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# Relationships among skin conditions, mood, and polyunsaturated fatty acids of RBCs in healthy women

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**Synopsis**

Little is known about nonpathological facial skin problems at present. The aim of the present study was to investigate the relationships among facial skin conditions, mood, and the fatty acid composition of red blood cells (RBCs) in women. One hundred and thirty-two apparently healthy Japanese women aged between 20 and 60 years were recruited. Facial skin conditions were analyzed using a Robo Skin Analyzer, and the RBC fatty acid composition was also determined. Questionnaires concerning mood were administered. Forehead pigmentation was more mood-dependent (in 20s group) and less arachidonic acid (AA)-dependent (in all participants) than that in other areas of the face. Actually there was no correlation in pigmentation between the forehead and other areas of the face when adjusted for age, smoking, and drinking. Skin conditions were adversely correlated with a negative mood. α-Linolenic acid concentrations were negatively correlated with negative mood scores. Pigmentation characteristics in the forehead were independent from other areas of the face. Negative mood and AA were adversely correlated with skin conditions.

## INTRODUCTION

The facial skin problems are very important concern especially for women even when these are within normal limits. Facial skin problems can lead to deterioration in mood regardless of whether there are pathological changes or not. However, little is known about nonpathological skin conditions. Reasons for the paucity of studies in this area include (i) the supposed lack of a relationship with disease, (ii) the methodological inability to analyze normal skin conditions until recently, and (iii) possible underestimation of the effects of skin condition on mood.

Mood has long been recognized as an important factor in pathological dermatological conditions (1), and the reverse also holds true (2). This relationship might further be explained

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in part by a third factor, namely nutrition (3). Unfortunately, nonpathological conditions such as the development of wrinkles and pigmentation have rarely been documented from a nutritional point of view. This point is important since *n*–3 fatty acids are anti-inflammatory mainly as a result of competition with arachidonic acid (AA) metabolism (4). Furthermore, administration of *n*–3 fatty acids has been shown to ameliorate symptoms of mood disorders (5–7), while antistress effects of fish oils have also been investigated (8,9).

Owing to recent IT revolutions, it is now possible to digitally determine skin conditions (10). In the present study, we determined the relationships among facial skin conditions, mood, and blood fatty acid composition.

## MATERIALS AND METHODS

### STUDY PROTOCOL

One hundred and thirty-two healthy Japanese women aged between 20 and 60 years were recruited from three local hospitals in Toyama-city ($n$ = 35 in their 20s, $n$ = 30 in their 30s, $n$ = 36 in their 40s, and $n$ = 31 in their 50s or aged 60). All underwent routine medical checkups within 6 months before the recruitment and were free from any serious diseases. Sun exposure of all participants was rather limited, as they all worked inside hospitals. None of the volunteers were using pills at the time of the present study. Study participants were asked to come to their hospital at 8 a.m. when they were off from work. They were asked to wash their face with soap, and 15 min later their facial skin condition was analyzed with a Robo Skin Analyzer (MM&Niic Co., Ltd., Tokyo, Japan). The analyzer is able to take digital pictures of the face from three angles (left, front, and right) while calculating the degree of pigmentation, presence of wrinkles (total length and area of crow's feet), and texture and brightness of the facial skin. More details were reported by Kawada *et al.* (10). In the present study, scores of texture and brightness did not have any marked correlations with mood or fatty acid levels. For this reason, we focused on pigmentation and wrinkles. The degree of pigmentation on various parts of the face was calculated as pixel counts with the help of Image J software (11). We also found that the forehead, defined as the area between the hair and the eyebrows, observed from the front angle was the only important area of the face in regression analysis of pigmentation and mood. The face was, therefore, divided into two parts: the forehead and others. The study protocol was approved by the ethics committee of One K Corporation (Tokyo, Japan), and written informed consent was obtained from each participant.

### FATTY ACID ANALYSIS

Blood samples were taken before skin analysis. Red blood cells (RBCs) were separated from ethylenediaminetetraacetic acid–anticoagulated blood samples, washed twice with saline and then frozen at −80°C until analysis. The fatty acid composition of the total phospholipid (PL) fraction was analyzed as previously described (12) with slight modifications.

### QUESTIONNAIRES

Two psychological tests, the Arousal Checklist (SACL) (13) and Profile of Mood States (POMS) (14), were administered on the day of skin analysis when the participants were

free from other analytical tests. SACL is used to measure stress and arousal levels. It consists of 25 adjectives that describe feelings and moods. Participants use a four-point scale to indicate how well each adjective matches their current state. Scores range from 0 to 14 for stress and 0 to 11 for arousal (13). POMS is another self-rating questionnaire used for measuring six mood states: tension-anxiety, depression-dejection, anger-hostility, vigor, fatigue, and confusion. A total of 65 questions were included to which participants responded on a five-point scale, indicating how well the adjective given described their current mood (14). Japanese versions of SACL (J-SACL) (15) and POMS (16) were employed.

