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ORIGINAL REPORT

The hockey-stick method to estimate evening dim light melatonin onset (DLMO) in humans

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The onset of melatonin secretion in the evening is the most reliable and most widely used index of circadian timing in humans. Saliva (or plasma) is usually sampled every 0.5–1 hours under dim-light conditions in the evening 5–6 hours before usual bedtime to assess the dim-light melatonin onset (DLMO). For many years, attempts have been made to find a reliable objective determination of melatonin onset time either by fixed or dynamic threshold approaches. The here-developed hockey-stick algorithm, used as an interactive computer-based approach, fits the evening melatonin profile by a piecewise linear-parabolic function represented as a straight line switching to the branch of a parabola. The switch point is considered to reliably estimate melatonin rise time. We applied the hockey-stick method to 109 half-hourly melatonin profiles to assess the DLMOs and compared these estimates to visual ratings from three experts in the field. The DLMOs of 103 profiles were considered to be clearly quantifiable. The hockey-stick DLMO estimates were on average 4 minutes earlier than the experts' estimates, with a range of −27 to +13 minutes; in 47% of the cases the difference fell within ±5 minutes, in 98% within −20 to +13 minutes. The raters' and hockey-stick estimates showed poor accordance with DLMOs defined by threshold methods. Thus, the hockey-stick algorithm is a reliable objective method to estimate melatonin rise time, which does not depend on a threshold value and is free from errors arising from differences in subjective circadian phase estimates. The method is available as a computerized program that can be easily used in research settings and clinical practice either for salivary or plasma melatonin values.

Keywords: DLMO, hockey-stick algorithm, melatonin

INTRODUCTION

Melatonin is the best and most commonly used index of circadian timing in humans (Benloucif et al., 2005; Klerman et al., 2002; Lewy et al., 1989). In practice, sampling saliva in half-hourly to hourly intervals under dim light in the evening (when melatonin levels usually begin to rise) is sufficient to estimate circadian phase, the dim-light melatonin onset (DLMO) (Benloucif et al., 2008; Lewy et al., 1989). The estimate is often based on either a fixed threshold method (time of attaining a 1- or 3-pg/ml level; e.g. Paul et al., 2009) or a dynamic threshold method (so-called ''2SD'' or ''3 k'': two standard deviations above the mean of three or more pre-rise values; Voultsios et al., 1997). However, based on the large inter-individual differences in melatonin production (i.e. low and high melatonin producers; Burgess & Fogg, 2008) the fixed method may be problematic for inter-individual comparisons. The dynamic method is difficult to apply when too few (less than 3) or inconsistent values form the baseline part of the profile.

Another approach is a so-called physiologically-based mathematical model considering the average rate of secretion and clearance of melatonin in human plasma or saliva. The method was developed for the full-night melatonin rhythm, but may also be used for the evening part of the profile as if the rest of the profile were known (Klerman et al., 2012; St Hilaire et al., 2007). However, as far as we know, the method is not yet widely used in clinical practice. We developed a computerized, easy to use, interactive algorithm (''hockey-stick'' method) to estimate the most probable switching time from ''no-rise'' to ''rise'' in the evening

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melatonin profiles, and compared it to subjective DLMO estimations of three experts in human circadian melatonin phase assessments.

METHODS

Algorithm description

The method name ''hockey stick'' was first coined by Marijke Gordijn and Marina Gimenez (personal communication). We formulated and tested different approaches to approximate the switching timepoint on the melatonin profile, using MATLAB software. For development of the algorithm, 364 evening salivary melatonin profiles were available for testing. An investigator's visual assumption as to when the melatonin rise most likely occurred was a judgment criterion and initially used for validation. The final built-in algorithm included 3 steps.

