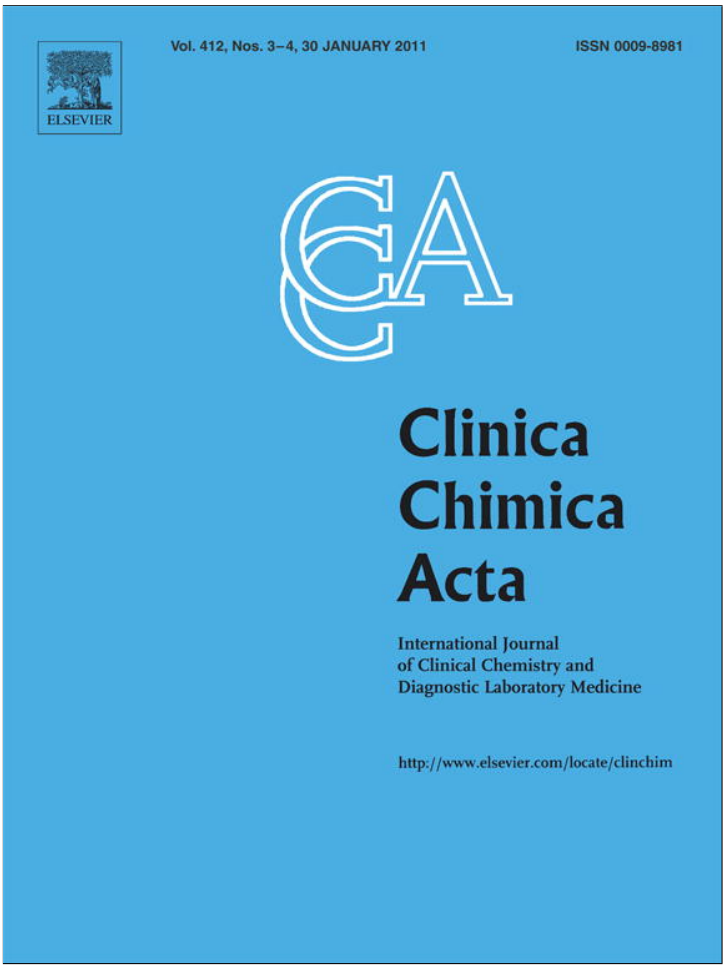

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Serotonin level and serotonin uptake in human platelets: A variable interrelation under marked physiological influences ☆

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ABSTRACT

Background: Although it is known that platelet serotonin level (PSL) depends directly on platelet serotonin uptake (PSU) through the plasma membrane, reports on their interrelation are inconsistent. The aim of this study was to systematically explore the relationship between these two platelet serotonin parameters in large human population.
Methods: PSL and full-kinetics of PSU were determined on 318 blood donors (276 males, 42 females; 20–67 years).
Results: The overall correlation coefficient between PSL and maximal velocity of PSU was highly significant but unexpectedly low ($r = 0.269$). Further analyses revealed lack of correlation among females, and variable association among males, depending on the subject age and season of measurements. Highly significant correlations were observed in spring–winter, while association was absent during summer–autumn. Lowering of PSL–PSU correlation with increased age was also demonstrated, showing modest interrelation among younger men and no interrelation in older population. By multiple regression analyses season was identified as the only independent predictor of PSL–PSU relationship.
Conclusions: The results show prominent influence of biological (sex, age) and, especially, environmental (seasons) physiology on the intraindividual relationship between PSL and PSU. Although serotonin transporter activity plays an important role in determining PSL, the observed correlations indicate that other factors may predominate.

1. Introduction

Serotonin (5-hydroxytryptamine, 5HT) is biogenic amine with a number of effects in the central nervous system (CNS) and periphery. Within the CNS, 5HT is synthesized and stored mainly in serotonergic neurons, while in periphery, the main site of 5HT production are enterochromaffin cells of the gastrointestinal tract. Newly synthesized 5HT is packaged into granules/vesicles, from where it is released upon stimuli, and can interact with nerve terminals or enters the blood plasma [1]. The majority of 5HT released from the intestinal mucosa into portal circulation is rapidly cleared by the liver and lungs [2] while small portion of free amine is actively taken up into blood platelets.

