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Effects of psychological stress on skin and hair pigmentation in Polish adolescents

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Abstract: The effects of psychological stress, gender and age on hair and skin pigmentation levels were evaluated in the reported study. The material included Polish high-school and university students aged 18–22 (in the age range 17.50–22.49). All subjects who had sunbathed or used tanning beds or lamps, skin tanning agents, tanning extenders and/or medical agents affecting skin pigmentation during the 60 days preceding the beginning of the study were excluded. The use of hormonal contraceptives within a month prior to the study was also an excluding factor. Stress levels were evaluated by the Perceived Stress Scale (PSS–10) in the Polish adaptation, while hair and skin pigmentation levels were assessed with a dermaspectrometer (Cortex Technology®, Denmark, 2007). The study was carried out with the exclusion of the summer period. Skin pigmentation was evaluated in 395 subjects (264 women and 131 men). Hair pigmentation was analyzed in a smaller group of 351 subjects (223 women and 128 men), as some had had their hair dyed within 12 months prior to the study while in some others the hair was too short to be correctly measured.

Regardless of their age, the studied women felt much more stress related to their life situation and were characterized by stronger skin pigmentation than the examined men. No sex differences were identified with regard to hair pigmentation. In the studied period of ontogenesis (18–22 years of age), hair pigmentation levels increased with age, while skin melanization remained stable. Disregarding the effects of age and sex, the level of perceived stress was negatively correlated with skin pigmentation levels; no such relationship was found for hair melanization.

Key words: skin melanin index, hair melanin index, perceived stress scale, PSS–10, dermaspectrometer

For many years, it has been assumed that the main function of melanocytes is melanin synthesis and distribution. However, substantial recent evidence indicates that these cells have also a number of other functions as they transform exo- and endogenous signals into an organized control network to maintain skin and systemic homeostasis (Slominski et al. 2004; Slominski 2009; Slominski et al. 2012a). This concept has been proven, demonstrating that melanocytes are not only *target action structures*, but also *sites of synthesis* of many compounds participating in immune functions and systemic reactions to stress (Slominski et al.

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2012b). Melanocytes are, among others, part of the so-called skin immune system (SIS) (Smit et al. 1993) and produce classical stress neurotransmitters, neuropeptides and neurohormones, thus acting as neuroendocrine cells (Slominski 2009; Slominski et al. 2012a). Interestingly, the production of these substances is hierarchical and conforms with classical organizational structures, such as the hypothalamic-pituitary-adrenal (HPA) axis (Slominski and Mihm 1996; Slominski et al. 2005c,d; 2007; Ito et al. 2005; Slominski 2009) as well as the serotoninergic (Slominski et al. 2005b) and catecholaminergic systems (Schallreuter 1997), which participate in the biological reaction to stress (Landowski 2007). A hypothesis has been put forward that melanocytes are elements linking the skin with the central nervous system (CNS) (Slominski et al. 1993; Ruiz-Maldonado and Orozco-Covarrubias 1997; Slominski and Wortsman 2000). Thus, melanocytes are also called "skin neurons" (Aaron B. Lerner, cited in Slominski 2009).

According to numerous reports, both systemic and local stressors associated with CNS functions induce or exacerbate various diseases of the skin and its appendages (e.g., hair). Psychological stress is now regarded to be an important etiological factor in psoriasis, atopic dermatitis, pruritus and urticaria (e.g., Kimyai-Asadi and Usman 2001; Kmieć and Broniarczyk-Dyła 2008). Stress may also affect hair growth or even cause its loss (York et al. 1998; Arck et al. 2001, 2003). Stress effects on the skin and its appendages are mediated, among others, via neurohormones and neuropeptides, which modulate the functions of keratinocytes, Langerhans cells, mast cells, endothelial cells, and immune cells (Grando 1997; Schallreuter 1997; Scholzen et al. 1998; Misery 2000; Slominski and Wortsman 2000; Slominski et al. 2000; Arck et al. 2001; 2003; Kmieć and Broniarczyk-Dyła 2008; Slominski et al. 2012b).

