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## VARIABILITY OF SKIN AND HAIR MELANIN INDICES AND OF ERYTHEMA INDEX IN POLISH PREPUBERTAL CHILDREN

**ABSTRACT:** Spectrophotometric analysis of human pigmentation characteristics plays an important role in medicine and cosmetology. However, to correctly interpret the results, one must know the normal range of variability of pigmentation and erythema in the population. This is of utmost importance for quantitative assessment of melanocytic nevi, hemangiomas, and allergic reactions. The objective of the present work was to determine the physiological range of variability of skin and hair pigmentation and of erythema in Polish prepubertal children. The study encompassed Polish children aged 7 to 10 years without any abnormalities in skin or hair pigmentation. A total of 802 children were examined. Constitutive skin and hair pigmentation and skin erythema were evaluated using a dermaspectrometer. The LMS method (Cole 1990, Pan, Cole 2004) was used for the construction of percentile charts presenting the distribution of skin melanin index (SMI), hair melanin index (HMI), and erythema index (EI) values in the various age and sex groups. The presented normal ranges of variability of SMI, HMI, and EI are the first Polish reference standards for these indices. The manner in which the data are presented makes it possible to graphically assess these characteristics (percentile charts) as well as to conduct their normalization using L, M, and S parameters. The normalized values may also be converted to percentiles. Standards may be used in clinical practice, e.g., for evaluating pigmentation abnormalities and skin reactions to inflammations, irritations, and allergies. Furthermore, they may be applied in designing dermatological and pharmacological research.

**KEY WORDS:** Melanin index - Erythema index - Pigmentation

### INTRODUCTION

Advances in spectrophotometry have enabled precise, objective quantitative evaluation of pigmentation characteristics, and, by the same token, identification of very subtle changes that could not be captured using

earlier descriptive methods (the von Luschan and Fitzpatrick scales for skin color assessment or the Fischer-Saller scale for hair color description). Some of these methods (Fitzpatrick phototypes and the Fischer-Saller scale) are still successfully used in examinations where the variability of the studied traits

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within particular categories is not of major interest. Nevertheless, quantitative evaluation methods are better suited for identifying components of variance than subjective, categorical qualitative methods.

Spectrophotometric analysis of human pigmentation characteristics plays an important role in medicine and cosmetology. Precise quantitative evaluation of melanocytic nevi, hemangiomas, and allergic reactions in healthy individuals is practically impossible without reference to the normal range of variability of pigmentation in a population. In the literature, quantitative pigmentation characteristics are usually given for adults (Kleesz *et al.* 2012, Matsubara 2012), and even if they include children or adolescents (Firooz *et al.* 2012, Pezic *et al.* 2013, Yun *et al.* 2010), then they are provided for age groups that are broader than one year. So far, no reference data concerning quantitative skin and hair color assessment have been produced for Polish children and adolescents. The only study in this field has been conducted by Michałowska (1996), who discusses variability in skin pigmentation in children and adolescents between 10 and 18 years of age (probably in the Polish population, but that is not clearly stated). However, due to the fact that the work cited above was not aimed at developing reference standards, it cannot be used for comparative purposes, as the data are presented only in the form of a chart showing mean values.

The objective of the present work was to determine the normal range of variability of skin and hair pigmentation and of erythema in Polish children aged 7–10 years. While the reported results concern only a narrow age range of children, they may be used as a frame of reference for evaluating individual or collective constitutive skin and hair pigmentation and erythema in children at this stage of ontogenesis.

## MATERIAL AND METHODS

The study was approved by the Bioethics Committee of the University of Łódź. It was conducted from

November 2009 to May 2012 (excluding the summer months of June, July, and August) at 15 randomly selected primary schools representing all the districts of the city of Łódź. At each school, the parents or legal guardians of the children were informed about the objective and methodology of the study and were requested to express written consent for examination of their children. The study encompassed Polish children aged 7–10 years (6.50–10.49) without abnormalities in skin or hair pigmentation, who have not tanned within one month preceding the examination. Taking into account the fact that the primary goal of the study was a development of pigmentation feature standards for children in prepubertal age, amenorrhoea was accepted as a qualification condition to participate in the study. A total of 802 children were examined (*Table 1*).