Step 1 determines whether the melatonin profile meets the criteria for proper DLMO analysis and which part of the profile will be included in the fitting procedure. For that, melatonin raw data have to be entered into the module (time in hours and decimals, melatonin values in pg/ml; Figure 1). To start the program, a melatonin level should be assigned to divide the profile in a lower and a higher (ascending) part. This ascending level is set to 5 pg/ml by default, but can be changed interactively. This is sometimes required if melatonin profiles are below 5 pg/ml or higher than 5 pg/ml (e.g. for melatonin levels in plasma). A change in the ascending level is also useful in ambiguous profiles (Figure 2c–e). Importantly, a change in the ascending level does not influence the DLMO estimation in unambiguous profiles (out of the 103 profiles analyzed and presented in the Results section, the change of the level from 5 to 2.3 pg/ml led to difference in DLMO in two cases, for only 0.05

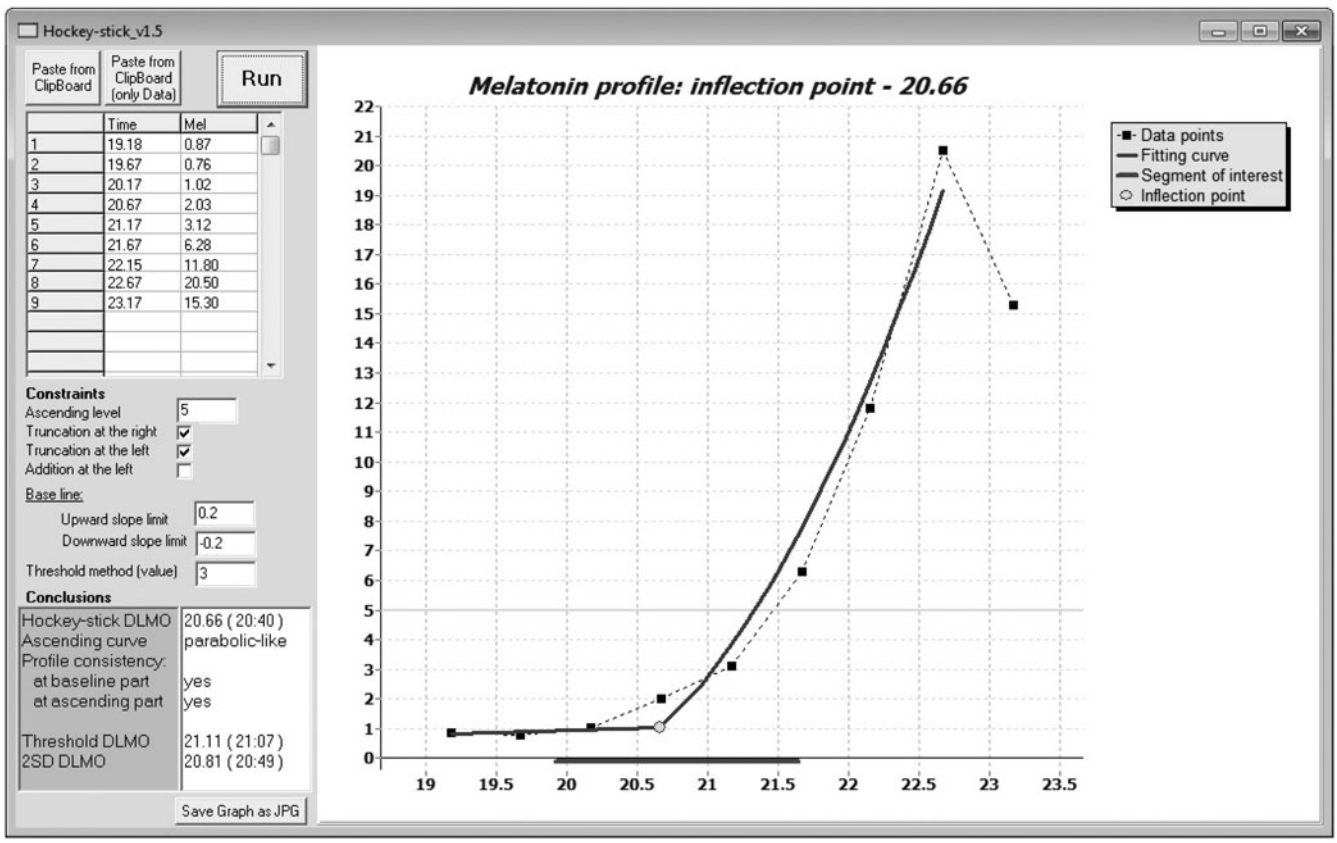

FIGURE 1. Hockey-stick program module. Example of the fitting to a melatonin profile with an accelerating parabolic-like rise. Tabs and windows on the left hand side: ''ascending level'' is set to 5 pg/ml (represented by a horizontal line on the graph) to divide the profile into a left-hand (lower) and a right-hand (higher) part to start the program. ''Truncation at the right'' tab is ticked off to automatically determine the ascending part of the profile to be considered in the computations. ''Truncation at the left'' tab excludes high (above the ascending level) initial baseline values of the profile from the computations, if they exist. The ''Addition at the left'' option allows addition of a left-sided node if the baseline part is constituted by one data point only. ''Base line'' slope limits constrain a slope of the straight fitting line (lying to the left of inflection point) and the default is ±0.2. ''Threshold method value'' is automatically set to 3 pg/ml to estimate the threshold DLMO in addition to the hockey-stick value, and may be changed interactively. The ''Conclusions'' window includes the hockey-stick DLMO result, categorization of the ascending fitting curve as parabolic-like or straight-line-like, a report on profile consistency at baseline part and at ascending part (''no'' would indicate a diversity of melatonin data points, suggesting that DLMO may not be reliable, and should be checked for outliers to be removed). ''Threshold DLMO'' and ''2SD DLMO'' are included for users who want this estimate as well as the hockey-stick DLMO.

