0.4 J cm^{-2} fluence, melanosomes were regenerated from intact melanophores. At 0.9 J cm^{-2} fluence, destroyed melanophores should be generated from stem cells, and they therefore required more time to regenerate in our study.

Melanosomes in melanophores of adult zebrafish normally have a round or slightly ellipsoid shape (Hirata *et al.*, 2005). In contrast, the damaged melanosomes in our study appeared shrunken or cracked after laser irradiation (Supplementary Figure S2 online). With the exception of the melanosomes, no other cells were observed to be damaged by transmission electron microscopy.

Xanthophores—the source of the yellow stripes on zebrafish skin—are hypothesized to act as a barrier delimiting the area that can be occupied by melanophores. The interactions between these two types of chromatophores are an important focus in the study of zebrafish pigmentation patterns (Takahashi and Kondo, 2008). After Q-switched Nd:YAG laser irradiation, intact xanthophores were detected (Supplementary Figure S3 online).

Recently, attempts have been made to use the 1064-nm Q-switched Nd:YAG laser with short pulse duration and low fluence for the treatment of melasma (Jeong *et al.*, 2008; Polnikorn, 2008; Chung *et al.*, 2009; Kim *et al.*, 2009). With short pulse duration and low fluence, the laser could selectively photothermolyse melanosomes without killing melanocytes. This might prevent postinflammatory hyperpigmentation after melanocyte destruction, which is especially common in Asians (Ho and Chan, 2009).

In addition, our study offers a convenient technique to destroy melanosomes in adult zebrafish. The Q-switched Nd:YAG laser can be used to remove melanosomes without affecting melanophores or other cells at the following settings : 1064 nm wavelength, 5–7 ns pulse duration, 7 mm spot size, and 0.4 J cm^{-2} fluence. Although they are very specific settings, and subtle changes might therefore invalidate the use of this model, the approaches used here may be helpful for the study of melanocyte biology with zebrafish.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This study was presented, in part, at the 29th Annual Meeting of the American Society for Laser Medicine and Surgery.

Jae Hwan Kim[1], Ho Kim[2], Hae Chul Park[2] and Il-Hwan Kim[1]

[1]Department of Dermatology, College of Medicine, Korea University, Seoul, Korea and [2]Laboratory of Neurodevelopmental Genetics, Graduate School of Medicine, Korea University, Ansan, Korea
E-mail: kumcihk@korea.ac.kr

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Ceol CJ, Houvras Y, White RM *et al.* (2008) Melanoma biology and the promise of zebrafish. *Zebrafish* 5:247–55

Choi TY, Kim JH, Ko DH *et al.* (2007) Zebrafish as a new model for phenotype-based screening of melanogenic regulatory compounds. *Pigment Cell Res* 20: 120–7

Chung WK, Yang JH, Lee DW *et al.* (2009) Paradoxical darkening of unperceived tattoo ink after relatively low fluence from a Q-switched Nd:YAG (1064-nm) laser in the course of treatment for melasma. *Clin Exp Dermatol* 34:e555–7

Goldberg DJ, Berlin AL, Phelps R (2008) Histologic and ultrastructural analysis of melasma after fractional resurfacing. *Lasers Surg Med* 40:134–8

Hirata M, Nakamura K, Kondo S (2005) Pigment cell distributions in different tissues of the zebrafish, with special reference to the striped pigment pattern. *Dev Dyn* 234:293–300

Ho SG, Chan HH (2009) The Asian dermatologic patient: review of common pigmentary disorders and cutaneous diseases. *Am J Clin Dermatol* 10:153–68

Hruza GJ, Dover JS, Flotte TJ *et al.* (1991) Q-switched ruby laser irradiation of normal human skin. Histologic and ultrastructural findings. *Arch Dermatol* 127:1799–805

Jeong SY, Chang SE, Bak H *et al.* (2008) New melasma treatment by collimated low fluence Q-switched Nd:YAG laser. *Korean J Dermatol* 46:1163–70

Kim MJ, Kim JS, Cho SB (2009) Punctate leucoderma after melasma treatment using 1064-nm Q-switched Nd:YAG laser with low pulse energy. *J Eur Acad Dermatol Venereol* 23:960–2

Parrish JA, Anderson RR, Harrist T *et al.* (1983) Selective thermal effects with pulsed irradiation from lasers: from organ to organelle. *J Invest Dermatol* 80(Suppl):75s–80s

Polnikorn N (2008) Treatment of refractory dermal melasma with the MedLite C6 Q-switched Nd:YAG laser: two case reports. *J Cosmet Laser Ther* 10:167–73