Constitutive skin and hair pigmentation and erythema were evaluated using a DSM II dermaspectrometer (Cortex Technology®, Denmark). This device detects differences in the absorption spectra of melanin and hemoglobin. While melanin absorbs light with different wavelengths, it exhibits the strongest absorption for short waves in marked contrast to long waves. In turn, hemoglobin has a wide absorption spectrum for green light (490–560), followed by a steep decline, so it absorbs little red light (630–780) (*Figure 1*). Therefore, red light absorption is used for evaluation of melanin content in the skin, while erythema is evaluated by determining the difference between green and red light absorption. The dermaspectrometer measures the intensity of reflected light at the wavelengths specified above and returns melanin and erythema indices (SMI and EI) calculated according to the formulas given below (Fullerton *et al.* 1996, Shriver, Parra 2000, Wagner *et al.* 2002):

$$SMI = 100 \times \log \frac{1}{I_{red}}$$

$$EI = 100 \times \log \frac{I_{red}}{I_{green}}$$

where: *I* – intensity of reflected light.

TABLE 1. Study group broken down into age and sex categories.

| Sex   | Age (Years)  |      |              |      |              |      |               |      | Total<br>n = 802 |      |
|-------|--------------|------|--------------|------|--------------|------|---------------|------|------------------|------|
|       | 7<br>n = 139 |      | 8<br>n = 251 |      | 9<br>n = 246 |      | 10<br>n = 166 |      |                  |      |
|       | n            | %    | n            | %    | n            | %    | n             | %    | n                | %    |
| Boys  | 67           | 48.2 | 115          | 45.8 | 105          | 42.7 | 84            | 50.6 | 371              | 46.3 |
| Girls | 72           | 51.8 | 136          | 54.2 | 141          | 57.3 | 82            | 49.4 | 431              | 53.7 |

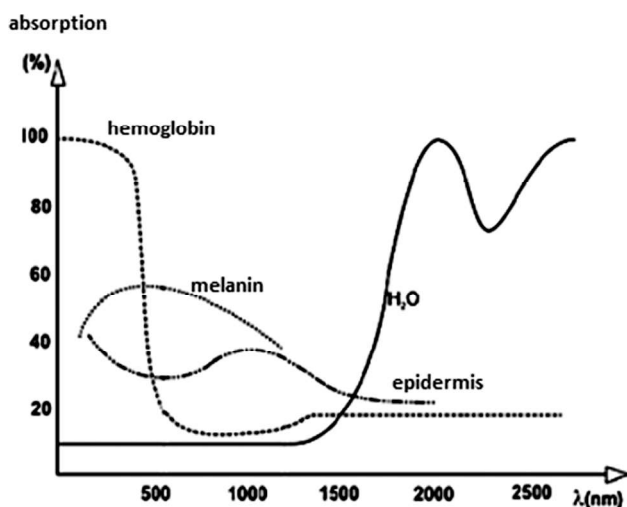


FIGURE 1. Absorption spectra for melanin and hemoglobin.

The higher the values of the indices, the stronger the pigmentation and erythema are. In contrast to the color description system *CIELAB* ( $L^*a^*b^*$ ), these indices are designed in such a way as to be linearly correlated with the content of melanin and hemoglobin in the skin (Takiwaki 2006).

The dermaspectrometer was calibrated before conducting examinations on a given day according to the manufacturer's instructions. Skin pigmentation was measured on the inner upper arm (right and left). This region of the body is commonly used in studies of constitutive skin pigmentation due to its low exposure to sunlight (Duffy *et al.* 2004, González *et al.* 2010, Manning *et al.* 2004, Manning, Caswell 2004, Shriver, Parra 2000, Wagner *et al.* 2002). Measurements were done in triplicate for both arms, each time applying the dermaspectrometer sensor to a different spot within the skin region defined above, excluding nevi and visible discolorations. The input data for statistical analysis were arithmetic means from all measurements on both arms because no statistically significant differences were found in SMI ( $p = 0.8572$ ) and EI ( $p = 0.2421$ ) values between the right and left arm.

