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Can skin wrinkling in a site that has received limited sun exposure be used as a marker of health status and biological age?

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Abstract

Objectives: to determine if skin wrinkling in a site that had received limited sun exposure may be a marker of health status and biological age.
Design: population-based, cross-sectional study.
Participants: we evaluated the health status of representative samples of elderly Greek-born people living in Melbourne, Greeks living in rural Greece, Anglo-Celtic Australians living in Melbourne and Swedes living in Sweden. We carried out microtopographic assessment of their skin and measured plasma dehydroepiandrosterone concentrations.
Methods: we derived activities of daily living, well-being, memory and general health status scores from a cross-cultural questionnaire. We measured skin wrinkling using cutaneous microtopographic methods and plasma dehydroepiandrosterone by enzyme immuno-assay.
Results: skin wrinkling was positively correlated with age ($r_s = 0.27$, $P < 0.0001$) and negatively with body mass index ($r_s = -0.19$, $P < 0.0001$). Therefore, all analyses were controlled for these variables. Plasma dehydroepiandrosterone was higher in smokers than non-smokers (2.86 *vs* 2.08; $P < 0.001$) and men had significantly higher plasma dehydroepiandrosterone than women (2.74 *vs* 1.69; $P < 0.0001$). In the pooled data, skin wrinkling was negatively associated with general health score ($r_s = -0.13$, $P < 0.01$) and activities of daily living score ($r_s = -0.14$, $P < 0.05$) after controlling for age, body mass index and smoking. These associations were more pronounced in women. Finally, those with the least skin wrinkling had the highest dehydroepiandrosterone level ($r_s = -0.12$, $P = 0.06$) after adjusting for age, smoking and sex.
Conclusion: skin wrinkling in a site with limited sun exposure might be used as a marker of health status and, to some extent, biological age—particularly for women.

Keywords: *activities of daily living, biological age, cutaneous microtopography, dehydroepiandrosterone, elderly, general health status, memory, skin wrinkling, well-being*

Introduction

Geriatric research tends to be on age-associated, chronic and life-threatening disorders. Less attention is given to the skin [1], which signals biological ageing as well as having a cosmetic significance.

Various intrinsic (genetic) and extrinsic factors (e.g. sunlight, diet, smoking and physical activity) may contribute to skin health in later life [2–4]. Most research in cutaneous gerontology has focused on the deleterious effect of ultraviolet radiation [5–8]. Instead of time-consuming questionnaires or more invasive tests,

skin microtopography might provide a quick and easy non-invasive marker of overall health status which complements other instruments. There have been no published studies investigating skin wrinkling as an indicator of health status in older people.

Another requirement is to describe people in relation to their biological (as distinct from their chronological) age. Biological age is a measure of how well the body is ageing. It is inversely related to the time left of the genetically determined life span (genotype).

Dependable markers of biological age are few: examples include specific organ performance [9, 10], voice analysis [11] and steroid hormone profile [9]. Dehydroepiandrosterone (DHEA), an adrenal steroid hormone, has been proposed as a marker of biological age. Plasma DHEA and its sulphate ester (DHEAS) are mostly adrenally-secreted steroids which decline with age [12–14] to about 10–20% of the values in young adults by the eighth and ninth decades. DHEA decreases more markedly with age than any other sex hormone, in both men and women [15, 16].

An integrative index, which provides a global measure of biological age, is not available. The skin may provide a non-invasive, relatively integrative way to assess biological age and influencing factors. Skin appearance and properties change with age [17]. The surface layer, being most exposed to environmental insult, requires extensive defence and reparative capacity. Its anti-oxidative activity might be expected to be greater than that of internal organs [18].

People may wish to slow or even maintain biological age as chronological age progresses. This may be commensurate with reduced morbidity in later life and be reflected in a more youthful appearance in old age [19]. With the measurement of biological age and manipulation of extrinsic factors affecting it, we may be able to alter the course of biological ageing.