### STATISTICAL ANALYSIS

Data are expressed as means ± S.D. Regression analysis between questionnaire scores, fatty acid concentrations in the RBC PL fraction, and skin problems were performed with (where indicated) or without adjustment for age, smoking, and drinking; $p < 0.05$ was considered significant. When separate age groups were calculated, $p < 0.0125$ was considered significant (Bonferroni's adjustment for the four age groups). Statview (Japanese version 5; SAS Institute, San Francisco, CA) was used for statistical calculations.

## RESULTS

### FATTY ACIDS

Table I shows the fatty acid composition of the RBCs in all participants. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were correlated with age; the older the participants, the higher the DHA concentration, whereas AA was inversely correlated with age. These trends were exactly as in our previous cross-sectional study of 456 Japanese participants. This is because elderly Japanese people eat more fish than younger generations (17). We focused on essential fatty acids for the sake of simplicity.

### SKIN CONDITIONS

Four people whose results confused hair or wrinkles as pigmentation were excluded from analysis. As expected, skin conditions were strongly associated with age. The total length

**Table I**
Fatty Acid Composition of the PL Fraction of Red Blood Cells ($n$ = 132)

| | | Mean ± S.D. |
|---|---|---|
| 16:0 | | 24.6 ± 1.5 |
| 18:0 | | 13.6 ± 1.0 |
| 18:1 | $n$–9 | 13.7 ± 0.9 |
| 18:2 | $n$–6 | 10.2 ± 1.1 |
| 18:3 | $n$–3 | 0.2 ± 0.05 |
| 20:4 | $n$–6 | 11.1 ± 1.1 |
| 20:5 | $n$–3 | 1.3 ± 0.5 |
| 22:6 | $n$–3 | 6.4 ± 1.0 |

of wrinkles ($r = 0.65$; $p < 0.0001$), pigmentation of the forehead ($r = 0.31$; $p = 0.0005$), and that areas other than the forehead ($r = 0.49$; $p < 0.0001$) were positively related with age after adjustment for smoking and drinking. Interestingly, pigmentation levels in the two different areas (the forehead and other areas) showed no significant correlation after adjustment for age, smoking, and drinking ($r = -0.07$; $p = 0.4$). Table II shows the regression between wrinkles and mood in all participants. Depression-dejection and confusion (POMS) were positively associated with the total length of wrinkles in all participants, although the correlations were weak. Table III shows the regression analyses between forehead pigmentation and mood in participants in their 20s only; all negative moods of POMS were positively associated with forehead pigmentation. There were no significant correlations between wrinkles (length or area) and mood scores in any age group except for the finding that the total length of wrinkles was positively associated with confusion in participants in their 50s and 60 ($r = 0.47$; $p = 0.01$).

Two significant correlations were found between skin conditions and fatty acids after adjustment. AA was positively correlated with pigmentation in areas other than the forehead ($r = 0.24$; $p = 0.006$) in all participants, but not with that in the forehead. AA was also positively correlated with the total length of wrinkles in participants in their 30s ($r = 0.47$; $p = 0.006$).

### CORRELATION BETWEEN FATTY ACIDS AND PSYCHOLOGICAL SCORES

Tables IV and V show the results of regression analyses between fatty acids and psychological test scores in all participants and those in their 50s and 60, respectively. α-Linolenic

**Table II**
Regression Coefficients Between Skin Conditions and Mood ($n = 131$)

| | Standardized regression coefficient | |
|---|---|---|
| | Depression-dejection | Confusion |
| Total length of wrinkles (crow's feet) | 0.14 | 0.19 |
| | $p = 0.04$ | $p = 0.004$ |
| Total area of wrinkles (crow's feet) | ns | 0.16 |
| | | $p = 0.03$ |

Only significant correlations ($p < 0.05$ after adjustment) are shown. Pigmentation was not significantly correlated with mood. Data were adjusted for age, smoking, and drinking. ns: No significance.

**Table III**
Regression Coefficients Between Skin Conditions and Mood Among Subjects in Their 20s ($n = 34$)

| | Depression-dejection | Anger-hostility | Fatigue | Tension-anxiety | Confusion |
|---|---|---|---|---|---|
| Forehead pigmentation | 0.48 | 0.43 | 0.54 | 0.62 | 0.58 |
| | $p = 0.005$ | $p = 0.009$ | $p = 0.0007$ | $p < 0.0001$ | $p = 0.0006$ |

Only significant correlations ($p < 0.0125$ after adjustment) are shown. Adjusted for age, smoking, and drinking. Interestingly, no correlations were observed between negative mood and pigmentation in other areas of the face in any age groups.