Hair pigmentation was measured in triplicate on hair close to the scalp in the occipital region (Shriver, Parra 2000) to avoid evaluation of facultative pigmentation (induced by the action of solar radiation). An arithmetic mean from all three measurements was calculated for each child.

The LMS method proposed by T. J. Cole was used for the construction of percentile charts presenting the

distribution of skin melanin index (SMI), hair melanin index (HMI), and erythema index (EI) values for the various age and sex groups, as this method enables normalization of skewed distributions (based on the lambda parameter, or  $L$ , in Box-Cox transformations) as well as optimum smoothing of the percentile curves thanks to the application of the median ( $M$ ) and the generalized coefficient of variation ( $S$ ) (Cole 1990, Pan, Cole 2004). Calculations were made using LMSChartMaker Pro version 2.3 software (Medical Research Council, UK) (Cole TJ, Pan H, *LMSChartmaker*, <http://www.healthforallchildren.co.uk>, 2005). For each of the studied variables, equivalent degrees of freedom (edf) models were constructed (edfL/edfM/edfS) with an appropriate degree of  $L$ ,  $M$ , and  $S$  curve smoothing. The LMS method makes it possible to fit percentile curves to reference data by increasing or decreasing the edf for the  $L$  curve (edfL). If the distribution of a variable is normal, then at edfL = 0 the  $L$  parameter has a constant value of 1 for all age groups. In this way, the skewness falling within normal distribution is not corrected and the percentile curves are determined by  $M$  and  $S$  curve smoothing. If a distribution significantly differs from normal, but exhibits the same direction of skewness in the various age groups, then edfL = 1 smoothing is used to correct skewness by a constant (mean) value for all age groups, without accounting for changes in the direction of skewness with age. In the case of variables with distributions significantly deviating from normal and the direction of skewness differing with age, the same transformation may not be used for all age groups. Here, modeling percentile curves requires edfL  $\geq 3$  for  $L$  curve smoothing. It was assumed that the best fitted models are those with the lowest deviation from the chi-square probability distribution and the Q-test.

In practical applications of the developed standards, e.g., in the normalization of individual children's anthropometric characteristics in reference to the presented data, one should use the following formula:

$$Z_{ind} = \frac{(Anth/M)^L - 1}{LS}$$

where:  $Z_{ind}$  – normalized value of the characteristic; Anth – value of the characteristic for a given child;  $M$ ,  $L$ , and  $S$  – parameters for the characteristic in question (Anth) as well as for the child's sex and age (provided in the tables).

Normalized values ( $Z_{ind}$ ) may be transformed to percentile values using a normal distribution table or the *NORMSDIST* function in the MS Excel

spreadsheet. The exact position of values falling beyond the  $\pm 3$  SD range ( $Z_{ind} < -3$  or  $Z_{ind} > 3$ ), is determined using the ( $Z_{ind}^*$ ) formulas recommended by the World Health Organization (WHO, <http://www.who.int/growthref/computation.pdf> 2008).

A standard interpretation is recommended for evaluation of the pigmentation features in child, using the presented centile values, determined for a given sex and age class: 25–75 centile – normal value; below the 25<sup>th</sup> centile – low feature values; above 75<sup>th</sup> centiles – high feature values. The values for the  $Z_{ind}$  (SD score) correspond to some commonly used centiles (Cole 1990).

## RESULTS

SMI, EI, and HMI statistics for the four studied age groups of children are presented in Table 2. Among the studied characteristics, the highest variation in all the age groups of boys and girls was found for HMI (19.1%–24.3%), followed by EI (11.9%–16.9%) and SMI (8.2%–11.7%), as can be seen from Table 2.