We have measured skin wrinkling by cutaneous microtopography in a body region with limited sun exposure and examined its inter-relationship with measures of general health status. We also explored the relationship between skin wrinkling and DHEA, and their relative merits as surrogate markers of biological age.

Subjects and methods

The study population was drawn from those recruited for the Food Habits and Health in Later Life cross-cultural study [20, 21]. This study was established from 1988 to 1992 by the International Union of Nutritional Sciences Subcommittee on Nutrition and Ageing in co-operation with Monash University. The study covered about 2000 subjects from Australia, Greece, Sweden, China, Japan, the Philippines and Indonesia, with a defined age of entry of 70 years and over. All subjects lived at home. The study protocol was approved by the standing committee on ethics in research on humans of Monash University.

In this report we used the baseline data from four different communities. We recruited representative subjects using the telephone directory for those living in Melbourne, and electoral rolls for those living in Greece and Sweden. The overall response rate was 75%. Representativeness of the samples is not a precondition for internal validity in a cohort investigation.

Of the 189 Greek-born elderly people living in Melbourne who participated in the study, 177 had their skin tested. We measured skin wrinkling in 69 of 104 Greek subjects living in rural Greece and 48 of 140 Anglo-Celtic Australian elders living in Melbourne, while 159 of the 217 Swedish subjects had the skin test. Blood DHEA levels were analysed in 166 Greek-born people living in Melbourne, 61 Greeks living in Greece and 83 Anglo-Celtic Australians. Memory and well-being scores of subjects who had the skin test were no different to those who did not, but those who had the test had a significantly higher activities of daily living (ADL) score and body mass index (BMI). In addition, subjects who had the DHEA test had a significantly higher score of general health status and BMI than those who did not.

Cutaneous microtopography

We assessed the degree of wrinkling using a validated cutaneous microtopography method, developed by Holman [22, 23] to measure skin wrinkling in sun-exposed sites. As well as using the method to assess a sun-exposed site, we investigated its use in a site that had received limited sun exposure. We used a silicon rubber impression material (Optosil Flussig, Bayer Leverkusen, Germany). A catalyst was mixed into the viscous white liquid (which started the hardening process) and applied to the skin. When the silicon material set (3–5 min after the addition of the catalyst), it was stripped slowly and steadily from the skin. This procedure did not cause any pain to the subjects. The silicon rubber material was placed on two areas of the body: the dorsum of the hand (which is highly exposed to the sun) and the inner aspect of the left forearm midway between the wrist and elbow (which receives limited sun exposure).

We then assessed the microtopograph using a binocular microscope ($\times 10$ magnification), and graded it using a 6-step scale (developed by Beagly and Gibson [24]). We gave a score according to the degree of skin wrinkling; a high score of 6 indicating extensive wrinkling. An inter-observer study between two observers in Melbourne and one in Sweden showed that the measurements of the two observers in Melbourne did not differ, while the Swedish observer showed significantly lower values. Thus, an analysis of the differences and similarities between the scores should be made with caution.

DHEA measurement

Plasma DHEA was measured by a commercial automated chemiluminescent enzyme immuno-assay using an immulite analyser (Diagnostic Products Corporation, Los Angeles, CA, USA) and assay kit (catalogue number LKDS1). This is a competitive immuno-assay using alkaline phosphatase-labelled DHEAS as tracer and adamantyl dioxetane as luminescent substrate for the alkaline phosphatase. The analysis was carried out in the Biochemistry Laboratories at Monash Medical Centre, Melbourne, Australia.

Markers of health status

An interviewer-administered questionnaire was developed for the International Union of Nutritional Sciences study to measure health and nutritional status, demographic characteristics, lifestyle, and other variables such as ADL, physical activity, sleeping pattern, smoking, social activities, social relation, economic resources [20, 21].

ADL

We assessed ADL by a series of questions on the degree of difficulty in performing tasks and coping with basic bodily functions and self care. The score ranged from 15 to 62; a higher score indicating better functional status. We took the ADL questions from instruments used in the World Health Organisation 11-country study on elderly people (Heikinnen, 1987) and from the Euronut–Seneca study [25]. These questions were originally adapted from the validated instrument developed by Katz and Akpom [26].

