METHODS

Age Dependence of Skin Autofluorescence

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The nontryptophane intrinsic fluorescence of finger pad skin was studied in men aged 20-70 years in order to evaluate the possibility of using this parameter as a biomarker of aging. Linear correlation coefficients (r) between the fluorescence intensity and age varied from 0.50 to 0.66 for various skin sites. Age dependence of the mean value of fluorescence (F) measured on 4 fingers can be approximated by a second-degree polynomial: $F=2.82-0.083T+0.0014T^2$ ($r_1=0.64$, $r_2=0.71$), where T is chronological age in years. The proposed measurement of skin autofluorescence is a simple, noninvasive, rapid test for evaluating the aging of the skin in subjects over 40 with a high age-related determination ($r^2=0.5$).

Key Words: *skin fluorescence; aging; lipofuscin; biological age*

Accumulation of lipofuscins (pigments of aging) is an easily detected characteristic shift observed in aging. A linear relationship between accumulation of lipofuscin and age has been demonstrated for *D. melanogaster* tissues [5]. Accumulation of lipofuscin in various tissues of mammals also increases with age [1,2]. Nontryptophane intrinsic fluorescence (FL) of the skin can be used as a biological marker of aging. Measurements of FL of subcutaneous collagen removed during intervention for vascular diseases showed a high correlation $r=0.99$ ($n=26$) between FL intensity (FLI) and age (42-78 years) [9], the age-standardized FLI did not correlate with sex, weight, or vascular pathology. Study of laser-induced intrinsic FL of the skin at a wavelength 325 nm in skin areas exposed and not exposed to sunlidht showed no correlation with age, but revealed a notable decrease in FLI of exposed skin sites, particularly in elderly subjects [8].

The problem is to choose the conditions of FL recording at which skin FL correlates with chronological age. We measured the nontryptophane FL of the skin of finger pads of men aged 20-70 years.

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MATERIALS AND METHODS

Skin FLI was measured in spring (April-May) in 44 healthy men aged 20-70 years. The device for measuring human skin FL was based on a Spekol fluorimeter equipped with an element for measuring FL of solid specimens (recording of FL from the anterior surface through a 5-mm opening) and UFS (at 365 nm excitation) and ZhS-5 filters (emission of more than 420 nm). FLI was measured on the third and fourth fingers of both hands after degreasing with ethanol.

The reference was white paper with intrinsic FL in this range. When recording the emission spectra, the FL beam was delivered to the monochromator through an optic fiber.

The sampling coefficients of correlations were evaluated using Fisher's z transformations: $z=0.5[\ln(1+r)-\ln(1+r)]$, with the following ratio as the reliability test for z: $t_z=z(n-3)^{-0.5}$, which, in turn, was compared to the critical t point determined from the critical point tables of Student's test [4].

RESULTS

The maximum FL in subjects of different age is about 435 nm, which is typical of aging pigments (Fig. 1).

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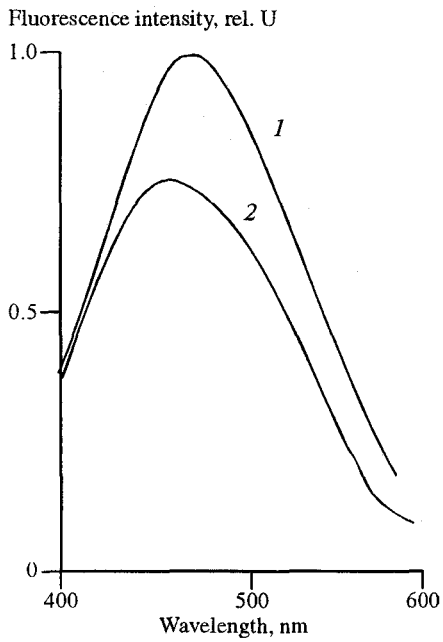

Fig. 1. Fluorescence emission spectra (at 365 nm excitation) from the fourth left finger pad surface in men aged 48.5 (*1*) and 38 (*2*) years.

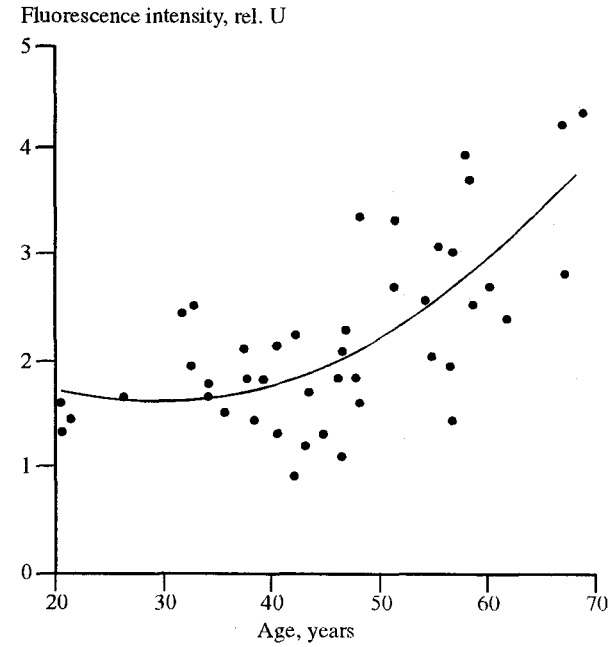

Fig. 2. Second-degree polynomial approximation of age dependence of mean intrinsic skin fluorescence (F). F=2.82-0.083T+0.0014T^2, where T is chronological age in years.

Evaluation of the linear correlation coefficients (r) for age dependence of FLI of the finger pads of both hands in men ($t_{crit.}$=2.02) showed higher correlations for the fourth fingers than for the middle fingers (Table 1). The arithmetic mean of FLI value for all fingers measured (mean intensity) is a more informative parameter of FL, because it levels the probable lateralization caused by the left or right predominance and the occupation.

The age dependence of the mean FL can be approximated by a second-degree polynomial with the correlation coefficients for the first and second degree terms of 0.64 and 0.71, respectively, which is higher than the linear correlation coefficient. The resultant relationship is characterized by a high increment, for example, at the age of 40-50 years the FL values increase by 25% (Fig. 2).

TABLE 1. Linear Correlation Coefficients (r) for Age Dependence of FLI of Finger Pad Skin on Both Hands in Men Aged 20-70 Years (n=44, $t_{crit.}$=2.02)

Area	r	t_2
Right hand fingers		
III	0.50	3.51
IV	0.56	4.05
Left hand fingers		
III	0.51	3.60
IV	0.66	5.08
Mean...	0.64	4.85

Measurements of intrinsic skin FL yield sufficiently high coefficients of age determination (square coefficient of correlations $D=r^2$): about 0.5. Metacarpal osteoporosis, hair grayness, and skin elasticity indexes are characterized by the highest age determination (D=0.6, 0.5, and 0.4, respectively) [6,7].

Previous studies revealed no relationship between laser-induced skin FL at 325 nm and age [8], which can be due to stimulation at this wavelength of fluorophore, whose concentration does not depend on age.

Therefore, measurement of intrinsic skin FL is a simple, noninvasive, and rapid (about 2 min) test for evaluating skin aging in subjects over 40 years (in normal aging) with a sufficiently high age determination (0.5).

Measurements of the skin FL and the rate of acoustic wave conduction, which may reflect collagen rigidity in the same subjects (n=16), showed no correlation between age-related shifts evaluated by FL and skin elasticity. The age-related shift was regarded as the difference between the biological (the value derived from approximation curves) and chronological age [3]. These two methods appear to be mutually complementary.

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