Due to the fact that the distribution of SMI, EI, and HMI values for boys and girls aged 6.50–10.49 years did not significantly deviate from normal (Shapiro-Wilk test results ranged from  $p = 0.0738$  for HMI in boys to  $p = 0.6891$  for SMI in boys), the constructed percentile charts are based on a constant  $L$  parameter ( $L = 1$ ) with a 0/5/3 transformation model.

The percentile values of the studied characteristics and the values of  $L$  (lambda in Box-Cox transformations),  $M$  (the median), and  $S$  (the coefficient of variation), obtained using the LMS method, are given separately for each sex in yearly age groups (Tables 3–5). They are also presented graphically in the form of percentile charts in Figures 2–7.

## DISCUSSION

The human eye is not a spectral organ, so perceptions of pigmentation and erythema are highly subjective. The use of spectrophotometric methods

TABLE 2. Statistics of skin melanin index (SMI), erythema index (EI) and hair melanin index (HMI). Note:  $\bar{x}$  – arithmetic mean, SD – standard deviation, CV – coefficient of variation.

| Traits                   | Age (years) | Boys      |       |      |       |        | Girls     |       |      |       |        | Total     |       |      |
|--------------------------|-------------|-----------|-------|------|-------|--------|-----------|-------|------|-------|--------|-----------|-------|------|
|                          |             | $\bar{x}$ | SD    | CV%  | min   | max    | $\bar{x}$ | SD    | CV%  | min   | max    | $\bar{x}$ | SD    | CV%  |
| Skin melanin index (SMI) | 7           | 29.51     | 3.09  | 10.5 | 22.45 | 36.20  | 30.11     | 2.81  | 9.3  | 23.70 | 35.60  | 29.82     | 2.96  | 9.9  |
|                          | 8           | 30.03     | 3.37  | 11.2 | 23.05 | 38.59  | 30.78     | 3.61  | 11.7 | 22.15 | 39.17  | 30.44     | 3.52  | 11.6 |
|                          | 9           | 30.07     | 2.87  | 9.5  | 23.49 | 36.01  | 31.01     | 3.24  | 10.4 | 23.49 | 38.57  | 30.63     | 3.12  | 10.2 |
|                          | 10          | 30.43     | 2.49  | 8.2  | 25.60 | 36.70  | 32.11     | 2.72  | 8.5  | 26.99 | 37.75  | 31.26     | 2.73  | 8.7  |
|                          | Total       | 30.04     | 3.00  | 10.0 | 22.45 | 38.59  | 31.00     | 3.26  | 10.5 | 22.15 | 39.17  | 30.56     | 3.18  | 10.4 |
| Erythema index (EI)      | 7           | 9.49      | 1.43  | 15.1 | 6.21  | 12.36  | 9.60      | 1.14  | 11.9 | 7.23  | 12.81  | 9.54      | 1.29  | 13.5 |
|                          | 8           | 9.40      | 1.48  | 15.7 | 6.05  | 13.23  | 9.57      | 1.50  | 15.7 | 6.38  | 12.80  | 9.49      | 1.49  | 15.7 |
|                          | 9           | 9.35      | 1.32  | 14.1 | 6.46  | 12.31  | 9.39      | 1.46  | 15.5 | 5.92  | 13.06  | 9.37      | 1.40  | 14.9 |
|                          | 10          | 9.33      | 1.51  | 16.2 | 6.45  | 13.29  | 9.28      | 1.57  | 16.9 | 5.54  | 12.53  | 9.30      | 1.53  | 16.5 |
|                          | Total       | 9.38      | 1.43  | 15.2 | 6.05  | 13.29  | 9.46      | 1.45  | 15.3 | 5.54  | 13.06  | 9.43      | 1.44  | 15.3 |
| Hair melanin index (HMI) | 7           | 76.06     | 17.80 | 23.4 | 40.47 | 118.09 | 85.29     | 16.49 | 19.3 | 53.32 | 118.58 | 80.71     | 17.70 | 21.9 |
|                          | 8           | 80.57     | 18.88 | 23.4 | 35.44 | 125.77 | 88.42     | 21.39 | 24.2 | 45.31 | 135.39 | 84.82     | 20.62 | 24.3 |
|                          | 9           | 84.23     | 20.17 | 23.9 | 44.06 | 132.44 | 91.81     | 19.22 | 20.9 | 48.09 | 142.00 | 88.57     | 19.95 | 22.5 |
|                          | 10          | 87.31     | 19.39 | 22.2 | 48.96 | 138.13 | 96.58     | 18.47 | 19.1 | 56.75 | 139.41 | 91.89     | 19.45 | 21.2 |
|                          | Total       | 82.32     | 19.49 | 23.7 | 35.44 | 138.13 | 90.61     | 19.69 | 21.7 | 45.31 | 142.00 | 86.75     | 20.02 | 23.1 |