Takahashi G, Kondo S (2008) Melanophores in the stripes of adult zebrafish do not have the nature to gather, but disperse when they have the space to move. *Pigment Cell Melanoma Res* 21:677–86

Yamaguchi M, Yoshimoto E, Kondo S (2007) Pattern regulation in the stripe of zebrafish suggests an underlying dynamic and autonomous mechanism. *Proc Natl Acad Sci USA* 104:4790–3

Development of Solar UVR-Related Pigmentation Begins as Early as the First Summer of Life

Journal of Investigative Dermatology (2010) **130,** 2335–2338; doi:10.1038/jid.2010.104; published online 29 April 2010

TO THE EDITOR

Parametrization of infant skin has been a research focus: development of the water barrier, acidification of the stratum corneum (SC), and normalization of SC hydration have been documented in infant populations of various ages (Hanley *et al.*, 1997; Hoeger and Enzmann, 2002; Agren *et al.*, 2006; Nikolovski *et al.*, 2008; Stamatas *et al.*, 2009). Few studies have followed

Abbreviations: SC, stratum corneum; TEWL, transepidermal water loss

skin development over a significant period of time (Johnke *et al.*, 2006; Harrison *et al.*, 2008), and the responses of infant skin to the environment require further study. Facultative pigmentation, defined as the increase in pigment above constitutive levels (Quevedo *et al.*, 1975), may be used to indicate skin adaptation to the environment, specifically exposure to solar UVR, and has also been proposed to be a marker to record the history of exposure (Lock-Andersen *et al.*, 1998). Although the melanogenic capacity of neonatal melanocytes in response to UVR is well known (Friedmann and Gilchrest, 1987), the kinetics of melanogenesis *in vivo* in infants is not well understood and the age at which skin adaptation to solar UVR becomes perceptible has not been defined.

The purpose of this preliminary study was to investigate the seasonal changes in pigmentation in infants starting with the first summer of life and to determine whether other biophysical parameters may relate to these changes in pigmentation. A longitudinal study was performed to investigate changes in skin properties over 1 year in healthy Caucasian male and female infants aged 6–24 months and female adults aged 30–40 years, in Skillman, NJ. Skin properties measured included transepidermal water loss (TEWL), SC conductance, color, and chromophores.

Infants were divided on the basis of their age into those experiencing their first summer of life (first summer, $n=10$, aged 6–12 months at the beginning of the study) and those experiencing their second summer of life (second summer, $n=10$, aged 16–24 months at the beginning of the study). Biological mothers of the infants were also enrolled in the study ($n=19$) and instructed to follow their normal skin care routines for both their infants and themselves. The study was performed following approval from the Allendale Investigational Review Board (Old Lyme, CT) and in compliance with the Helsinki Declaration. Adults and parents of infants signed a written informed consent before the start of the study. Timepoints were scheduled in spring (May 2006), fall (September 2006), winter (January 2007), and the following spring (April 2007). A total of 15 infants (first summer, $n=7$; second summer $n=8$) and 11 adults completed the study at these four timepoints. The total time spent in outdoor activities and the use of sunscreens were monitored through weekly diaries.

Apparent concentrations of skin chromophores (melanin, oxy-hemoglobin, deoxy-hemoglobin) were determined using diffuse-reflectance spectroscopy, by calculating the chromophore apparent concentration value from an acquired reflectance spectrum of the skin (Kollias and Baqer, 1986; Stamatas *et al.*, 2008). Skin color was measured using the Chromameter CR-300 (Konica Minolta, Tokyo, Japan), TEWL was measured using the Vapometer (Delfin Technologies, Kuopio, Finland), and SC conductance was measured using the Skicon-200 (IBS, Hamamatsu, Japan). Facultative pigmentation was calculated by subtracting the melanin apparent concentration values on the upper ventral arm, assumed to be primarily constitutive pigmentation, from the melanin apparent concentration values on the lower dorsal forearm (Quevedo *et al.*, 1975). Facultative changes in the tristimulus skin-color parameters were calculated similarly. Student's *t*-tests were performed in Excel 2007 (Microsoft, Seattle, WA) to determine the significance ($P<0.05$) of differences in skin properties between age groups at the baseline timepoint and within each age group across the timepoints.