In platelets, which are the major storage site for 5HT outside the CNS, serotonin is deposited in dense granules, similarly to vesicular 5HT in neurons. In contrast to neurons, platelets do not synthesize 5HT and their amine content is, *in toto*, a result of transporter-mediated uptake from the surrounding plasma. Since other blood cells, as well as blood plasma, contain only negligible amounts of this indolamine, as compared to platelets [3], 5HT level in blood directly reflects 5HT content of platelets.

A key molecule in determining the amount of 5HT in platelets is 5HT transporter (5HTt), a transmembrane protein that mediates active transport of 5HT across the cell membrane by Na^+/Cl^--dependent mechanism [4]. Through this process, 5HT is taken up from the plasma into platelets, stored in their dense granules and released upon stimuli contributing to aggregative response [5], as well as to platelet impact on the cardiovascular, immune and other functions.

5HTt activity is a process that obeys the Michaelis–Menten kinetics, described by maximal velocity, V_{max}, and the Michaelis constant, K_m. The same transporter protein is also the main regulator of central serotonergic synapse. In the brain, 5HTt is expressed primarily by the serotonergic neurons, and regulates serotonergic

Abbreviations: 5HT, serotonin (5-hydroxytryptamine); CNS, central nervous system; 5HTt, serotonin transporter; V_{max}, maximal velocity; K_m, Michaelis constant; PSL, platelet serotonin level; PSU, platelet serotonin uptake; ACD, acid-citrate dextrose; PRP, platelet-rich plasma; OPT, ortophtaldialdehyde; SAD, seasonal affective disorder.

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neurotransmission by removing 5HT from the synaptic cleft into the presynaptic terminal neuron. In the periphery 5HTt is, beside in platelets, expressed in several other tissues, notably in membranes of lung endothelial cells, placenta, gastrointestinal epithelium, adrenal gland, as well as in lymphocytes [6]. Among peripheral tissues which express 5HTt, platelets are of particular interest because they express, in addition to 5HTt, two other 5HT synaptic proteins: 5HT-2A receptor and 5HT-degrading enzyme, monoamine oxidase B. Due to this peculiarity, as well as to their accessibility, platelets are often used as a peripheral model in neuropsychiatric research, although it is still unclear whether 5HTt function in platelets correlates with that in serotonergic neurons [7,8].

Since platelet 5HT level depends completely on the platelet uptake of 5HT from the surrounding plasma, close association between these two parameters should have been expected. However, among many studies dealing with the platelet 5HT system, there are only few that have examined more than one platelet 5HT measures at a time. Accordingly, reports on interrelation of platelet serotonin level (PSL) and platelet serotonin uptake (PSU) are scarce, and, more importantly, their results are highly variable.

We have previously studied physiological characteristics of PSL in a large human population [9], and recently we have examined physiological influences on kinetic parameters of PSU [10]. As an extension of these investigations, in the present study we focused on the relationship between these two platelet 5HT parameters, with specific attention given to the influence of gender and age of subjects, as well as to seasonal variations of the PSL–PSU relationship.

2. Materials and methods

2.1. Subjects and blood sampling

Studies were performed on 318 healthy volunteers (blood donors) of both sexes (276 males and 42 females) aged between 20 and 67 years, recruited at the Croatian Institute of Transfusion Medicine in the course of 14 consecutive months. All participants were asked about possible somatic or neuropsychiatric illnesses through a written questionnaire, and informed consent was obtained from all of them. The study was approved by the Ethics Committee of the Medical faculty, University of Zagreb, and carried out in accordance with Declaration of Helsinki. All blood samples were obtained between 7:30 and 10:00 a.m. From each person a sample of 16 mL of venous blood was collected in a plastic syringe preloaded with 4 mL acid–citrate–dextrose (ACD) anticoagulant. The content of the syringe was thoroughly mixed by repeated gentle inversions.