TABLE 3. Percentiles and LMS parameters for the skin melanin index (SMI) in boys and girls. Note: *L* (lambda in Box-Cox transformations), *M* (the median), and *S* (the coefficient of variation).

| Sex   | LMS model 0/5/3 |          |          | Age (years) | Percentiles |       |       |       |       |       |       |       |       |
|-------|-----------------|----------|----------|-------------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|
|       | <i>L</i>        | <i>M</i> | <i>S</i> |             | 3           | 5     | 10    | 25    | 50    | 75    | 90    | 95    | 97    |
| Boys  | 1               | 29.59    | 0.104    | 7           | 23.82       | 24.55 | 25.66 | 27.52 | 29.59 | 31.66 | 33.52 | 34.64 | 35.36 |
|       | 1               | 29.84    | 0.108    | 8           | 23.78       | 24.54 | 25.71 | 27.67 | 29.84 | 32.01 | 33.97 | 35.14 | 35.90 |
|       | 1               | 30.16    | 0.098    | 9           | 24.60       | 25.30 | 26.37 | 28.17 | 30.16 | 32.16 | 33.95 | 35.02 | 35.72 |
|       | 1               | 30.60    | 0.078    | 10          | 26.10       | 26.66 | 27.53 | 28.98 | 30.60 | 32.21 | 33.66 | 34.53 | 35.09 |
| Girls | 1               | 30.04    | 0.097    | 7           | 24.57       | 25.26 | 26.32 | 28.08 | 30.04 | 32.00 | 33.76 | 34.82 | 35.50 |
|       | 1               | 30.85    | 0.111    | 8           | 24.41       | 25.22 | 26.46 | 28.54 | 30.85 | 33.16 | 35.24 | 36.48 | 37.29 |
|       | 1               | 31.07    | 0.104    | 9           | 25.00       | 25.76 | 26.93 | 28.89 | 31.07 | 33.25 | 35.21 | 36.39 | 37.15 |
|       | 1               | 32.11    | 0.085    | 10          | 26.97       | 27.62 | 28.61 | 30.27 | 32.11 | 33.95 | 35.61 | 36.60 | 37.25 |

TABLE 4. Percentiles and LMS parameters for the erythema index (EI) in boys and girls. Note: *L* (lambda in Box-Cox transformations), *M* (the median), and *S* (the coefficient of variation).

| Sex   | LMS model 0/5/3 |          |          | Age (years) | Percentiles |      |      |      |      |       |       |       |       |
|-------|-----------------|----------|----------|-------------|-------------|------|------|------|------|-------|-------|-------|-------|
|       | <i>L</i>        | <i>M</i> | <i>S</i> |             | 3           | 5    | 10   | 25   | 50   | 75    | 90    | 95    | 97    |
| Boys  | 1               | 9.47     | 0.155    | 7           | 6.71        | 7.05 | 7.59 | 8.48 | 9.47 | 10.46 | 11.36 | 11.89 | 12.24 |
|       | 1               | 9.38     | 0.150    | 8           | 6.74        | 7.07 | 7.58 | 8.43 | 9.38 | 10.33 | 11.18 | 11.69 | 12.02 |
|       | 1               | 9.37     | 0.149    | 9           | 6.74        | 7.07 | 7.58 | 8.43 | 9.37 | 10.32 | 11.17 | 11.67 | 12.01 |
|       | 1               | 9.30     | 0.156    | 10          | 6.57        | 6.91 | 7.44 | 8.32 | 9.30 | 10.29 | 11.17 | 11.70 | 12.04 |
| Girls | 1               | 9.55     | 0.124    | 7           | 7.32        | 7.60 | 8.03 | 8.75 | 9.55 | 10.36 | 11.08 | 11.51 | 11.79 |
|       | 1               | 9.62     | 0.147    | 8           | 6.96        | 7.29 | 7.80 | 8.66 | 9.62 | 10.57 | 11.43 | 11.94 | 12.28 |
|       | 1               | 9.39     | 0.159    | 9           | 6.59        | 6.94 | 7.48 | 8.39 | 9.39 | 10.40 | 11.31 | 11.85 | 12.20 |
|       | 1               | 9.23     | 0.169    | 10          | 6.30        | 6.66 | 7.23 | 8.18 | 9.23 | 10.28 | 11.22 | 11.79 | 12.16 |