Baseline-facultative pigmentation was indistinguishable from constitutive pigmentation for infants in the first summer of life and was significantly higher than constitutive pigmentation for infants in the second summer of life and for adults (Figure 1). The seasonal changes in facultative pigmentation were similar in all three age groups (Figure 2). Facultative pigmentation was significantly greater at the fall timepoint (September 2006) and returned to values statistically similar to presummer values (May 2006) by the winter season (January 2007). Skin color parameters (L^*, a^*, b^*) showed changes consistent with those observed in facultative pigmentation (facultative L^* decreased on average by 3.5 ± 3.0 units after summer and facultative b^* increased on average by 2.6 ± 2.1 units after summer). Diary data indicated that 60% of first summer infants and 70% of second summer infants experienced on average greater than 5 hours of sun exposure per week (there were no reported instances of sunburn in the infants); all mothers used sunscreen on their infants during the summer and 60% of mothers used sunscreen on their infants at least three times per week. The development of significant levels of facultative pigmentation in infants whose mothers practiced sun protection may indicate that infants experience significant environmental UVR exposure during events that their caregivers do not associate with a need for sun protection.

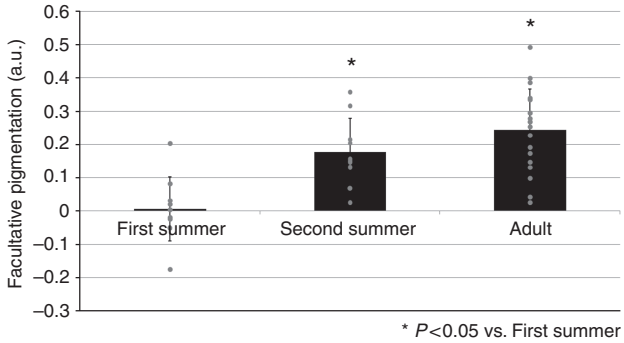

Figure 1. Facultative pigmentation is negligible before the first summer of life and increases with age. Facultative pigmentation (calculated by subtracting melanin apparent concentration (A.C.) value on the upper inner arm from melanin A.C. value on the dorsal forearm) is negligible in the first-summer infants. Significant levels of facultative pigmentation are observed in the second-summer infants and adults. Bars represent means and error bars represent SDs. Values for each subject are represented by filled circles. An asterisk represents a statistical difference from first-summer infants at a significance level of $P<0.05$ (Student's *t*-test).

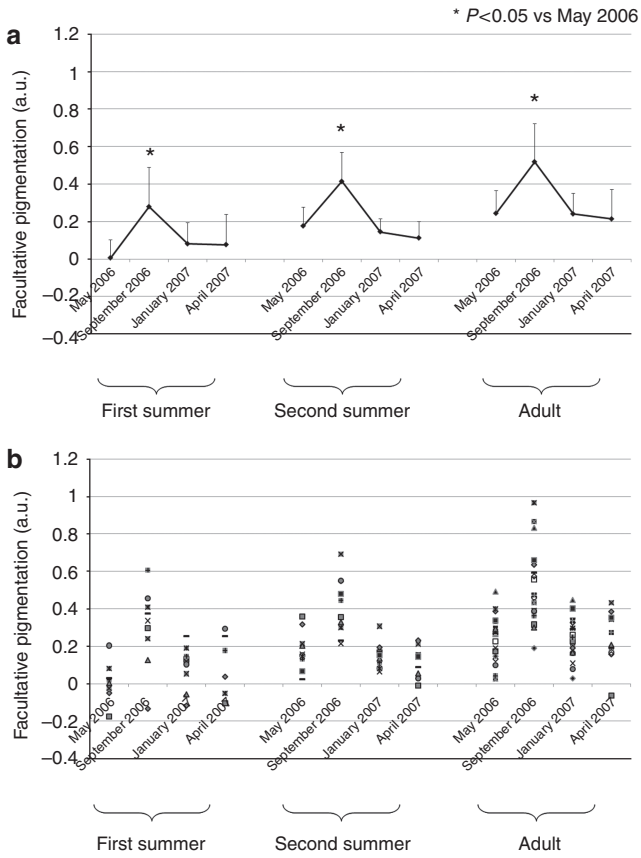

Figure 2. Facultative pigmentation changes with season are similar in infants and adults. (**a**) In all three age groups, facultative pigmentation increases after summer. By the winter season (January 2007), facultative pigmentation has returned to values statistically similar to the pre-summer (May 2006) values. Data represents means and error bars represent SDs. An asterisk represents a statistical difference from May 2006 at a significance level of $P<0.05$ (Student's *t*-test). (**b**) Changes in facultative pigmentation values for each subject show the same trends, which are observed in the mean values.

Values of TEWL and SC conductance fell within the range of previously published values, and similar trends of greater TEWL and SC conductance and greater variability in these parameters in infants compared with adults were observed (Nikolovski *et al.*, 2008). We did not observe statistically significant changes over season in TEWL or SC conductance in infants or adults, perhaps because of the small number of subjects or the large variability observed in infant water-handling properties.

