of covariates were selected a priori given our sample size and based on the previous literature on variables possibly affecting coagulation function [34]. We employed multiple linear regression analysis to investigate the independent relationship between plasma melatonin levels and coagulation measures after forced entry of age, BMI, MAP, HR, and norepinephrine levels. Effect sizes are expressed as partial eta squared ( $\eta_p^2$ ) in MANOVA and MANCOVA and as  $R^2$  change in regression analysis.

### Results

Melatonin levels were expectedly higher in subjects who received melatonin than in those under placebo medication (Table 1). Of note, plasma melatonin levels varied largely within the melatonin group (17.3–2036.4 pg/mL) as opposed to the placebo group (1.3–8.6 pg/mL). Heart rate was marginally higher in the group who received melatonin compared with the group with placebo medication. In contrast, age, BMI, MAP, and plasma norepinephrine levels were not significantly different between groups.

In all subjects, FVIII:C levels correlated with mean arterial pressure ( $r = 0.30$ ,  $P = 0.047$ ) and norepinephrine levels ( $r = -0.31$ ,  $P = 0.037$ ). Fibrinogen levels correlated with levels of FVIII:C ( $r = 0.34$ ,  $P = 0.022$ ), and D-dimer ( $r = 0.50$ ,  $P < 0.001$ ). There was also a bivariate correlation reaching borderline significance between FVIII:C and D-dimer levels ( $r = 0.25$ ,  $P = 0.093$ ) and between fibrinogen and FVII:C ( $r = 0.26$ ,  $P = 0.081$ ).

Multivariate ANOVA with medication (melatonin versus placebo) as the between-group factor and the four coagulation measures as the dependent variables showed a trend towards statistical significance for a medication effect ( $F_{4,41} = 2.27$ ,  $P = 0.078$ ;  $\eta_p^2 = 0.181$ ). Table 2 shows that FVIII:C levels were significantly lower in subjects with melatonin than in those with placebo ( $\eta_p^2 = 0.087$ ). There was also a trend towards statistical significance for lower fibrinogen levels in subjects with melatonin relative to those with placebo ( $\eta_p^2 = 0.072$ ).

Multivariate ANCOVA controlling for age, BMI, MAP, HR, and norepinephrine levels showed a significant main effect for medication ( $F_{4,36} = 4.13$ ,  $P = 0.007$ ;  $\eta_p^2 = 0.314$ ). As shown in Table 2, subjects who received melatonin had significantly lower levels of FVIII:C ( $\eta_p^2 =$

Table 2. Multivariate analysis of variance and covariance for coagulation factor levels as per medication group (n = 46)

	Melatonin (n = 25)	Placebo (n = 21)	<i>P</i> -MANOVA	Melatonin (n = 25)	Placebo (n = 21)	<i>P</i> -MANCOVA
Factor VII:C (%)	84 (77–91)	88 (80–95)	0.435	83 (76–91)	88 (80–96)	0.389
Factor VIII:C (%)	82 (72–94)	101 (87–117)	0.047	81 (71–92)	103 (90–119)	0.018
Fibrinogen (g/L)	1.98 (1.82–2.14)	2.20 (2.02–2.37)	0.071	1.92 (1.76–2.08)	2.26 (2.09–2.43)	0.007
D-dimer (ng/mL)	125 (102–154)	119 (95–148)	0.717	123 (99–154)	121 (95–153)	0.883

Values are given as mean values (FVII:C, fibrinogen) and as geometric means (FVIII:C, D-dimer) with 95% confidence interval. Multivariate analysis of covariance (MANCOVA) controlled for age, body mass index, mean arterial pressure, heart rate, and norepinephrine levels.

Variables entered	Partial correlations	Standardized $\beta$ -coefficient	<i>P</i> -value	<i>R</i> <sup>2</sup> change
Dependent variable: Factor VIII clotting activity				
Age	-0.024	-0.021	0.882	< 0.001
Body mass index	0.060	0.053	0.711	0.003
Mean arterial pressure	0.261	0.245	0.100	0.053
Heart rate	0.014	0.013	0.929	< 0.001
Plasma norepinephrine	-0.339	-0.330	0.030	0.094
Plasma melatonin	-0.326	-0.310	0.037	0.086
Dependent variable: fibrinogen level				
Age	0.186	0.168	0.245	0.027
Body mass index	0.120	0.112	0.453	0.011
Mean arterial pressure	-0.160	-0.152	0.318	0.020
Heart rate	0.365	0.377	0.019	0.118
Plasma norepinephrine	-0.243	-0.236	0.126	0.048
Plasma melatonin	-0.357	-0.355	0.022	0.112

Table 3. Multiple linear regression analysis of coagulation measures

The *R*<sup>2</sup> change value refers to the variance in FVIII:C and fibrinogen levels explained by an individual variable after controlling for the influence of all other variables of the model.