Note: *L* (lambda in Box-Cox transformations), *M* (the median), and *S* (the coefficient of variation)

ensures objectivity and comparability of results as well as phenotypic identification of subtle color differences.

Pigmentation measurements are currently used in clinical practice for evaluation of pigmentation abnormalities as well as skin reactions to infections, irritations, and allergies. They can also be helpful in estimating the risk of developing certain skin diseases (melanoma and non-melanoma skin cancer, undesirable skin reactions), determining PUVA dosage, or administering laser therapy (González *et al.* 2010). Such measurements are also used for evaluation of

therapeutic outcomes in the course of discoloration treatment (e.g., Eimpunth *et al.* 2014). Knowledge of the physiological variability of skin and hair pigmentation and of erythema is important not only for its own sake, but is also essential for designing dermatological and pharmacological studies and developing tools for evaluating pigmentation phenotypes. Using the presented reference standards, one can evaluate individual or collective skin and hair pigmentation and erythema. The obtained normal ranges of variability of SMI, HMI, and EI will also be

TABLE 5. Percentiles and LMS parameters for the hair melanin index (HMI) in boys and girls. Note: *L* (lambda in Box-Cox transformations), *M* (the median), and *S* (the coefficient of variation).

| Sex   | LMS model 0/5/3 |          |          | Age (years) | Percentiles |       |       |       |       |        |        |        |        |
|-------|-----------------|----------|----------|-------------|-------------|-------|-------|-------|-------|--------|--------|--------|--------|
|       | <i>L</i>        | <i>M</i> | <i>S</i> |             | 3           | 5     | 10    | 25    | 50    | 75     | 90     | 95     | 97     |
| Boys  | 1               | 74.54    | 0.231    | 7           | 42.09       | 46.16 | 52.43 | 62.90 | 74.54 | 86.18  | 96.65  | 102.92 | 106.99 |
|       | 1               | 80.55    | 0.232    | 8           | 45.33       | 49.75 | 56.55 | 67.92 | 80.55 | 93.18  | 104.55 | 111.35 | 115.77 |
|       | 1               | 84.30    | 0.232    | 9           | 47.53       | 52.14 | 59.25 | 71.12 | 84.30 | 97.49  | 109.36 | 116.47 | 121.08 |
|       | 1               | 88.10    | 0.221    | 10          | 51.41       | 56.02 | 63.10 | 74.94 | 88.10 | 101.26 | 113.10 | 120.19 | 124.79 |
| Girls | 1               | 83.86    | 0.203    | 7           | 51.78       | 55.81 | 62.00 | 72.35 | 83.86 | 95.36  | 105.71 | 111.90 | 115.93 |
|       | 1               | 88.53    | 0.228    | 8           | 50.64       | 55.39 | 62.71 | 74.94 | 88.53 | 102.12 | 114.35 | 121.67 | 126.42 |
|       | 1               | 91.78    | 0.217    | 9           | 54.32       | 59.02 | 66.26 | 78.35 | 91.78 | 105.22 | 117.31 | 124.55 | 129.25 |
|       | 1               | 98.15    | 0.169    | 10          | 66.96       | 70.87 | 76.90 | 86.96 | 98.15 | 109.33 | 119.40 | 125.42 | 129.33 |