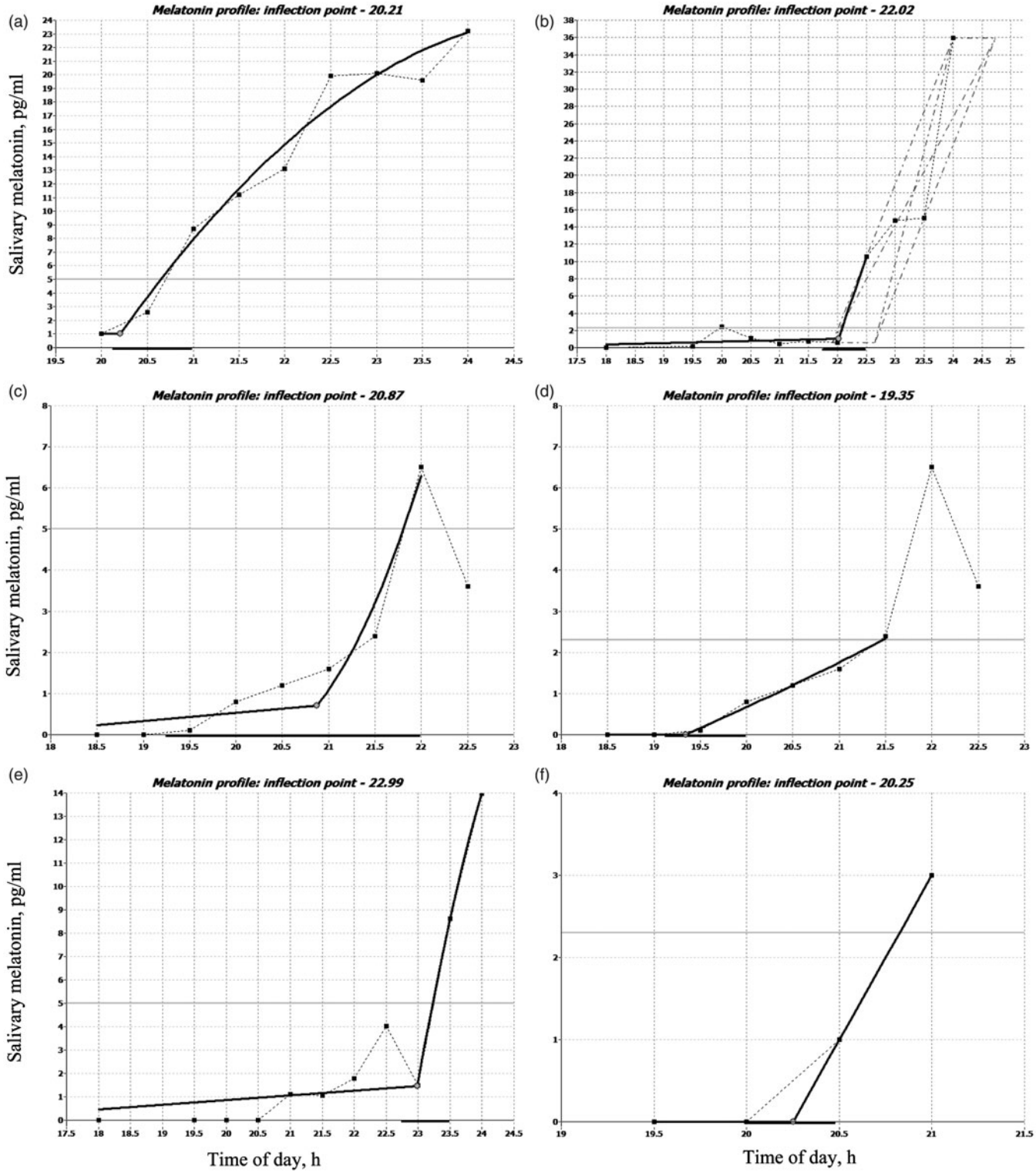

FIGURE 2. Examples of the hockey-stick fitting. (a) Melatonin profile with a decelerating parabolic-like rise. (b) ''Parallelogram rule'' defining whether the ascending data points lie within a sufficiently narrow ''corridor'' (least-squares parallelogram), i.e. constitute a straightforward rise, to be included in the fitting. Here the slope ratio of diagonals in the parallelogram is more than 2 (2.08), therefore, the rise is not straightforward; only the first segment of the ascent was finally included in the fitting. (c) and (d) is the same data set. In this ambiguous profile a change of the ascending level setting in the program from 5 pg/ml (left profile) to 2.3 pg/ml (right profile) changes the DLMO estimate from 20.87 h to 19.35 h. This latter profile was included in the analysis, as all raters estimated the DLMO around 19.80 to be correct (the high data point at 22 may be an artifact). This profile also demonstrates the greatest difference between the hockey stick estimation and the raters' estimation, −27.3 min. (e) An ambiguous melatonin profile that was not included in the statistical analysis: a 1.67-hour earlier DLMO may be also considered at 21.32 h (when lowering the default ascending level from 5 to 1.4 pg/ml). (f) Fitting of a 3-data-point melatonin profile (theoretical data set). The left-hand baseline point is added by the program.

and 0.09 h). The ascending level is also necessary for step 3 (see below).

Baseline, ascending and intermediate parts of the profile are defined based on an algorithm developed by us that considers the ascending level setting, but mainly the slope sign (positive, zero or negative) and the tangent ratio (''a two-times ratio'' criterion) of the profile segments. The profile is truncated on the right if it does not satisfy a triple condition, which includes our specially developed ''parallelogram rule'' (Figure 2b). In turn, the baseline part, if constituted by one profile data point only, may be automatically added with a left-sided node (Figure 2f) lying two times below and 30 minutes earlier than this baseline data point by ticking the ''Addition at the left'' option, and the computation is continued. The computation is stopped if the program reports ''no baseline part'' or ''no ascending part''.

Step 2 defines a time segment during which the melatonin rise would occur. The algorithm (description omitted) is based on the baseline, ascending and intermediate parts division done during step 1. The obtained ''segment of interest'' is represented by a thick line on the *X*-axis and is used for step 3.