Skin and hair pigmentation systems are also significant elements of the response to stress (Tobin and Kauser 2005). Substances which correlate the mechanism of biological response to stress with pigmentation-controlling processes include the following: corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (MSH), serotonin, β-endorphins, as well as catecholamines (dopamine, adrenaline, noradrenaline) and corticosteroids (mainly cortisol) (Slominski et et al. 2000; 2001; 2007). CRH, ACTH, MSH and β-endorphins clearly play a melanogenic function (Slominski et al. 2000; 2004; Kauser et al. 2004; 2005;Hirobe 2005; Paus 2011), while serotonin (O'Malley 1960; Slominski et al. 2004), dopamine (Slominski et al. 2004), adrenaline and noradrenaline (O'Malley 1960) exert an inhibiting effect on melanogenesis. Regarding corticosteroids, some authors are of the opinion that they stimulate the biosynthesis of melanin (Shibata et al. 1993), while others have demonstrated their depigmentation effects (Arnold et al. 1975).

The role of ACTH and MSH peptides in skin and hair pigmentation processes has been proven clinically in patients with proopiomelanocortin (POMC) gene mutations (POMC is a precursor of ACTH and MSH), who – besides other symptoms – reveal light skin pigmentation and red hair (Krude et al. 1998; Krude and Gruters 2000). In turn, pathologically elevated POMC/ACTH plasma levels in Addison's disease or an exces-

sive production of ACTH by tumour cells (Nelson's syndrome) are associated with hyperpigmentation of skin and hair (Slominski and Wortsman 2000; Stenn and Paus 2001; Paus 2011). However, it should be noted here that in a study on mice by Slominski et al. (2005a) no changes in melanogenesis were observed after POMC gene inactivation, thus the effects of the gene are not absolute.

The above-mentioned neurohormones and neurotransmitters are not the only factors which affect skin and hair pigmentation. Important controllers of the stress-related functions of melanocytes include also cytokines, growth factors, prostaglandins, histamine, substance P, neuropeptide Y and prolactin (e.g., Slominski et et al .2004; Arck et al. 2006a,b; Costin and Hearing 2007; Tam and Stępień 2007; Kmieć and Broniarczyk-Dyła 2008; Paus 2011; Slominski et al. 2012b).

Following the above-mentioned findings, it was decided to examine whether psychological stress modifies the phenotypic effect of hair and skin pigmentation in Polish adolescents, and, if so, in what way.

Materials and methods

The present study was approved by the Institutional Bioethical Committee (KBBN-UŁ/II/11/2010). The material comprised Polish high-school and university students aged 18–22 (in the age range 17.50–22.49). The data were collected during the period from March 2010 to December 2011, disregarding the May–September summer periods in 2010 and 2011. Only those subjects were examined who agreed to fill in a questionnaire form containing questions regarding gender, date of birth, as well as information about sunbathing, the use of tanning beds or lamps, skin tanning agents or tanning extenders, and/or medical agents affecting skin pigmentation during 60 days prior to the beginning of the study. The questionnaire also asked whether and when the subjects dyed their hair and women were questioned about the use of hormonal contraceptives. Hair dyeing within 12 months and hormonal contraception within 30 days preceding the beginning of the study were exclusion factors.

Additionally, the questionnaire contained a psychometric tool assessing the level of perceived stress, the so-called Perceived Stress Scale (PSS–10) designed by Cohen et al. (1983) and adapted to Polish conditions by Juczyński and Ogińska-Bulik (2009). This tool was created to evaluate stress intensity related to the patient's life situation during the last month. Stress intensity corresponds here not to the number of stressful events but to their subjective evaluation. In its Polish adaptation, the tool includes ten (10) questions. The interviewed person answers these questions by choosing a number: 0 – never, 1 – almost never, 2 – occasionally, 3 – fairly often, 4 – very often. Prior to calculating of the general index of perceived stress, it is necessary to change scores in answers to positively-formulated questions (for detailed guidelines, see: Juczyński and Ogińska-Bulik 2009). The total score is the sum of all partial scores and falls in the range 0–40. The higher the PSS–10 score value, the higher the perceived stress intensity. The reliability of the Polish adaptation of PSS–10 was evaluated by its authors following a thorough assessment of its internal consistency and absolute stability. Internal consistency was assessed in a study of a group of 120 adults, which revealed a Cronbach's alpha coefficient of

0.86. The reliability of the Polish adaptation as determined by a repeated examination of a 30-person group of students at a 2-day interval was 0.90, and 0.72 at a 4-week interval (Juczyński and Ogińska-Bulik 2009). The authors of the Polish adaptation of the PSS–10 tool indicate that the scale accurately measures subjective perceptions associated with personal events and problems and with coping abilities and mechanisms.