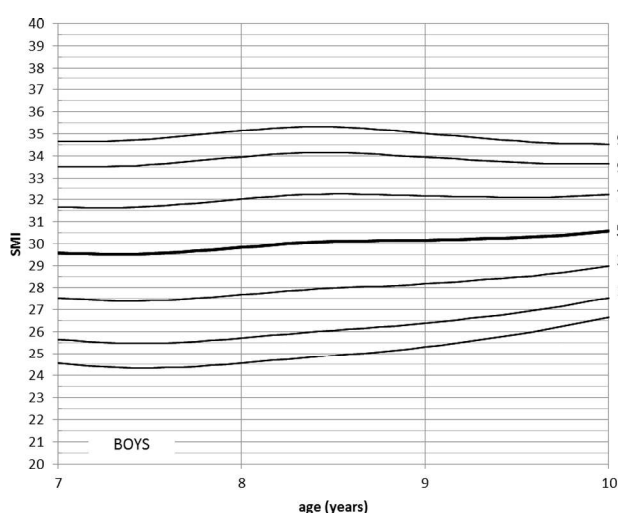


FIGURE 2. Skin melanin index (SMI) percentile chart for boys.

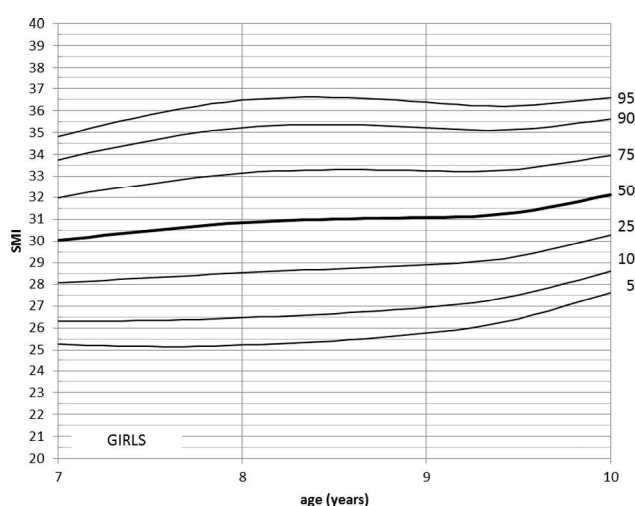


FIGURE 3. Skin melanin index (SMI) percentile chart for girls.

helpful in categorizing these variables (e.g., based on the 25<sup>th</sup> and 75<sup>th</sup> percentiles).

The standards were defined separately for girls and boys since the level of melanisation in prepubertal phase of ontogenesis demonstrates sexual dysmorphism – the girls are more strongly pigmented than boys (regarding both skin and hair). The enhanced melanisation of the skin is one of the first morphological signals of puberty, associated with the increased levels of sex hormones in girls. This change

precedes the pubertal leap of body height and menarche (Sitek *et al.* 2013). In boys, skin pigmentation gets more intensive probably later, since the onset of their puberty comes later, while the effect of skin darkening may be enhanced by androgens which influence the process of melanogenesis much more strongly than estrogens (Edwards, Duntley 1949).

The presented standards are limited to the Polish population only and to the narrow scope of ontogenesis, encompassing the prepubertal age only. It

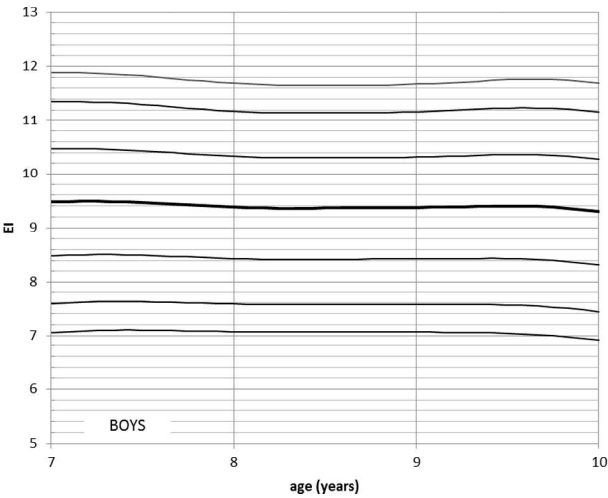


FIGURE 4. Erythema index (EI) percentile chart for boys.