Step 3 fits the melatonin profile using certain conditions and constraints. The profile is fitted by a piecewise function: $F(t) = f_1(t) * \theta(t_0 - t) + f_2(t) * \theta(t - t_0)$, where $f_1(t)$ is a straight line: $f_1(t) = a_1 t + b_1$, $f_2(t)$ is a parabola: $f_2(t) = a_2 t^2 + b_2 t + c_2$, t_0 is the time of the inflection point (see definition below) and $\theta(x)$ is the Heaviside step function:

$$\theta(x) = \begin{cases} 0, & x < 0 \\ \frac{1}{2}, & x = 0 \\ 1, & x > 0. \end{cases}$$

The function is graphically represented by a straight line switching to a branch of parabola (either accelerating or decelerating) or to another straight line (Figures 1 and 2). The switch is hereafter called the ''inflection point''. During the fitting, the inflection point ''travels'' across the points of a virtual grid confined within the segment of interest along the *X*-axis and between the lowest profile node and the ascending level (set by default to 5-pg/ml) along the *Y*-axis; the shortest step of the grid is set to 0.01 along each axis. The greater the area of interest, the greater the computation time (usually not more than 1–10 seconds).

The fitting is performed using orthogonal (the shortest distance) least-squares method applied separately for the data points lying to the left and to the right of the inflection point. The distances on the left are diminished twice to lower the influence of baseline nodes variability and to improve *X*–*Y* scaling comparability. Our testing showed that a change of the scaling (by increasing or decreasing the melatonin values twofold) did not significantly influence the DLMO estimates in the majority of cases.

The tangent of the parabola at its beginning (= at inflection point) was restricted to be no less than half the slope of a straight line virtually fitting the same data points. This prevents a definition of a too early DLMO in profiles with slowly accelerating melatonin rise (Figure 1). Such an accelerating ascent was signaled to be ''parabolic-like'' in the conclusions window of the module, otherwise the rise was classified as ''straight-line-like''.

Baseline was restricted to have a slope between −0.2 and 0.2 (Figure 2c and e). These limits were based on an assumption that melatonin concentration follows a ''corridor'' 0–2.3 pg/ml over ~11.5 daytime hours (2.3/11.5 = 0.2). The 2.3 pg/ml level was chosen based on the calculation that no more than 5% of the salivary melatonin values occasionally exceeded 2.3 pg/ml during pre-rise portion. Remarkably, the same level was obtained in the study by Molina & Burgess (2011) for a dynamic threshold (see Introduction section) when they defined DLMO in their salivary melatonin profiles. The usefulness of the ±0.2 window versus 0 (=horizontal base line) or wider than ±0.2 was supported during testing. Only in some cases (e.g. in low melatonin producers) the limit constraints may be advised to be set to 0 by changing upward and downward slope limits interactively.

The method may run even if only 4 or 3 melatonin data points are present for the profile (Figure 2f). However, it is not designed to judge between two alternative decisions when, for some reason, melatonin levels rise twice during the evening (Figure 2e).

In addition to the hockey-stick DLMO, the module also computes fixed and dynamic threshold DLMOs to meet requirements for some users (Figure 1). These DLMOs are determined by linear interpolation when the melatonin profile segment crosses a certain concentration level. The default fixed threshold (i.e. 3 pg/ml) can be changed interactively. The dynamic threshold is a mean of melatonin values comprising the non-ascending part of the profile (defined during step 1 of the algorithm) and the first value in the ascending part (if it does not exceed the non-ascending values), plus two standard deviations (SD) of the mean.

Profiles analyzed

Taking into account individual reproducibility of the melatonin profiles (''almost like a hormonal fingerprint''; Arendt, 1998), one profile from each of the 109 subjects who participated in 8 studies performed in Novosibirsk and Basel was chosen for the analysis (Table 1). For all but seven study participants, it was the first profile during the study; in seven, another profile was taken since the first one was not analyzable (no melatonin rise, rise without baseline or ''W''-like pattern). The profiles comprised 8–14 data points (median = 9) in half-hourly intervals (rarely greater).

TABLE 1. Number of salivary melatonin profiles analyzed.