Constitutive pigmentation of hair and skin was studied with a dermaspectrometer (Cortex Technology®, Denmark, 2007). Taking advantage of the differences in the absorption spectra of melanin and haemoglobin, this instrument returns index values for these two pigments. The higher the values of the melanin index (MI) and the erythema index (EI), the darker and ruddier the skin tone. Taking into consideration the goal of the present study, only melanin index (MI) data were used in the analysis. Skin pigmentation measurements (SMI) were repeated three times on the inner part of the left and the right arm, each time changing the sensor location, avoiding moles and visible melanoderma spots (Shriver and Parra 2000, Wagner et al. 2002). Due to the lack of statistically significant differences between the mean values of the melanin index on the right and left arm, individual arithmetic means of six measurements were used for statistical analysis. In each examined subject, hair pigmentation (HMI) was evaluated three times on deeper hair strands in the occipital region (Shriver and Parra 2000), following which an arithmetic mean was calculated from the obtained three results.

Having excluded subjects who reported sunbathing or the use of tanning beds or lamps, skin tanning agents or tanning extenders, and/or medical agents affecting skin pigmentation during the 60 days preceding the beginning of the study, as well as women who had used hormonal contraceptives, 401 Polish subjects (268 females and 133 males) aged 18–22 were qualified for the study. Due to the elimination of observations atypical for linear regression and a lack of hair pigmentation measurements in some of the subjects (dyed hair in women and too short hair in men), the final study group consisted of 395 subjects (264 females and 131 males) for skin pigmentation analysis and 351 subjects for hair pigmentation evaluation (223 females and 128 males).

Table 1 presents the number of subjects in particular classes for each pigmentation variable.

Statistical analysis of the data was conducted by means of the Statistica 10 software package. In the first stage, using two-way ANOVA, the influence of sex and age, as well as of the sex*age interaction was evaluated with regard to the perceived stress level (PSS–10), the skin melanin index (SMI) and the hair melanin index (HMI). The effects of independent variables and of their interactions exerted on particular metric features were assessed by the following coefficients: sample effect size (eta square, η^2) and population effect size (omega square, ω^2). In the second stage, the above-mentioned variables were simultaneously standardized for sex and age to obtain values independent of the influence of the above-mentioned factors (PSS–10 (Z), SMI (Z), HMI (Z)). Since the distributions of such transformed variables did not differ in any significant way from the normal distribution (SMI (Z) SW-W=0.994, p=0.1238; HMI (Z) SW-W=0.993; p=0.1098; PSS–10 (Z)

Table 1. The number of examined subjects (n (%)) with consideration of sex and age

Age (years)	Age interval	SMI			HMI		
		Females	Males	Total	Females	Males	Total
18	17.50–18.49	40	29	69 (17.5)	36	26	62 (17.7)
19	18.50–19.49	91	59	150 (37.9)	86	59	145 (41.3)
20	19.50–20.49	78	23	101 (25.6)	62	23	85 (24.2)
21	20.50–21.49	39	13	52 (13.2)	26	13	39 (11.1)
22	21.50–22.29	16	7	23 (5.8)	13	7	20 (5.7)
	Total	264 (66.8)	131 (33.2)	395 (100)	223 (63.5)	128 (36.5)	351 (100)

SW-W=0.993, p=0.0713), the correlation between stress intensity (PSS–10 (Z)), the skin melanin index (SMI (Z)) and the hair melanin index (HMI (Z)) was evaluated by Pearson's linear correlation.

Results

The mean level of perceived stress in the study group was 18.91 (SD=5.90) – see Table 2. On the whole, the intensity of perceived stress was sex-dependent (F=11.96; p=0.0006); women perceived stress related to one's life situation during the previous month much more intensely than men (see Fig. 1). The "sex" factor explained 6.3% of the variability of stress perception in the study group; this variability index for the population was only slightly lower, namely 6.1%. The analyzed dependent variable did not change with the age of the subjects (F=1.15; p=0.3334), and the "sex*age" interaction was not statistically significant, either (F=1.91; p=0.1088). This means that sex differences regarding the intensity of perceived stress were similar in each age class (see Fig. 1).