2.2. Preparation of platelet-rich plasma (PRP) and platelet counting

PRP was obtained by a method described earlier [9]. Briefly, after centrifuging blood in adapted plastic syringes at $1200 \times g$ for 2 min in a swing-out rotor, PRP was quantitatively transferred into a plastic tube by the smooth upward movement of the syringe plunger and thoroughly mixed in order to obtain a homogenous platelet suspension. Aliquots of PRP were separated for platelet counting and for determination of PSL and PSU.

Platelet pellets for determination of PSL were obtained by centrifugation (10 min at $8500 \times g$) of diluted PRP aliquots (1 mL PRP + 3 mL saline), followed by washing and were stored at −20 °C until further use. Determination of PSU was performed within 2 h after PRP separation. Platelet number and platelet volume were determined in aliquots of PRP samples (Coulter Counter® ZM) and in whole blood (WB).

2.3. Determination of platelet serotonin level (PSL)

PSL was determined by orthophthaldialdehyde (OPT)–enhanced fluorometry, according to the previously reported method [9]. Briefly, after homogenising the platelet pellets in deionised water (1 mL) by ultrasonication (30 s, 20 kHz, 8 μm), proteins were precipitated by $ZnSO_4$ (10%,1 mL) and NaOH (1 N, 0.5 mL) followed by centrifugation ($1200 \times g$, 15 min). Supernatant (1.5 mL) was transferred into a glass tube, L-cysteine (1%, 100 μL) and OPT (0.01%, 2 mL) were added and the reaction mixture was boiled for 10 min. After cooling, the fluorescence of samples was read at 485 nm with the excitation at 345 nm (Perkin Elmer LS50). Standard (100–300 ng/mL) and blank (deionised water) samples were processed in the same way. Results were expressed as ng 5HT per unit number of platelets.

2.4. Determination of platelet serotonin uptake (PSU)

PSU was assayed by the use of radioisotopic method described previously [10]. Shortly, 100 μL of PRP was preincubated (37 °C, 10 min) in 800 μL of Krebs–Ringer phosphate buffer (without $CaCl_2$, pH 7.4). Incubation was started by the addition of 100 μL of radioactive substrate (^{14}C-5HT creatinine sulphate, specific activity 57 mCi/mmol, six final concentrations ranging from 0.15 to 2.00 μM). After 60 s, incubation was terminated by the addition of the ice-cold saline and immediate vacuum filtration (500 mmHg) over glass fibre filters (Whatman GF/C) followed by washing (2×3-mL saline). Radioactivity retained in the filters was assayed by liquid scintillation counting (Tri-Carb® Liquid Scintillation Analyzer, PerkinElmer). The values of internalized ^{14}C-5HT were corrected for blanks measured by the same procedure, but at 0 °C (ice bath).

Kinetic parameters, maximal velocity (V_{max}) and Michaelis constant (K_m) were calculated from Eadie–Hofstee plots and results were expressed as pmol 5HT/10^8 platelets/min and μM of 5HT, respectively. In addition, 5HT transporter efficiency (V_{max}/K_m) which takes into account both transporter velocity and its affinity ($1/K_m$), was calculated.

2.5. Statistical analysis

Data were analyzed using GraphPad Prism, version 5.02, GraphPad Instat, version 3.01 (GraphPad Software, San Diego, CA) and AnalystSoft, StatPlus, version 2007 (http://www.analystsoft.com). Continuous variables were tested for a normal distribution by the Kolmogorov–Smirnov test. Non-normally distributed data were logarithmically transformed before the statistical analysis was performed. Mean values and standard deviations (mean ± SD) were calculated for all platelet parameters. Student *t*-test or one-way ANOVA were used to make group(s) comparisons. Pearson correlation coefficient (r) was used to quantify the overall association between platelet 5HT parameters. The stepwise multiple regression analysis was used to identify independent variables that have an influence on PSL. Additional regression analyses were conducted with either PSU or ratio of PSL/PSU as the dependent variable. Polynomial regression was used for modelling curvation in the relationship between PSU velocity and months of the year. Statistical power of the sample was calculated by power and sample size calculations, version 2.1.31. Statistical significance was set at $P<0.05$.