City	Study acronym	Reference	Setting	Assay method	Number of profiles analyzed
Novosibirsk	Dawn-1	Danilenko et al. (2000a)	lab	RIA	9
	Dawn-2	Danilenko et al. (2000a)	lab	RIA	9
	Dawn-3	Danilenko et al. (2000b)	lab	RIA	8
	Sleep-advance	Danilenko et al. (2003)	lab	RIA	10
	ERG in DD	Danilenko et al. (2009)	lab	RIA	7
	Shift-work	Sergeeva et al. (2010)	lab	RIA	9
	Dusk	Danilenko & Hommes (2013)	home	ELISA	26
Basel	LAD	Wirz-Justice et al. (2011)	home	RIA	31

Melatonin was assayed using the Bühlmann RIA (radioimmunoassay) or ELISA (enzyme-linked immunosorbent assay) kits (http://www.buhlmannlabs.ch; Weber et al., 1997). During the DLMO estimation with the hockey-stick method, the method user (KVD) also registered whether there could be an ambiguity in the profile estimation leading to clearly different solutions (≥0.5-hour difference; see an example in Figure 2e).

At the same time, four independent experts were invited to visually classify the most certain time of melatonin rise according to their own experience. They had to provide two figures after the decimal point in each of the estimations (e.g. 21.67 hours) and also indicate if there was considerable difficulty (ambiguity) in judging the inflection time and if yes, they might classify an alternate inflection time. Besides these instructions, we explained to the raters that the hockey-stick algorithm ''is not aimed to define the earliest or the latest increase, but rather the most probable one, a time of the most rapid transition between no-rise and rise (inflection time)''. Each of the raters and the hockey-stick user (KVD) emailed their estimations to an independent investigator of the study (CC) to maintain ''blindness''. After receiving all estimations, the investigator performed a preliminary statistical analysis of the data. StatView 5.0.1 software (SAS Institute Inc., Cary, NC) was used for the statistical analysis (Student's *t*-test, equality of variances *F*-test and Pearson correlation test).

RESULTS

Three raters provided very similar DLMO estimations. Estimations of the other rater, though internally consistent, were approximately 0.5 hours later. This was subsequently explained by unclear instructions, which made the rater apply a threshold method instead. Therefore, these data could neither be included in the analysis, nor could the rater be asked to repeat the estimations as the rater was no longer ''blind''.

Four of the 109 profiles were considered difficult (ambiguous) by at least two out of the four raters (hockey-stick user included) and yielded an inter-rater DLMO difference of more than 0.5 hours. Thus, they were not included in the analysis (4–14 profiles were considered to be difficult by raters). One of these melatonin profiles is presented in Figure 2(e). Two further profiles were not included because step 1 in our algorithm discovered a ''no baseline part''. No default parameters in the program were changed during the computations except for a decrease of the ascending level setting 5 pg/ml in profiles with melatonin values lower than 5 pg/ml at the ascending part.

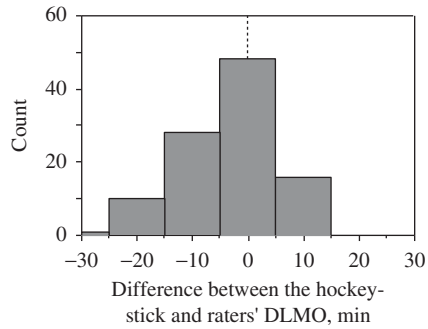

FIGURE 3. Histogram of the difference in minutes between the hockey-stick and raters' DLMO ($N = 103$).

Mean DLMOs estimates of the three raters fell within an 8-minute window: 20.93, 21.00 and 21.06 hours, and were thus rather similar though significantly different ($p < 0.001$, Student's *t*-test). The average hockey-stick DLMO 20.94 ± 1.16 (SD) hours was 3.6 ± 8.0 minutes earlier than the average raters' DLMO estimate 21.00 ± 1.12 hours ($p < 0.0001$, Student's *t*-test). The distribution of the differences was normal (Figure 3). In 48 profiles (47%), the difference between the hockey-stick and raters' estimations fell within ±5 minutes. In 92 cases (89%) the deviation did not exceed 15 minutes. In only 2 profiles (<2%) did the difference exceed 20 minutes: −20.6 and −27.3 minutes (the latter profile is shown in Figure 2c and d).

The result did not depend on whether the profile rise was categorized as parabolic-like ($N = 25$) or straight-line-like ($N = 78$; $p = 0.41$ by unpaired Student's *t*-test); whether the saliva was collected in the laboratory ($N = 50$) or at home ($N = 53$; $p = 0.22$); or whether the assay was performed using ELISA ($N = 23$) or RIA ($N = 80$; $p = 0.50$).