The SMI, similarly as the perceived stress level, varied according to sex only (F=15.02; p=0.0001) – see Table 3. Women were on average characterized by darker skin pigmentation than men (see Fig. 2); however, the effect of gender on that feature was weaker than in the case of stress, amounting to 3.8% for the sample and 3.5% for the population. Neither age (F=1.28; p=0.2778) nor the "sex*age" interaction was correlated in any significant way with the skin pigmentation level, which indicates the stability of skin melanization levels during the studied period of ontogenesis (18–22 years) both in women and men. Thus, the extent of sex differences was not age-related (see Fig. 2).

Entirely different results were obtained for hair pigmentation (see Table 4), which increased with age (F=6.81; p<0.0001) but was not sex-related (F=2.06; p=0.1525). The "age" factor

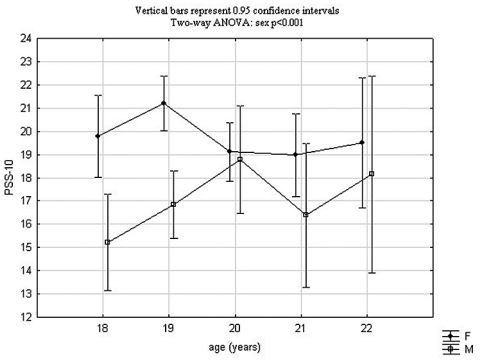

Fig. 1. Mean values of perceived stress (PSS–10) for women (F) and men (M) in the analyzed age classes

Table 2. Results of perceived stress in the study group (PSS–10), n=395

Age (years)	Total		Females		Males		Two-way ANOVA
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
Total	18.91	5.90	19.94	5.66	16.84	5.84	**Sex: F= 11.96 p=0.0006 η_p^2=6.3% ω^2=6.1%**
18	17.86	6.40	19.78	5.92	15.21	6.17	
19	19.48	6.02	21.20	5.44	16.83	5.96	
20	19.04	5.26	19.12	5.39	18.78	4.91	
21	18.33	6.28	18.97	6.29	16.38	6.09	
22	19.09	5.16	19.50	5.28	18.14	5.15	
Two-way Anova	Age: F= 1.15 p=0.3334 η_p^2=1.3% ω^2=0.3%		Sex*Age: F= 1.91 p=0.1088 η_p^2=1.9% ω^2=0.9%				

explained 7.4% of HMI variability in the study group and 6.3% of the variability in the population. No sex differences were noted in hair melanization levels and the "sex*age" interaction was not significant, either (F=1.26; p=0.2870) (Fig. 3).

Taking into account the fact that the level of perceived stress and the level of skin and hair pigmentation were sex- or age-related in the study group, the final statistical analysis was carried out on the values of variables standardized for sex and age: PSS–10 (Z), SMI (Z), HMI (Z).

Having eliminated the effects of sex and age, the obtained linear correlation ([r] Pearson's correlation) indicated that subjects with higher stress perception levels had a lighter skin tone than

Table 3. Skin melanin index (SMI) in the study group, n=395

Age (years)	Total		Females		Males		Two-way ANOVA
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
Total	30.09	2.32	30.50	2.33	29.28	2.08	**Sex: F= 15.02 p=0.0001 η_p^2=3.8% ω^2=3.5%**
18	29.58	2.91	29.70	3.29	29.41	2.34	
19	30.32	2.33	30.89	2.43	29.43	1.85	
20	30.25	2.02	30.57	1.78	29.18	2.42	
21	30.04	1.88	30.51	1.61	28.62	1.96	
22	29.62	2.27	29.87	2.31	29.06	2.22	
Two-way Anova	Age: F= 1.28 p=0.2778 η_p^2=1.3% ω^2=0.3%		Sex*Age: F= 1.12 p=0.3477 η_p^2=1.1% ω^2=0.1%				

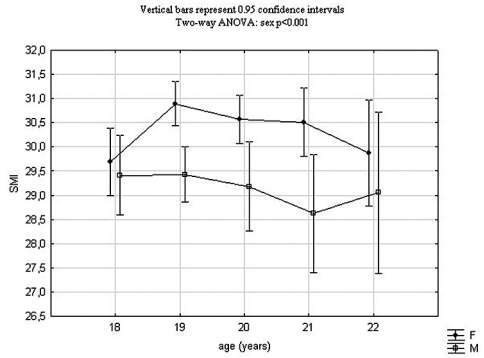

Fig. 2. Mean values of the skin melanin index (SMI) for women (F) and men (M) in the analyzed age classes