3. Results

Mean values of measured platelet parameters are presented in Table 1. Platelet recovery of approximately 70% was obtained in all subgroups, and platelet volume index (calculated as a ratio between PRP and WB) ranged from 0.96 to 0.99, both suggesting that isolated platelets well represent population of platelets in the whole blood. Correlation analysis showed low but significant negative association between PSL and platelet count (WB: $r=-0.199$, $P=0.0003$; PRP: $r=-0.127$, $P=0.023$) whereas positive correlation, highly significant and moderate by value (WB: $r=0.320$, $P<0.0001$; PRP: $r=0.321$, $P<0.0001$), was shown between PSL and platelet volume. Multiple

Table 1
Platelet measures in population of healthy individuals and various subgroups according to gender and age.

	N	Platelet count ($\times 10^6$/mL)		Platelet volume (fL)		PSL (ng/10^9 platelets)	V_{max} (pmol/10^8plt/min)	K_m (nM)
		WB	PRP	WB	PRP			
All subjects	318	235 ± 50	329 ± 81	7.25 ± 0.74	7.09 ± 0.78	562 ± 166	142 ± 25	404 ± 86
Females, all	42	243 ± 47	310 ± 75	7.39 ± 0.74	7.12 ± 0.65	573 ± 162	138 ± 23	396 ± 85
Males, all	276	233 ± 45	331 ± 82	7.22 ± 0.74	7.09 ± 0.79	560 ± 166	143 ± 25	405 ± 86
Males, <30 years	36	241 ± 48	342 ± 84	7.18 ± 0.68	6.97 ± 0.67	586 ± 190	148 ± 25	412 ± 76
Males, 31–50 years	176	232 ± 50	332 ± 84	7.24 ± 0.75	7.11 ± 0.82	578 ± 165	144 ± 27	408 ± 89
Males, >50 years	57	231 ± 52	322 ± 76	7.07 ± 0.73	6.99 ± 0.79	504 ± 133*	136 ± 22	390 ± 85

Means ± standard deviations are given. WB = whole blood, PR*P* = platelet-rich plasma, PSL = platelet serotonin level, V_{max} = maximal velocity , K_m = Michaelis constant.
*P<0.05 vs. two other age groups, one-way ANOVA followed by Tukey post-hoc test.

regression analysis demonstrated that the effects of platelet count and platelet volume could explained 4.76% of the total variance in PSL ($R^2 = 0.0476$, adjusted $R^2 = 0.0415$; $F = 7.869$; $P = 0.0005$), with significant effect in platelet volume ($\beta = 0.188$; $P = 0.0007$). There were no differences in platelet parameters between men and women, while a slight decrease of PSL values (15%, $P<0.01$) was observed in males older than 50 years (Table 1).

Relationship between PSL and PSU was firstly determined by simple correlation and then variables potentially influencing this relationship, i.e. sex, age and seasonality, were evaluated by use of stepwise multiple regression.

Intraindividual correlation between PSL and V_{max} of PSU on the total sample of 318 subjects is shown in Fig. 1. The strength of this relationship, as calculated by Pearson correlation coefficient, was rather small ($r = 0.269$), although highly significant ($P<0.0001$). For reliable detection of this correlation, the statistical power of our sample was sufficiently high (~95%). It should be noted that stronger association between PSL and PSU was found when uptake velocities at lower substrate concentrations were used in correlation analysis instead of V_{max}. Thus, at concentration of 0.15 μM 5HT in incubating medium correlation coefficient between PSL and PSU was 0.517. Association between PSL and efficiency of PSU (i.e. V_{max}/K_m) was essentially the same as for the V_{max} ($r = 0.262$, $P<0.0001$), whereas no association was observed between PSL and Michaelis constant ($r = -0.029$).

Separate analysis by gender (Table 2) revealed that highly significant association between PSL and V_{max} of PSU exists only in men and is of modest value ($r = 0.314$, $P<0.0001$, $N = 276$), whereas in women analogous correlation was virtually absent ($r = -0.053$, $N = 42$).