Memory score

Responses to the questions of orientation and memory generated a memory score ranging from 0 to 5, where a score of 5 represented good memory. We took these questions from the questionnaire used in developing countries (the World Health Organisation Western Pacific Study [27]).

Well-being and health status

We used the validated Multi-Level Assessment Instrument [28] to generate the general health score, which includes self-reported health conditions (such as diabetes mellitus, heart disease and hypertension), self-rated health and frequency of use of health services. These health conditions were cross-checked with the use of medications. The general health score ranged from 33 to 74. We derived well-being status from questions about depression, feelings of worry, tiredness, sleeplessness and contentedness with life. We modified these questions from the World Health Organisation Western Pacific Study [27]. The responses to the questions on well-being were summed to generate the well-being score ranging from 0 to 7. Higher scores indicated a better overall health and sense of well-being.

Data analysis

We used the SAS statistical analysis package [29] for data analysis. To compare age, skin wrinkling, well-being, ADL, memory and general health status and BMI among the three populations studied, we performed the Wilcoxon rank test. We calculated non-parametric Spearman correlation coefficients to assess the associations between skin wrinkling and well-being, ADL, memory and general health scores. We made adjustments for age and BMI because these variables were significantly correlated with skin wrinkling. Even though there were no significant differences in wrinkling by sex and smoking status, all analyses controlled for the latter because of the results of published findings [30].

We calculated the agreement [31] between skin wrinkling of the forearm (limited sun exposure) and that of the back of the hand (maximum sun exposure) using the Excel 97 program. The purpose of this analysis was to determine whether the forearm could be assumed to be a limited sun-exposed site.

Results

Demographic and health characteristics of the study populations are shown in Table 1. The relationship between skin wrinkling and DHEA levels are shown in Table 2.

Greek-born elderly people living in Melbourne had the highest mean BMI and the lowest memory score after controlling for age. Swedish elderly people had the highest mean age, the most skin wrinkling in the limited sun-exposed site, the least disability (i.e. high ADL score), but the lowest BMI, degree of skin wrinkling in the sun-exposed site and general health score. Greek elderly subjects living in rural Greece had the least skin wrinkling in the limited sun-exposed site, but the most disability, the lowest well-being score and the lowest DHEA levels. Anglo-Celtic Australian elderly people had the lowest mean age, but the highest skin wrinkling in the sun-exposed site; they also had the highest scores for well-being, memory and general health, and the highest DHEA level (Tables 1 and 2).

We found that skin wrinkling on the back of the hand and the inner forearm was significantly correlated among the four populations (data not shown). However, disagreement between the two measurements [31] indicates that the wrinkling at the two sites does not measure the same thing. The hand is more sun-exposed than the arm. It was possible to deduce from the statistical analysis that the wrinkling on the hand was caused mainly by sun exposure, whereas the causes of

Table 1. Demographic and health characteristic of the study population (age-adjusted)