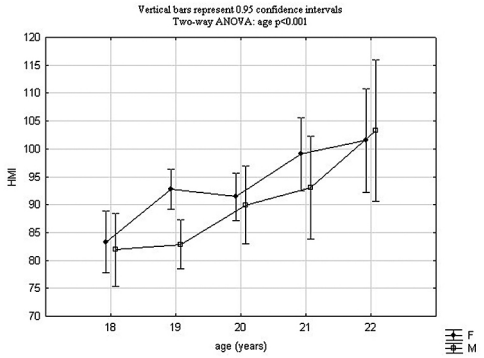

Fig. 3. Mean values of the hair melanin index (HMI) for women (F) and men (M) in the analyzed age classes

those who scored lower on the PSS–10 scale (see Fig. 4). Even though the relationship between these two variables is admittedly weak (r=–0.11), it was still statistically significant (p=0.028).

Unlike the SMI, HMI values (see Fig. 5) did not depend on stress perception levels (r=–0.02; p=0.778), although the sign of the correlation coefficient (negative) was the same as that for SMI results.

Discussion

Stress may be interpreted as a stimulus, a reaction (Selye's concept of stress, 1978) or an interaction between an individual and the environment (the concept of Lazarius and Folkman, 1984). The latter way of understanding stress has received the highest recognition in the psychological literature, since it is based

Table 4. Hair melanin index (HMI) in the study group, n=351

Age (years)	Total		Females		Males		Two-way ANOVA
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
Total	89.93	17.77	92.12	18.45	86.10	15.87	Sex: F= 2.06 p=0.1525 η_p^2=0.6% ω^2=0.3%
18	82.71	15.98	83.30	17.18	81.89	14.46	
19	88.77	16.82	92.80	17.80	82.90	13.37	
20	91.01	16.56	91.41	16.23	89.93	17.75	
21	97.07	20.91	99.08	21.98	93.06	18.77	
22	102.13	17.82	101.50	20.18	103.29	13.74	
Two-way Anova	**Age: F= 6.81 p<0.0001 η_p^2=7.4% ω^2=6.3%**		Sex*Age: F= 1.26 p=0.2870 η_p^2=1.5% ω^2=0.3%				

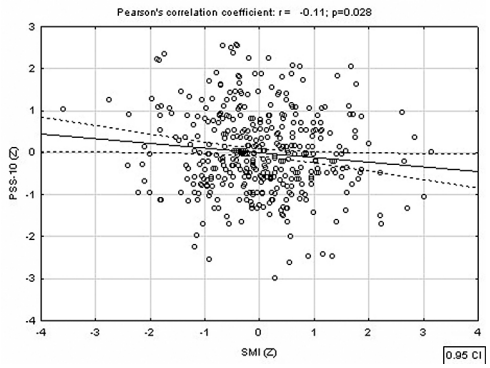

Fig. 4. Linear correlation of perceived stress values (PSS–10) and of the skin melanin index (SMI), standardized for sex and age

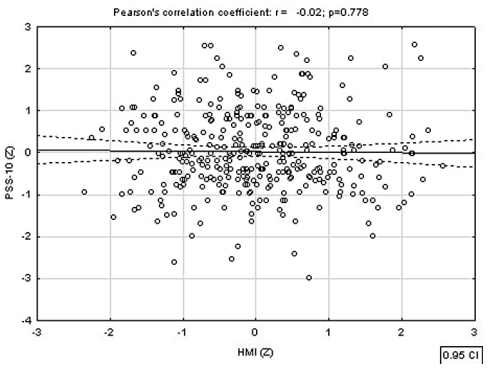

Fig. 5. Linear correlation of perceived stress values (PSS–10) and of the hair melanin index (HMI), standardized for sex and age

mainly on an individual's appraisal of the experienced event. Only a personal, subjective evaluation of a given situation, perceived as a threat, harm/loss or challenge, induces specific psychological and physiological reactions (Juczyński and Ogińska-Bulik 2009). The Perceived Stress Scale (PSS) refers to stress understood in such a way that it does not take into account the experienced event but the way how it is perceived and interpreted by the affected individual.