Age influence on the PSL–PSU association was studied by correlation analysis in males from three age groups: younger than 30 years, 31–50 years and older than 50 years. Results showed highly significant correlation between PSL and PSU variables among younger individuals ($r = 0.437$), while correlation coefficient decreased ($r = 0.182$) toward older age (Table 2).

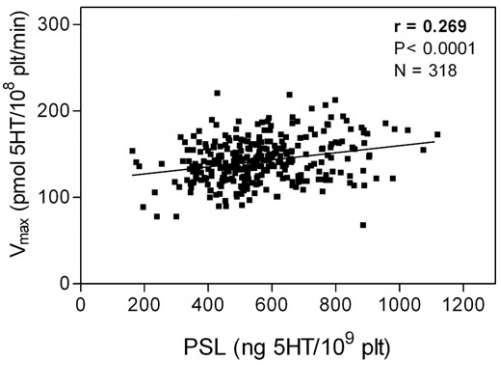

Fig. 1. Correlation between platelet serotonin level (PSL) and maximal velocity (V_{max}) of platelet serotonin uptake in the whole population studied ($N = 318$). r = coefficient of correlation, N = number of subjects.

Further analysis explored potential influence of seasonal variations on the association between PSL and PSU. The respective results are presented in Fig. 2. Highly significant correlation coefficients, modest in their values, were observed in spring ($r = 0.469$, $P<0.0001$, $N = 80$) and winter ($r = 0.591$, $P<0.0001$, $N = 37$), i.e. in the course of period with lower ambient light. In summer, correlation was low and only marginally significant ($r = 0.216$, $P = 0.0546$, $N = 80$), whereas in autumn a lack of any significance was observed ($r = 0.139$, $N = 78$).

For analysing simultaneous effects of uptake rate, age, gender and season on PSL, stepwise multiple regression analysis was performed. Together these variables explained 8.65% of the total variance in PSL ($R^2 = 0.0865$, adjusted $R^2 = 0.0748$; $F = 7.41$; $P<0.0001$), with significant effects only for PSU ($\beta = 0.2677$, $P<0.0001$). Thus, V_{max} of PSU was identified as the only independent predictor of PSL, although it explained only a small proportion of its variance (7.2%). Neither sex nor age nor season entered into the final regression model, although lower PSL values have been significantly associated with older age ($r = -0.172$, $P = 0.0024$) and, in the regression of PSL, age was eliminated as last ($\beta = 0.10$; $P = 0.065$).

Regression analysis was then performed with PSU as a dependent variable and age, sex and season as independent variables ($R^2 = 0.0454$, adjusted $R^2 = 0.0363$; $F = 4.98$; $P = 0.0022$). In contrast to regression on PSL, here highly significant positive correlation was found for season ($\beta = 0.2056$, $P = 0.0002$). Again, neither sex nor age contributes significantly to the prediction. To model curvature in the relationship between V_{max} of PSU and months of the year (Fig. 3), polynomial regression method was used, and the best fitting was obtained with third order polynomial curve.

We also performed multiple regression analysis of PSL/PSU ratio, which was viewed as dependent measure, to determine whether season predicts this relationship, after controlling for age and sex. Results show significant effect of season ($\beta = -0.123$, $P = 0.0272$).

In all analyses, variance inflaction factors were small (ranged from 1.01 to 1.11), suggesting that collinearity among independent variables was not a problem.

4. Discussion

Almost all of the circulatory serotonin is transported and stored in platelets, which take it up from the blood plasma through the efficient uptake mechanism mediated by transmembrane 5HTt protein. Since platelets do not possess synthetic potential for 5HT, their

Table 2
Correlation between platelet serotonin level (PSL) and maximal velocity (V_{max}) of platelet serotonin uptake (PSU) in dependence of gender and age in healthy individuals.

	Number of subjects	Correlation coefficient	Significance
Females, all	42	−0.0527	n.s.
Males, all	276	0.3145	<0.0001
<30 years	36	0.4374	0.0076
31-50 years	176	0.3121	<0.0001
>50 years	57	0.1817	n.s.

n.s. = non significant.