This preliminary study showed that the adaptive response of the skin to solar UVR begins as early as the first summer of life. To our knowledge, quantitative documentation of the pigmentation response of infant skin to UVR exposure is previously unreported. At baseline, infants who were old enough to have experienced a summer season showed levels of facultative pigmentation similar to those observed in adult subjects. This observation raises an intriguing question on the reversibility of facultative-pigmentation changes after UVR exposure and the onset of permanent tanning. Seasonal increases in facultative pigmentation after summer were found in infants experiencing their first and second summers of life and in their mothers, indicating significant levels of exposure to UVR and the associated risk of photodamage (Gilchrest and Eller, 1999). Levels of facultative pigmentation in the first-summer infants were statistically similar at baseline (May 2006) and 1 year later (April 2007), but the numerical difference in facultative pigmentation before the first and second summers of life in these subjects is interesting to note. A definitive statement regarding the kinetics of melanogenesis and the skin's ability to recover after UVR exposure or the risks and benefits of sunscreen usage in infants and children cannot be made based on the results of this small study; however, these results highlight the need for further research in this area.

CONFLICT OF INTEREST

The authors are all employed by affiliates of Johnson & Johnson Group of Consumer Companies, a manufacturer of infant skin care products and sunscreens.

ACKNOWLEDGMENTS

We would like to thank Dr Paulo Bargo for his critical contribution to the data analysis.

M. Catherine Mack[1], Neena K. Tierney[2], Edvardo Ruvolo Jr[3], Georgios N. Stamatas[4], Katharine M. Martin[1] and Nikiforos Kollias[3]

[1]Baby Care Science & Technology, Johnson & Johnson Consumer Companies, Skillman, New Jersey, USA; [2]Baby Care Scientific Affairs, Johnson & Johnson Consumer Companies, Skillman, New Jersey, USA; [3]Models & Methods Development, Johnson & Johnson Consumer Companies, Skillman, New Jersey, USA and [4]Baby Care Science & Technology, Johnson & Johnson Consumer France, Issy-les-Moulineaux, France
E-mail: nkollia@its.jnj.com

REFERENCES

Agren J, Sjors G, Sedin G (2006) Ambient humidity influences the rate of skin barrier maturation in extremely preterm infants. *J Pediatr* 148:613–7

Friedmann PS, Gilchrest BA (1987) Ultraviolet radiation directly produces pigment production by cultured human melanocytes. *J Cell Physiol* 133:88–94

Gilchrest BA, Eller MS (1999) DNA photodamage stimulates melanogenesis and other photoprotective properties. *J Investig Dermatol Symp Proc* 4:35–40

Hanley K, Jiang Y, Elias PM *et al.* (1997) Acceleration of barrier ontogenesis *in vitro* through air exposure. *Pediatr Res* 41:293–9

Harrison SL, MacLennan R, Buettner PG (2008) Sun exposure and the incidence of melanocytic nevi in young Australian children. *Cancer Epidemiol Biomarkers Prev* 17:2318–24

Hoeger PH, Enzmann CC (2002) Skin physiology of the neonate and young infant: a prospective study of functional skin parameters during early infancy. *Pediatr Dermatol* 19:256–62

Johnke H, Norberg LA, Vach W *et al.* (2006) Patterns of sensitization in infants and its relation to atopic dermatitis. *Pediatr Allergy Immunol* 17:591–600

Kollias N, Baqer A (1986) On the assessment of melanin in human skin *in vivo*. *Photochem Photobiol* 43:49–54

Lock-Andersen J, Knudstorp ND, Wulf HC (1998) Facultative skin pigmentation in caucasians: an objective biological indicator of lifetime exposure to ultraviolet radiation? *Br J Dermatol* 138:826–32

Nikolovski J, Stamatas GN, Kollias N *et al.* (2008) Barrier function and water-holding and transport properties of infant stratum corneum are different from adult and continue to develop through the first year of life. *J Invest Dermatol* 128:1728–36

Quevedo WC, Fitzpatrick TB, Pathak MA *et al.* (1975) Role of light in human skin color viariation. *Am J Phys Anthropol* 43:393–408

Stamatas GN, Nikolovski J, Luedtke M *et al.* (2009) Infant skin microstructure assessed *in-vivo* differs from adult skin in organization and at the cellular level. *Pediatr Dermatol*; e-pub ahead of print 4 October 2009

Stamatas GN, Zmudzka BG, Kollias N *et al.* (2008) *In vivo* measurement of skin erythema and pigmentation: new means of implementation of diffuse reflectance spectroscopy with a commercial instrument. *Br J Dermatol* 159:683–90