The mean stress intensity in the studied adolescents ($\bar{x}$=18.91; SD=5.90) was close to the results for subjects of similar age in the Polish population, e.g., emergency medicine students at the Medical University in Lodz, Poland ($\bar{x}$=18.75) (Bartczak and Bartczak: www.kpsw.edu.pl), adult students of vocational schools, secondary technical schools and high schools in Otwock District ($\bar{x}$=19.41) (Walkiewicz 2012), as well as for other populations, e.g., Greeks under the age of 25 ($\bar{x}$=18.53) (Andreou et al. 2011). At the same time, the obtained results were statistically significantly higher ($p<0.0001$) than those obtained by Juczyński and Ogińska-Bulik (2009) for the population of healthy Poles ($\bar{x}$=16.62; SD=7.50), who were on average older than the subjects in the present study group. There may be several causes of this discrepancy. Bartczak and Bartczak indicate that young persons, with relatively little life experience may tap much greater emotional resources than older persons when they find themselves under the pressure of certain events. Another factor which may be responsible for higher subjectively perceived stress levels in high-school and university students may be frustration resulting from the necessity to make choices about one's future (high school students), engage in self-directed learning, often fend for oneself, and assume full responsibility for one's actions (university students). The results of studies conducted by Dudzińska et al. (2007) on second-year medicine students at the Medical University in Lublin and second-year political science students at the Maria Curie-Skłodowska University in Lublin confirm the above-mentioned suggestions. In both studies, the most stressful elements of student life included intensive learning, personal and financial problems, and family expectations. A decrease of stress with age was also confirmed by Andre-

ou et al. (2011), who analyzed persons in three age intervals (≤25, 26–36, >35). The fact that younger persons score higher on the PPS–10 scale was also indicated by Juczyński and Ogińska-Bulik (2009). These authors also showed that females are predisposed to higher levels of felt stress (Juczyński and Ogińska-Bulik 2009; Andreou et al. 2011; Walkiewicz 2012). Also in the present study women perceived the stress associated with their life situation much more intensely than men. The above-mentioned observations show that our results correspond to the literature data regarding the effects of sex and age on the intensity of perceived stress.

No reports have been found in medical literature on the relationship between perceived stress levels and spectrophotometrically measured skin pigmentation in healthy subjects. However, the existence of a relationship between psychological stress and skin pigmentation is indicated by papers reporting emotional trauma as a possible element of the etiopathogenesis of vitiligo (acquired leucoderma) (e.g., Papadopoulos et al. 1998; Firooz et al. 2004; Manolache and Benea 2007; Manolache et al. 2009). According to one theory, oxidative stress plays the key role in this pathological depigmentation (e.g., Bickers and Athar 2006; Namazi 2007). Studies by Cernak et al. (2000), Kupper et al. (2009), and others showed psychological stress to be one of the factors inducing the production of reactive oxygen forms (ROS), which indicates that oxidative stress could be an element linking emotions and the mechanisms responsible for pigmentation processes. Other authors are of the opinion that neuropeptides and nerve growth factors (NGF), which are the key controllers of emotional reactions, may induce vitiligo in subjects predisposed to the development of this medical condition (e.g. Yu et al. 2012). It is thought that also heat shock proteins (HSP 70), which induce autoimmune skin depigmentation, may be a factor linking the mechanism of biological response to stress with skin pigmentation processes (Denman et al. 2008). Another possible factor decreasing skin pigmentation is the fact that persons experiencing increased stress levels or suffering from depression may avoid exposure to sunshine.

According to the literature, emotional stress also affects hair pigmentation (e.g. Navarini et al. 2009; Weissmann 2009; Paus 2011). It is suggested that melanogenesis suppression in hair follicles may involve substance P (a stress-related neuropeptide), which may be responsible for the increased susceptibility of melanocytes to an autoimmune attack (Paus et al. 2006; Paus 2011). Alternatively, substance P may accelerate the catagen phase, inducing the premature termination of melanogenesis, which in hair is limited to the anagen only (Paus et al. 2006; Peters et al. 2007). Other researchers suggest that oxidative stress, which is generated by, among others, emotional stress, may be the underlying cause of the hair greying mechanism (Arck et al. 2006a,b; Inomata et al. 2009). Following this line of reasoning, reactive oxygen species (ROS) would have a cytotoxic effect not only on the already differentiated melanocytes of hair follicles (Arck et al. 2006a,b) but also on the entire pigmentation unit, including the stem cells of melanocytes (Inomata et al. 2009). However, the above-mentioned theories explain only cases of weakening or total suppression of pigment production at the level of the hair follicle, while mechanisms leading to depigmentation of al-

ready necrotic hair parts, i.e., "overnight greying," still remain unknown. Paus (2011) is of the opinion that in such radical and rapid hair greying situations all the key immune and hair pigmentation protecting mechanisms fail and are dysfunctional.