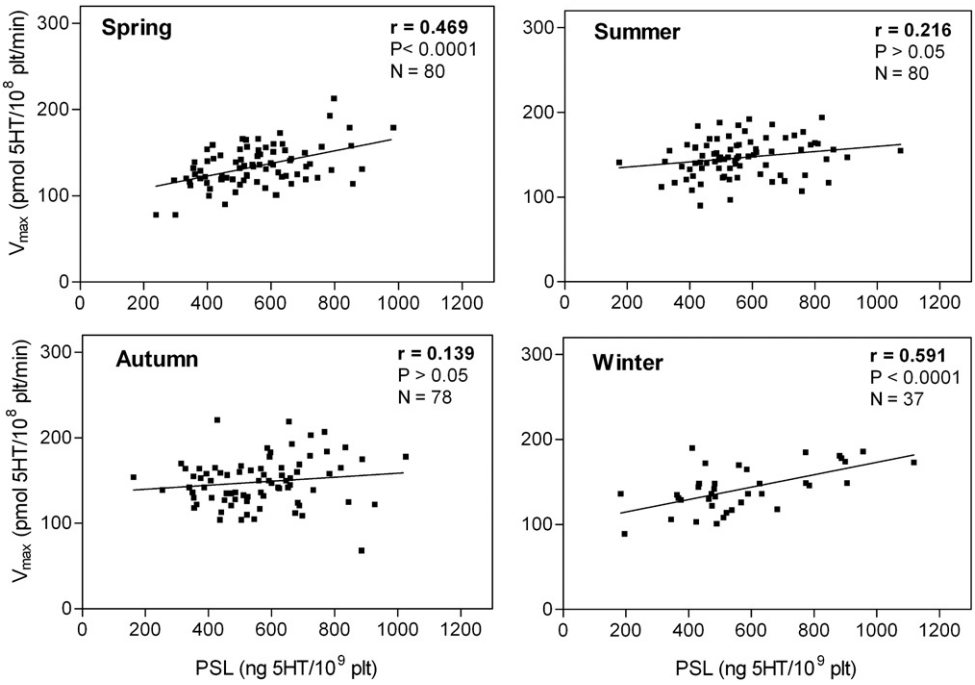

Fig. 2. Correlation between platelet serotonin level (PSL) and maximal velocity (V_{max}) of platelet serotonin uptake in male subjects across four seasons. r = coefficient of correlation, N = number of subjects.

concentration of this amine depends completely on the activity of membrane 5HTt. Therefore, a kind of relation between PSL and the activity of PSU mechanism would be expected. Indeed, our previous studies on rats have shown that the breeding selection of animals for extreme values of PSL resulted in clearly different values of V_{max} of PSU [11].

We have previously described characteristics of both PSL [9] and PSU [10] in a large human population, with specific consideration of personal (age, gender) and environmental (seasonal) physiological influences on both variables, and here we focus on their mutual relationship. Mean values of platelet 5HT parameters (Table 1) fit well in the reported literature, as discussed in our previous papers [9,10]. Search for literature on the PSL–PSU relationship, on the other hand, revealed very few studies, performed mostly on small samples and reporting variable strength and significance of their correlation (Table 3).

Our results on overall population of 318 healthy individuals demonstrated low, but highly significant association between PSL and V_{max} of PSU (Fig. 1). Similar correlation coefficient, but without statistical significance was observed in a previous study by Franke et al. [14].The same group demonstrated highly significant correlation between PSL and the efficiency of PSU uptake (V_{max}/K_m, $r = 0.627$, $P<0.001$), which is in agreement with our findings. The absence of association between PSL and K_m of PSU also accords with the mentioned study, whereas other studies referred in Table 3 did not report on their relationship. In fact, comparison of our data with literature is difficult to make, because studies reporting PSL–PSU association did not perform separate analysis for different sex, age or season, which in our hands appeared to be relevant for interpretation of the results. According to the results of multiple regression analyses, it seems that annual rhythm of PSU represents the major factor influencing this relationship.