	Greek-born people			
	Living in Australia ($n=177$)	Living in Greece ($n=69$)	Anglo-Celtic Australians ($n=48$)	Swedes in Sweden ($n=159$)
Age, years				
Mean ± SD	77.57 ± 0.33	77.56 ± 0.55[c]	74.11 ± 0.47[a]	78.15 ± 0.48[c]
Median (range)	77 (70–104)	76.5 (70–94)	73 (70–92)	78 (69–96)
No. (and %) aged ⩾80 years	64 (33.9)	41 (39.4)	16 (11.3)	85 (28.7)
Well-being score				
Mean ± SD	5.10 ± 0.10	4.65 ± 0.16[a]	5.70 ± 0.15[a,b,d]	5.62 ± 0.14[a,b]
Median (range)	6 (1–7)	5 (0–7)	6 (2–7)	6 (1–7)
Activities of daily living score				
Mean ± SD	56.31 ± 0.44	55.75 ± 0.73[d]	56.06 ± 0.68[d]	58.06 ± 0.65[a]
Median (range)	61 (28–62)	59 (25–62)	58 (50–62)	62 (22–62)
Memory score				
Mean ± SD	4.24 ± 0.06	4.40 ± 0.11	4.66 ± 0.15[a]	4.62 ± 0.09[a]
Median (range)	5 (0–5)	5 (0–5)	5 (1–6)	5 (0–5)
General health score				
Mean ± SD	65.54 ± 0.26[d]	65.14 ± 0.44[c,d]	66.76 ± 0.60[d]	61.82 ± 0.39
Median (range)	66 (50–74)	65 (54–74)	67 (60–73)	63 (50–69)
Body mass index				
Mean ± SD	28.36 ± 0.26[b,c,d]	27.19 ± 0.50[c,d]	25.63 ± 0.38[d]	24.23 ± 0.37
Median (range)	28.6 (16.4–50.9)	27.0 (18–39)	25.8 (17.3–33.3)	24.2 (16.4–36.9)

Significantly different ($P<0.05$) from [a]Greek-born people living in Australia, [b]Greek-born people living in Greece, [c]Anglo-Celtic Australians and [d]Swedish elders living in Sweden.

Table 2. Skin wrinkling and dehydroepiandrosterone levels in the study population (age-adjusted)

	Greek-born people			
	Living in Australia ($n=177$)	Living in Greece ($n=69$)	Anglo-Celtic Australians ($n=48$)	Swedes in Sweden ($n=159$)
Wrinkling on forearm				
Mean	2.28 ± 0.04	2.07 ± 0.08[a]	2.29 ± 0.10	2.43 ± 0.07[b]
Median	2.2 (1–5)	2.0 (1–3.7)	2.0 (1–4)	2.0 (1–5)
Wrinkling on hand				
Mean	4.87 ± 0.05	5.06 ± 0.10	5.14 ± 0.12[a]	4.74 ± 0.08[b,c]
Median	5.0 (2.7–6.0)	5.3 (3.7–6.0)	5.4 (3.5–6.0)	5.0 (3–6)
Dehydroepiandrosterone				
Mean	2.13 ± 0.13	1.89 ± 0.21[c]	2.81 ± 0.19[a]	NA
Median	1.9 (0.1–6.5)	1.5 (0.3–5.2)	2.2 (0.1–13.8)	NA

Significantly different ($P<0.05$) from [a]Greek-born people living in Australian, [b]Greek-born people living in Greece, [c]Anglo-Celtic Australians and [d]Swedish elders living in Sweden.
NA, not available.

Table 3. Spearman correlation coefficients between skin wrinkling, dehydroepiandrosterone (DHEA) level, age and body mass index for the pooled data

	Skin wrinkling ($n=453$)	DHEA level ($n=310$)
Age	0.27[a]	−0.26[a]
Body mass index	−0.19[a]	−0.03

[a]Significant difference, $P<0.0001$.

wrinkling of the inner part of the arm were less likely to be actinic. We therefore assumed in the analyses that the skin grades on the arm represented limited sun exposure.

General health status, well-being, memory and ADL

Age was positively correlated ($r_s=0.27$, $P<0.0001$) and BMI was negatively correlated ($r_s=-0.19$, $P<0.0001$) with skin wrinkling (Table 3). Therefore, all analyses with skin wrinkling and health variables were controlled

for age and BMI. There were no significant differences in skin wrinkling between smokers and non-smokers (2.25 ± 0.65 *versus* 2.29 ± 0.77) or between men and women (2.22 ± 0.72 *versus* 2.24 ± 0.71) in the pooled data. However, since other studies have shown that smoking influences skin wrinkling [30], we adjusted for this variable in all analyses.