In the present study, stress associated with the life situation of young adults did not modify their hair pigmentation, in contrast to skin pigmentation. It seems that such results for hair pigmentation may be linked to the many differences observed between epidermal and hair follicle melanocytes and, eventually, to differences in the melanogenesis microenvironment of skin and hair. While skin melanogenesis seems to be a continuous process (Nordlund and Ortonne 2006), hair follicle melanogenesis is linked with the hair growth cycle and is limited, as mentioned before, to the anagen phase only (Slominski and Paus 1993; Tobin et al. 1998; 1999). Clinical observations also provide a substantial body of evidence for the relative independence of epidermal and hair pigmentation; examples include white hair on black skin in elderly Africans and blue-black hair in white-skinned Europeans (Tobin 2010). The independence of these pigmentation units is also confirmed by the observed lack of hair pigmentation disturbance in the majority of vitiligo cases, as well as by undisturbed skin pigmentation in *alopecia areata*, also associated with premature hair greying (Tobin and Bystryn 1996; 2000). Some authors suggest that epidermal and hair follicle melanocytes have different antigen profiles; thus some medical agents may decrease skin melanogenesis, while simultaneously increasing the process in hair follicles (Campbell et al. 2009). Despite their common origin, the melanogenically active melanocytes of the hair follicle in the anagen may have larger and longer dendritic processes than melanogenically active skin melanocytes. They are also characterized by a larger Golgi apparatus and endoplasmic reticulum, producing bigger melanosomes than those produced by epidermal melanocytes (e.g., eumelanosomes of the hair follicle in Caucasians are 0.35–1.0 μm^2 in size, while epidermal eumelanosomes are 0.25–0.6 μm^2 in size –Tobin 2010). This parameter affects the nature of their uptake by recipient keratinocytes (Thong et al. 2003) and their relative susceptibility to enzymatic degradation (Chen et al. 2006). Melanin metabolism also differs. While melanin produced by epidermal melanocytes undergoes almost total degradation in the differentiating epidermal layers, in hair pigment granules are transferred to cortical keratinocytes and remain minimally digested; therefore, the hair shaft is similarly pigmented in its various sections (Tobin 2010).

The lack of any stress effects on hair pigmentation levels in our study could be attributed to several causes. First, stress analysis concerned the subjects' experience from the previous month. Thus, taking into account the physiology of hair growth, the pigmentation effect of this factor should be measured on the first centimetre of the hair shaft from the skin (the average hair growth rate is approximately 1 cm per month – Stenn and Paus 2001). During pigmentation measurements the probe was located in such a way as not to measure the pigmentation of the skin of the head, at a certain distance from the hair base. Thus, the obtained results may reflect stress that was felt much earlier than during the previous month, and so of unknown intensity. Obviously, accepting this ex-

planation, one should assume that the stress level associated with the life situation of the adolescents abruptly surged during the previous month, resulting in a prompt appearance of the "skin effect", while the "hair effect" will not emerge until some time later (when the hair has grown enough to carry out measurement of the appropriate section of the hair shaft). It seems, however, highly unlikely that the personal situation of the studied subjects changed so suddenly. Moreover, the mean stress intensity in the studied group does not differ from the values typical of adolescents in this age interval, which has already been discussed. Thus, it seems that the hair pigmentation effect in our study, which may in fact correspond to stress levels associated with the life situation of the studied subjects from 2 or even 3 months before, should not in general differ from the stress levels from the previous month. Therefore, it appears possible that the actual stress intensity was too weak to disturb the extremely effective immune mechanisms of the hair pigmentation unit. Skin pigmentation seems to be more sensitive and susceptible to the effects of modifiers (e.g., hormonal ones) than hair pigmentation, which was suggested in previous studies by the authors of this report (Sitek et al.[1]). Moreover, one should note that in the present study hair pigmentation was evaluated in a smaller number of young adults, either due to excessively short hair or reported dyeing. Therefore, the effect of stress, especially if weak, could have been blurred by an insufficient number of subjects in the study group, especially if subjects with extreme stress perceptions had been incidentally excluded for the above-mentioned reasons.