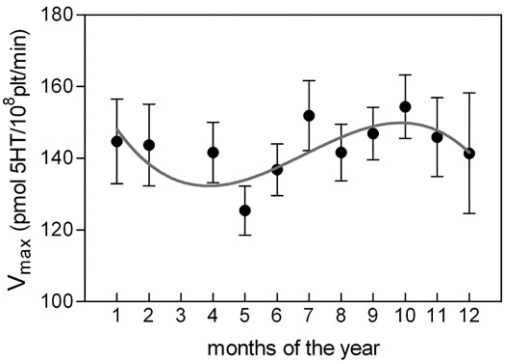

Fig. 3. The seasonal rhythm of maximal velocity (V_{max}) of serotonin uptake in human platelets. Data were fitted with third order polynomial curve to identify the peaks and troughs. Mean ± 95% CI. N = 16–45 per month.

Gender analysis of platelet 5HT parameters showed that positive correlation between PSL and PSU is present only in males, whereas is completely absent in females (Table 2), although according to the multiple regression analysis gender does not contribute to either PSL or PSU variability. From previous studies, it is known that platelets vary in size and age with menstrual cycle, which consequently causes variations in platelet 5HT measures in women [17,18]. Literature comparison of these 5HT parameters between sexes gave variable results [19–22], while our own studies demonstrated lower PSU kinetic parameters, higher PSU efficiency and a tendency toward increased PSL values in females as compared to males [9,10]. These results are in line with frequently reported sex-related differences in serotonergic homeostasis. According to the results reported here, it is

Table 3
Literature reports on correlation between platelet serotonin level (PSL) and maximal velocity (V_{max}) of platelet serotonin uptake (PSU).

Reference	Number of subjects (males/females)	Age of subjects (years)	Correlation coefficient	Significance
[8]	14 (6/8)	37.0 ± 12.1	0.175	n.s.
[12]	31 (19/12)	13.9 ± 4.9	0.55	0.001
[13]	18 (n.a.)	38.2 ± 6.7	0.56	0.020
[14]	57 (27/30)	41.1 ± 14.7	0.238	n.s.
[15]	15 (12/3)	41.7 ± 9.6	0.576	0.025
[16]	26 (n.a.)	31.9 ± 7.8	0.09	n.s.
this study	318 (276/42)	42.4 ± 9.9	0.269	0.0001

Data are given as mean ± SD; n.a.= not available; n.s. = not significant.

possible that hormonal influences, which are supposed to be responsible for sex differences in 5HT homeostasis, reflect also on the relationship of platelet 5HT parameters.

Regarding the age influence, intraindividual correlation between PSL and V_{max} values of PSU decreases with age, being the most significant in the youngest group (under 30 years), and without significance in the oldest one (over 50 years). This result can be compared to our earlier studies of age influence on platelet 5HT parameters, showing decrease of PSL in older individuals [9], but an absence of age effect on PSU parameters [10]. The observed decrease in correlation between PSL and V_{max} of PSU with increased age could probably be the consequence of age influence on PSL, but not on PSU. Here, significant interrelation was shown between PSL and age ($r=-0.172$, $P=0.0024$), although age was not included into final regression model ($P=0.065$). By our knowledge, in the literature there are no systematic investigations on this issue. An association between PSL and PSU V_{max} was shown in study dealing with teenager subjects of both sexes [12], which is consistent with our finding of higher correlation coefficient in younger subjects (see Table 2). Other studies, performed mostly on adult population between 30 and 40 years old, reported variable results with respect to the value and significance of the PSL–PSU correlation (Table 3).