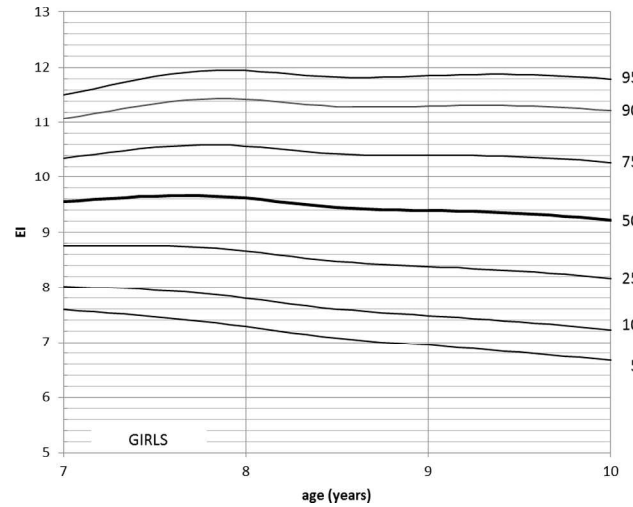


FIGURE 5. Erythema index (EI) percentile chart for girls.

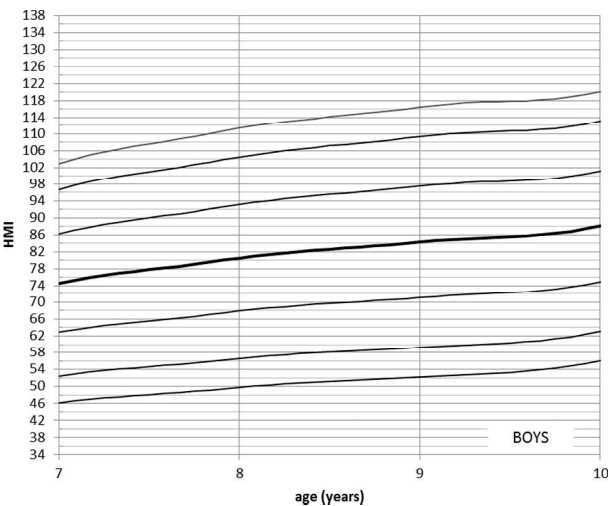


FIGURE 6. Hair melanin index (HMI) percentile chart for boys.

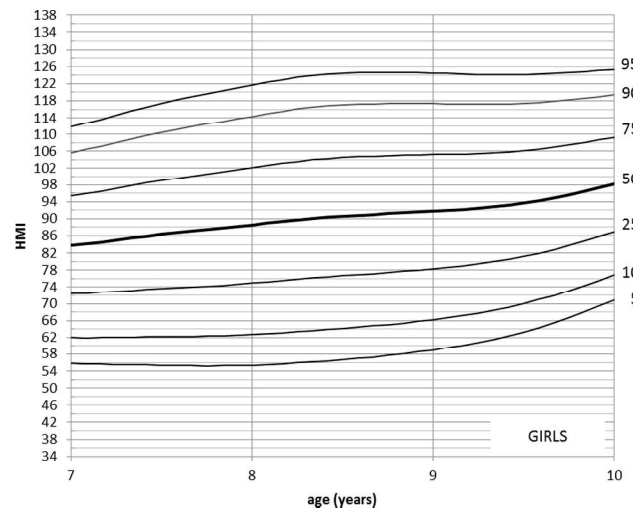


FIGURE 7. Hair melanin index (HMI) percentile chart for girls.

would be fairly justified to include reference data, encompassing the entire personal development and populations with different levels of constitutive skin and hair pigmentation.

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