In the majority of known populations, females are characterized by lighter pigmentation than males (e.g.,Van den Berghe and Frost 1986; Frost 1988; Norton et al. 2006; Madrigal and Kelly 2007), which most probably results from the necessity to synthesize a greater amount of vitamin D needed for the periods of pregnancy and lactation (e.g., Jablonski and Chaplin 2000; Parra 2007). This hypothesis is supported by a study of Mazess (1967), who states that female skin is the least pigmented during the reproductive period. Mazess further suggests that the sexual differentiation of skin pigmentation is associated with the volume of fat tissue, which is relatively greater in women than in men. In the fat tissue androgens are converted into estrogens under the action of the enzyme aromatase (Dunger et al. 2005). Since androgens are stronger stimulators of melanogenesis than estrogens (Edwards and Duntley 1949), Mazess (1967) argues that the increased volume of fat tissue observed in maturing girls is responsible for sex differences in skin pigmentation. It is also thought that sexual selection could enhance sex differences in skin pigmentation in populations where men prefer lighter-skinned women (Van den Berghe and Frost 1986; Jablonski and Chaplin 2000). A number of studies indicates that women have also lighter pigmented hair than men (e.g., Zemelman et al. 2002; Norton et al. 2006), although some authors indicate a reverse trend in dimorphic differences, albeit observed only during growth spurts (Reuer 1977).

In the present study, women were characterized by stronger skin melaniza-

[1] Sitek A, Żądzińska E, Rosset I, Antoszewski B. Is increased constitutive skin and hair pigmentation an early morphological sign of puberty? – under review.

tion than men. In light of the acknowledged evolution concepts, it is rather difficult to provide a plausible explanation for this finding; however, there are populations where females are characterized by more pigmented skin, as well as populations characterized by a lack of sexual dimorphism in this feature (Madrigal and Kelly 2007). In contrast to the skin, no dimorphic differences were found in hair pigmentation processes. The above-mentioned results may also be associated with the different proportions of females and males in the studied group of adolescents.

Some authors indicate that skin and hair pigmentation undergo change, regardless of one's sex, in the course of ontogenesis. The skin gradually darkens from childhood to the age of 25–30, and then gradually becomes lighter again (Ortonne 1990). After 30, pigment production in epidermal melanocytes decreases by about 10%–20% per decade (Quevedo et al. 1969; Whiteman et al. 1999). Age-related hair pigmentation changes are especially visible in the European population (Tobin 2009). In Caucasians, hair is the lightest in early childhood, then gradually darkens (this process sometimes begins before puberty) and continues to darken during puberty and early adulthood (Allende 1972; Costin and Hearing 2007) until the onset of hair greying (Tobin 2009). This process is associated with hormonal effects, mainly linked to sex steroids (Tobin 2009). The present results did not reveal any increase in skin pigmentation between the 18th and the 22nd year of life, which may indicate that the studied subjects may already have obtained the maximal values of that characteristic by individual response standards. In turn, an increase in hair melanization was observed in the analyzed period of ontogenesis (in both sexes), which is consistent with the above-cited reports. It seems that the observed (during the same phase of ontogenesis) stability of skin pigmentation and the simultaneous increase in hair pigmentation may corresponded to the aforementioned differences between the pigmentation units of skin and hair.

Conclusions

In Polish adolescents, psychological stress (the average intensity of which does not differ from that typical of the studied age interval) leads to decreased skin pigmentation but has no effect on hair pigmentation processes. The correlation of perceived stress levels with skin melanization processes seems to be sex- and age-independent. Hair melanization is probably more "stable" than skin melanization; thus, it may either not react at all to the effects of less potent factors or react to them with some delay with respect to the skin reaction.

Authors' contribution

AS conceived the concept, designed and performed the research project, performed statistical analysis, analysed data and drafted the manuscript; EŻ served as principal investigator for the research and drafted the manuscript; IR performed statistical analysis, analysed data and drafted the manuscript. All authors read and approved the final manuscript.

Conflicting interests

The authors declare that they have no conflicts of interest in the research.

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