Study of seasonal oscillations revealed cyclic pattern of PSL–PSU relationship, with the highest correlation in winter and spring, lower correlation in summer, and lack of their interrelation in autumn (Fig. 2). Seasonality in PSL–PSU relationship could be possibly better understood if we consider the finding that only PSU, and not PSL, is under significant influence of season. Number of literature data report seasonal variability in both investigated variables of the platelet 5HT system, but results, often obtained on small samples, are not unequivocal [23–28] and indicate different pattern of annual rhythm in PSL and PSU. In our hands, somewhat higher PSL values were found in spring [9], whereas PSU V_{max} values were highest in autumn [10]. Comparison of PSU across different months of the year presented here indicate that peak values in PSU occur in autumn equinox, and trough values on spring equinox (Fig. 3). This could probably relate to the seasonal changes in free-plasma 5HT reported previously [23]. It has been proposed recently that elevated plasma 5HT could limit its own uptake in platelets by down-regulating 5HTt [29]. Nevertheless, when looking at the relationship between these two platelet 5HT parameters, differences were found between periods of winter–spring and summer–autumn, which should not be neglected.

Based on our results, it could be hypothesized that 5HT transporter plays dominant role in determining PSL during the period with lower ambient light, whereas this influence is absent in the course of high daylength season. Here, it should be recalled that almost all physiological and behavioural function in humans occur on a rhythmic basis and that serotonergic activity is influenced by annual and circadian rhythmicity [30–32]. Moreover, serotonergic disturbances are implicated in aetiology of seasonal affective disorder (SAD) [33], where winter-SAD is suggested to be induced by the decreased ambient light, in contrast to the summer-SAD supposedly induced by high temperature [34]. In periphery, there is evidence that release of 5HT from platelets is under influence of melatonin, a hormone responsible for circadian and seasonal rhythmicity [35]. It seems therefore that seasonal changes in daylight exposure significantly affect serotonergic homeostasis, both central and peripheral.

It is not clear which mechanisms trigger observed associations. It could be supposed that factors affecting PSL and PSU, probably variation of daylight time, are subjected to different annual rhythm, leading finally to different rhythmicity also in their relationship. Although 5HTt is the only supplier of serotonin in platelets, there are oscillations in the free 5HT in plasma, which could influence the amine content in platelets [29]. Factors affecting plasma/platelet 5HT level include 5HT synthesis/release from enterochromaffin cells, volume of the gut wall, gut length, the rate of 5HT clearance by lungs and liver, body mass [36–38] etc. Seasonal variations, possibly daylength dependent, were shown in availability of plasma 5HT [23], which mainly depends on 5HT production and release from the gastrointestinal tract [5]. Since transport of 5HT into platelets is unsaturated process, they have the capacity for accumulating the additional amine from blood plasma if available [39], as can be seen, e.g. in carcinoid syndrome [40].

Understanding the relationship among various components of the peripheral 5HT system is important for better insights into etiology of disorders where altered function of peripheral 5HTt occurs. For example, relationship between platelet and plasma 5HT level and 5HT transport function seems to be important in regulation of the blood pressure [41,42] and peripheral circulation [43], while understanding the origin of platelet hyperserotoninemia in autism is expected to shed some more light on autistic brain [38].

Based on presented results, we could argue that, in human platelets, relationship between platelet 5HT content and platelet 5HT transporter activity is markedly influenced by physiological–biological and environmental variables. In contrast to low correlation between PSL and V_{max} of PSU in humans, the strength of this relationship is much higher in some other species, e.g. rat [11], horse (personal data, not published) and monkey [44] which may be relevant for comparative studies of (platelet) 5HT system in mammals. Additionally, our results showing stronger correlation between PSL and PSU at the lowest 5HT concentration indicate that, given the very low concentration of serotonin in blood plasma, measuring the uptake at lower 5HT concentration might be more reflective of *in vivo* platelet 5HT uptake than measuring the V_{max}. Also, it would be interesting to perform similar clinical study in northern latitudes where the difference between winter and summer daylight is much higher than in southern Europe.

In conclusion, our results demonstrated unexpectedly low correlation between two directly related platelet 5HT parameters: granular 5HT level and velocity of 5HT uptake, which argues against the use of platelet 5HT level as an indirect measure of platelet 5HT transporter activity in basal (non-pharmacological) conditions in human, and also against its use as peripheral measure of neuronal 5HT transporter functionality. Results also implicate gender importance in studies of platelet 5HT system, and point to the seasonality in peripheral serotonergic mechanisms that regulate platelet 5HT level and the activity of platelet 5HT transporter.

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