After adjusting for age, BMI and smoking status, less skin wrinkling was significantly associated with a better general health score and ADL score and, to a lesser extent, DHEA levels in the pooled data (Tables 4 and 5). The associations were, however, more pronounced in women. For the individual communities, skin wrinkling of the forearm was negatively associated with well-being, ADL and general health score in the Greek-born people living in Melbourne. Among Greeks living in Greece, skin wrinkling was inversely associated with DHEA level and general health score. In the Anglo-Celtic Australian elderly subjects, less skin wrinkling was significantly associated with less functional disabilities. Significant correlations were not seen in the Swedish group.

DHEA levels

DHEA levels were higher in smokers than non-smokers (2.08 *versus* 2.86; $P < 0.001$) and men had significantly higher DHEA levels than women (2.74 *versus* 1.69; $P < 0.0001$). DHEA levels were also negatively associated with age ($r_s = -0.26$, $P < 0.0001$), but were not correlated with BMI. Therefore all analyses with DHEA were controlled for age, sex and smoking (Table 2).

Skin wrinkling was negatively associated with DHEA in non-smokers ($r_s = -0.18$, $P < 0.01$) and women ($r_s = -0.20$, $P < 0.05$) after adjusting for age and BMI. Those with the least skin wrinkling in the pooled data had a higher DHEA level ($r_s = -0.12$, $P = 0.06$) after adjustment for age, smoking and sex (Table 5).

Table 4. Spearman correlation coefficients between skin wrinkling in a limited sun-exposed site (forearm) and markers of health status by sex and ethnicity (adjusted for age, body mass index and smoking status)

	Correlation coefficient, by group				
	Greek-born people				
	Living in Australia ($n=177$)	Living in Greece ($n=69$)	Anglo-Celtic Australians ($n=48$)	Swedes in Sweden ($n=159$)	Total ($n=453$)
All					
Well-being score	−0.22[b]	−0.06	−0.08	−0.00	−0.07
ADL score	−0.21[b]	−0.14	−0.32[a]	0.07	−0.05
Memory score	−0.05	0.06	−0.25	0.04	−0.03
General health score	−0.22[b]	−0.24[a]	−0.14	−0.02	−0.13[b]
Men					
Well-being score	0.17	0.06	−0.07	−0.02	−0.04
ADL score	−0.02	0.20	−0.06	0.20	0.09
Memory score	0.04	0.02	−0.35	0.10	−0.02
General health score	0.15	0.10	−0.45	0.03	−0.03
Women					
Well-being score	−0.28[b]	−0.11	0.10	0.03	−0.08
ADL score	−0.38[c]	−0.36	−0.38	0.00	−0.14[a]
Memory score	−0.12	0.23	−0.23	0.01	−0.02
General health score	−0.27[b]	−0.48[b]	0.21	−0.03	−0.19[b]

Significant differences: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.
ADL, activities of daily living.

Table 5. Spearman correlation coefficients between skin wrinkling in a limited sun-exposed site (forearm) and dehydroepiandrosterone level by sex and ethnicity (adjusted for age and smoking; pooled data also adjusted for sex)

	Correlation coefficient, by group			
	Greek-born people			
	Living in Australia ($n=166$)	Living in Greece ($n=61$)	Anglo-Celtic Australians ($n=83$)	Total ($n=310$)
All	−0.13	−0.26[a]	−0.24	−0.14[b]
Men	−0.15	−0.10	0.86	−0.05
Women	−0.11	−0.26	−0.07	−0.15

Significant differences: [a] $P < 0.05$; [b] $P = 0.06$.

Discussion

Skin ageing is the result of many factors, intrinsic and extrinsic. The rate at which different people age varies. Biological ageing is not homogeneous, but evolves with various accelerations and decelerations [32].