Comparison of the DLMO obtained using four methods (hockey stick, 3 pg/ml, 1 pg/ml and 2SD; see Methods section for definition), with the raters' DLMO,

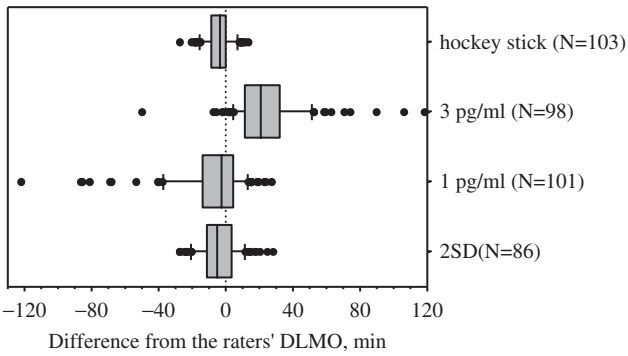

FIGURE 4. Difference between the dim light melatonin onset (DLMO) defined by various methods – hockey-stick, 3-pg/ml, 1-pg/ml or 2SD – and visually rated DLMO. Each box shows 25th and 75th percentiles, an intermediate vertical line marks the median. The end of the whiskers marks the 10th and 90th percentiles, the individual points are the cases outside the 10th to 90th percentiles range.

showed a great diversity in the differences (Figure 4). The distribution of the differences between the hockey-stick and raters' estimates was significantly narrower compared with the distributions of the differences between the other three estimates and raters' estimates ($p<0.0001$, equality of variances F-test). A similar result was obtained when the hockey-stick method was a reference for the other four methods ($p<0.0001$). Visually, the 2SD method did almost as well as the hockey-stick method compared to the visual inspection (Figure 4). Nevertheless, correlation analysis showed poor accordance between the differences (hockey-stick DLMO minus raters' DlMO vs. 2SD DLMO minus raters' DLMO; $r=0.44$, $p=0.44$, $N=86$, Pearson's test). Five profiles were not used for 3-pg/ml DLMO estimation as melatonin concentration did not attain the 3 pg/ml level. Two profiles were not used for 1 pg/ml DLMO estimation as melatonin concentration was consistently above the 1-pg/ml level. Seventeen (17%) profiles were missed in 2SD DLMO estimations as less than three melatonin values formed the pre-rise part of the profile. To be noted, the average difference between the 3-pg/ml threshold and hockey-stick method was 28 minutes.

DISCUSSION

A satisfactory mathematical algorithm (''hockey-stick'' method) has been developed to objectively approximate a ''real'' onset time of the evening melatonin concentration rise in human saliva or plasma. The deviation from the experts' estimations did not exceed 15 minutes in the majority of cases (89%), and never exceeded 28 minutes, which can be considered a very good result for melatonin profiles using half-hourly sampling windows.

The analysis showed, however, that the hockey-stick DLMO estimates did not match well with DLMO estimates obtained with the 3 pg/ml, 1 pg/ml or 2SD threshold methods, confirming previous suggestions that the widely used threshold DLMO may not reliably reveal an initial melatonin rise (Molina & Burgess, 2011; St Hilaire et al., 2007).

A further improvement of the algorithm could be an automatic division of the lower and higher parts of the melatonin profile without adherence to a somewhat arbitrary 5 pg/ml level. Without this, ''a two-times ratio'' criterion remains the single ''semi-arbitrary'' mathematical operation in the algorithm; the criterion categorizes melatonin segments into steep and non-steep segments, truncates the profile on the right and constrains the parabola tangent. We also consider programming an intermediate DLMO choice in case of equal straight line or parabola approximations of the rise when only 2 or 3 melatonin values constitute the ascending part, as well as reporting a profile ambiguity when alternative decisions (>0.5-hours difference) exist. These tasks are for the future, after we receive feedback from program users. The program is freely available for researchers via a personal request to KVD and may be passed on from one investigator to another without permission.

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DECLARATION OF INTEREST

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