0.136) and fibrinogen ( $\eta_p^2 = 0.172$ ) than those who received placebo medication.

In multivariate regression analysis, we computed the independent relationship between plasma levels of melatonin and coagulation measures adjusting for age, BMI, MAP, HR, and norepinephrine levels across all subjects. Table 3 shows that a lower melatonin concentration was significantly associated with higher levels of FVIII:C and fibrinogen explaining 9 and 11% of the respective variance. Fig. 1 illustrates the independent relationship between the plasma melatonin concentration and the levels of FVIII:C (Fig. 1A) and fibrinogen (Fig. 1B). Melatonin levels were not independently associated with D-dimer (*P* = 0.96) and FVII:C (*P* = 0.49) levels.

## Discussion

To address the hypothesis that, in humans, the administration of supraphysiologic doses of melatonin would be associated with decreased plasma levels of procoagulant measures, we investigated the effect of a single oral dose of 3 mg melatonin on plasma levels of FVII:C, FVIII:C, fibrinogen, and D-dimer in young and healthy men. We found that, 60 min after substance administration, subjects on melatonin had significantly lower mean levels of FVIII:C and of fibrinogen than those on placebo explaining 14 and 17% of the respective variance. These group differences are further supported by regression analyses in which increased plasma concentration of melatonin independently predicted lower levels of FVIII:C and fibrinogen explaining 9 and 11% of the respective variance. In other words, the higher the plasma melatonin concentration, the lower the plasma levels of FVIII:C and fibrinogen. Given that our placebo control group had higher levels of FVIII:C and fibrinogen compared with the melatonin group at a time when orally administered melatonin is known to reach maximum plasma levels [28], we may assume that melatonin was involved in mediating the decrease in these coagulation factors in the melatonin group. Particularly, there might be a dose-response relationship between the plasma concentration of melatonin and coagulation activity

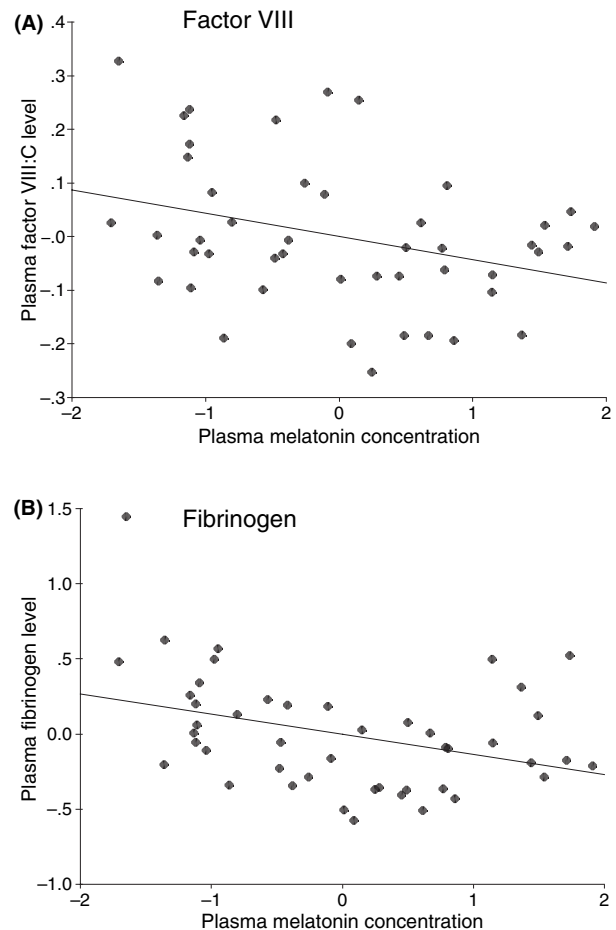


Fig. 1. Independent relationship between melatonin and coagulation factor levels. The partial residual plots depict the inverse and independent relationship between plasma melatonin levels and plasma levels of (A) clotting factor VIII activity (*P* = 0.037) and (B) fibrinogen (*P* = 0.022). Adjustment was made for age, body mass index, mean arterial pressure, heart rate, and plasma norepinephrine levels. All variables in the partial regression plots are residuals.

of FVIII and fibrinogen levels. Interestingly, melatonin intake did not affect the levels of D-dimer and FVII:C suggesting that melatonin administration might exert a selective effect on the plasma level of coagulation measures.