Our findings indicate that the blood DHEA levels of non-smokers are significantly lower than those of smokers and that men have a higher level of DHEA than women. Feldmen *et al.* [15] and Sulcova *et al.* [16] also reported that DHEA decreases more markedly than any other sex hormone both in men and women during ageing. Since most of the smokers in this study were men, this could explain why the DHEA level of smokers was higher than that of their non-smoking counterparts. We also found that subjects with less skin wrinkling had a higher DHEA concentration ($P < 0.06$, Table 4). Therefore, skin wrinkling in a limited sun-exposed site might be used as a surrogate marker of biological age. There is evidence that concentrations of both DHEA and DHEAS indicate how well the body is ageing [13–16]. Higher DHEA levels are suggestive of better health and organ performances. Results from the Massachusetts Male Aging Study indicate that serum DHEA levels have an inverse relationship to heart disease [15]. Administration of DHEA resulted in changes in electrophysiological indices of central nervous system in elderly men [14], and increased bone mineral density in post-menopausal women [33].

Our results show that subjects with a different genetic make-up and environment have a different degree of skin wrinkling. Subjects with Anglo-Celtic ancestry are more susceptible to actinic damage. Holman [22] also reported that constitutional traits related to skin pigmentation, such as Celtic ancestry, are associated with actinic damage.

Skin wrinkling differs amongst Greek people living in different environments. This might be because the rural Greek elderly subjects were mostly involved in farming, so they had been exposed to more sunlight. This may explain why the wrinkling at the back of the hand (sun-exposed site) of Greeks in rural Greece was higher than that of urbanized Greek-born Australians. The extrinsic factors of skin ageing are the result of an individual's habits, nutrition and exposure to deleterious factors, such as ultraviolet sunlight. A person can influence or control extrinsic factors, largely by avoidance and through the maintenance of good health habits. The Greeks in Melbourne had more skin wrinkling on the arm than Greeks in Greece, probably because of their higher prevalence of chronic diseases (especially heart disease and diabetes, although this is not reflected in the general health score). Also a move to Melbourne tends to result in reduced physical activity and a change in food habits, particularly an increased intake of animal foods, refined carbohydrates and ω-6 fatty acids and decreased intake of olive oil, which may contribute to their skin wrinkling.

This study revealed that a general health status score was negatively correlated with skin ageing at a site that had received limited sun exposure (Table 3). This is probably because the dermal changes that occur during ageing—such as skin being more easily damaged, delayed wound healing, decreased inflammatory response, decreased protection from ultraviolet light, decreased urticarial reaction, more stretching under low loads, loss of resilience, altered thermal regulation and decreased sensitivity to pain and pressure—have disparate physiological consequences [34].

The associations between skin wrinkling and well-being, ADL, memory and general health scores are weak and not statistically significant in men. This is probably because men have a thicker dermis than women [34], even though our results show that sex does not affect skin wrinkling. This may explain why female skin seems to deteriorate more readily with ageing. Thinner skin is more easily damaged by sun exposure and trauma. Another factor that may affect the association is that men tend to be outdoors more than women. For example, more of the Greek Australian men (80%) reported doing some gardening compared with the Greek Australian women (60%) [21]. Outdoor activities may affect their sun exposure, therefore the skin wrinkling grade on the arm may not be such a good site for men. The skin of the arm may have had more sun exposure and thus the grade of wrinkling at this site might not have been a good measure of biological age in men. Another site of measurement with even less sun exposure (e.g. closer to the armpit) may have given more significant results, particularly in men.

This study provides evidence that skin wrinkling in sites that have received limited sun exposure may possibly be used as a marker of health status and, to some extent, biological age. This is particularly true for women, because skin wrinkling is significantly correlated with measures of ADL and general health status. In future analyses we will investigate whether nutritional factors are correlated with skin wrinkling and therefore whether there is potential to reduce biological age with diet.

Key points

- In older people, skin wrinkling in a site that has received limited sun exposure is positively associated with age and negatively with body mass index.
- Older people with less skin wrinkling tend to have better health and fewer disabilities.
- Older people with the least skin wrinkling in a site that has received limited sun exposure have a higher blood dehydroepiandrosterone level.
- Skin wrinkling in a site that has received limited sun exposure is a possible marker of health status and, to some extent, biological age.

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