What are the clinical implications of our findings and how do they fit with the literature? Fibrinogen, which is converted to fibrin, the main component of a thrombus [26] has repeatedly emerged as a primary risk factor of cardiovascular disease [22, 35, 36]. Also FVIII:C, which becomes activated in the coagulation cascade by thrombin [25, 37], is associated with increased incidence of ischemic heart disease [21]. Our findings of lower levels of these cardiovascular risk factors after melatonin intake suggest a cardioprotective and antithrombotic effect of melatonin. Such reasoning is in support of studies suggesting cardio-protection from exogenous melatonin administration by favorably affecting inflammatory and prothrombotic mechanisms pertinent to the manifestation of acute coronary syndromes. In animal models, melatonin reduced leukocyte rolling and adhesion in the microcirculation during acute inflammation [16] and normalized shortened prothrombin time and elevated levels of fibrin degradation products inflicted by thermal injury [19]. Melatonin infusion also reduced arrhythmias induced by experimental ischemia of the isolated rat heart [38]. In humans, platelet aggregation *in vitro* was inhibited by melatonin suggesting a dose-response effect [17, 18]. Moreover, findings on lowered nocturnal melatonin excretion in CAD patients relative to healthy controls further support a cardioprotective effect of melatonin [8–12]. To the best of our knowledge, our study is the first human *in vivo* study to show that administration of a single oral dose of melatonin is associated with decreased plasma levels of the procoagulant measures fibrinogen and FVIII:C.

Which mechanisms could be involved in mediating this association? Previous findings suggest a role for catecholamines. We could previously show that acute systemic increase in catecholamines evokes a hypercoagulable state [39]. Particularly, catecholamine infusion to healthy individuals increases plasma levels of FVIII:C [39]. Also, the magnitude of norepinephrine surge during acute mental stress was directly associated with increases in thrombin/antithrombin III complex (i.e., a marker of thrombin formation) [40] and D-dimer [41]. To account for a potential lowering effect of melatonin on catecholamine secretion as observed in other animal and human studies [42–46], we measured in our study norepinephrine. However, in contrast to other placebo-controlled studies on healthy young men [44, 46], we did not observe lower plasma norepinephrine levels in the melatonin group compared with the placebo group 1 hr after intake of the medication. This could be because we obtained blood for catecholamine measurements while subjects were sitting, whereas previous studies found an effect of melatonin on plasma norepinephrine levels only when subjects were supine [44–46]. Although norepinephrine indeed turned out to be a significant predictor of fibrinogen and also showed a positive association with FVIII:C plasma levels, this catecholamine did not mediate the association between higher melatonin concentration and lower activity of these coagulation factors (Sobel test, data not shown). The

mechanisms underlying this association therefore still need to be elucidated.

Theoretically, it could be that by virtue of its anti-inflammatory and antioxidative properties [6] both of which are associated with coagulation activity [47, 48], melatonin could exert an indirect effect on plasma coagulation factor activities.

Several factors add to the clinical significance of our study: First, we performed a placebo-controlled design in which study participants were randomly assigned to the respective medication condition of which they were unaware. Second, we recruited a homogenous sample of healthy nonsmoking young men having reasonable health habits and being unmedicated. Third, we administered melatonin at a time when endogenous melatonin secretion is known to be low such that we could rule out confounding by endogenous melatonin production. Fourth, negligible confounding of melatonin effects on coagulation measures was further achieved by considering *a priori* a set of well-known demographic, metabolic, and neuroendocrine correlates of hemostatic function and of potential melatonin effects. Especially, controlling for norepinephrine levels under melatonin administration was important, given that norepinephrine has been shown to be lowered by oral melatonin administration in humans [44–46] and given that norepinephrine levels emerged as an independent predictor of some coagulation measures [40, 41]. Our study has also its limitations. The data cannot be generalized to women, elderly subjects, and patients with cardiovascular disease. However, it seemed prudent to first investigate the effect of melatonin on coagulation activation in a healthy young population before subjecting the patients with cardiovascular diseases to a study medication (i.e., melatonin) that has not been approved yet by Switzerland's regulatory agency for therapeutic products (Swissmedics). We did not determine coagulation measures immediately before melatonin administration to compare these levels with those obtained 60 min after intake of the medication.

Taken together, we found lower levels of the coagulation measures FVIII:C and fibrinogen 1 hr after oral intake of a single dose of 3 mg of melatonin compared with placebo medication. Moreover, at this time, higher melatonin plasma concentration predicted lower plasma levels of these coagulation measures. Such an effect may suggest potential implications for the use of melatonin as a therapeutic agent in patients at-risk of atherothrombotic events such as patients with CAD or systemic hypertension. However, future studies are needed to test the clinical significance of melatonin administration for coagulation activity in older age when the prevalence of CAD considerably increases and in patient populations. Moreover, the effect of repeated administration of melatonin on coagulation should be subject to further research.

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