**Table IV**
Regression Coefficients Between Fatty Acids and Mood ($n$ = 131)

| | Stress (SACL) | Depression-dejection | Fatigue | Tension-anxiety | Confusion |
|---|---|---|---|---|---|
| α-Linolenic acid (ALA) | −0.24 | −0.20 | ns | −0.17 | −0.19 |
| | $p$ = 0.007 | $p$ = 0.02 | | $p$ = 0.048 | $p$ = 0.04 |
| Docosahexaenoic acid | ns | ns | −0.18 | ns | ns |
| | | | $p$ = 0.046 | | |

Only significant correlations ($p < 0.05$ after adjustment) are shown. ALA was also marginally associated with anger-hostility scores ($r = -0.17$, $p = 0.06$). Other fatty acids were not significantly correlated with mood scores. Adjusted for age, smoking, and drinking. ns: No significance.

**Table V**
Regression Coefficients Between α-Linolenic Acid and Mood Among Those in Their 50s and 60 ($n$ = 31)

| | Stress (SACL) | Arousal (SACL) | Confusion |
|---|---|---|---|
| ALA | −0.61 | 0.52 | −0.55 |
| | $p$ = 0.0002 | $p$ = 0.003 | $p$ = 0.002 |

Only significant correlations ($p < 0.0125$ after adjustment) are shown. Other fatty acids were not significantly correlated with mood scores. Adjusted for age, smoking, and drinking.

acid (ALA) was inversely correlated with some of the negative moods in all participants; DHA was inversely associated with fatigue scores (Table IV). In participants in their 50s and 60, ALA was favorably associated with moods (Table V).

## DISCUSSION

One of the most striking findings in this study was the highly significant correlations between pigmentation in the forehead and negative mood scores among the participants in their 20s (Table III). No significant correlations between these parameters were found when all participants were combined. As described in the Results section, skin conditions were heavily affected by age; however, this age effect was variable from person to person and is probably particularly so among those in their 30s to 50s. It is likely that the effects of age on skin conditions have not fully appeared in the 20s group or are still too small to detect compared with the older groups. Consequently, other effects such as mood are thought to be more influential and noticeable in the 20s group.

Comparison of pigmentation between the forehead and other face areas deserves some discussion. At first, there was no correlation in pigmentation between two areas at all ($r = -0.07$). The correlation with negative mood scores in the 20s group was also completely different between the forehead and other areas (see Table III). In contrast, AA was significantly correlated with pigmentation in the other face areas only. It is not very clear why there were substantial differences between the forehead and other face areas. One reason could be the location of the forehead. Stress hormones and sunlight that possibly do more harm to the forehead than the other face areas might synergistically increase forehead pigmentation. However, the foreheads of most participants were covered by their front hair, and therefore, this reasoning appears insignificant.

The forehead is located in front of the forebrain. When the brain works hard especially during stress it must be cooled from the forebrain (selective brain cooling) (18,19). In men forehead sweating was shown to be maintained during exercise even when dehydrated (20). We recently found that water retention in the forehead skin of women at the start of the menstrual cycle was positively correlated with the degree of stress (unpublished data); this relationship was not found in the cheek areas. Furthermore, the beneficial effects on stratum corneum hydration and transepidermal water loss of an oral contraceptive containing chlormadinone and ethinylestradiol were shown to be different between the cheek and the forehead (21). Consequently, it is likely that the skin on the forehead is different from the skin on the cheeks, making the former particularly vulnerable to stress and negative mood.

ALA is generally thought to be cardioprotective and anti-inflammatory (22). Only a few documents have reported a relationship between ALA and mood/behavior/personality. Suzuki *et al.* (23) found that the odds ratios of depression in newly diagnosed lung cancer patients were about one-half in the highest quartile of daily ALA intake compared with the lowest. Moreover, Conklin *et al.* (24) observed an inverse correlation between serum ALA concentrations and impulsivity in 105 hypercholesterolemic patients. In a pilot study, Joshi *et al.* (25) also reported that ALA and vitamin C improved attention deficit hyperactivity disorder symptoms. Taken together, our findings showing the negative correlations between RBC ALA concentrations and negative mood (Tables IV and V) appear to follow the same conclusion.

In a cross-sectional study, Yoneyama *et al.* (26) found that serum C-reactive protein (CRP) levels were inversely related to the intake of ALA in 1461 women. In addition, Rallidis *et al.* (27) reported in a randomized controlled trial of 76 male dyslipidemic patients that dietary supplementation with ALA significantly decreased serum levels of CRP and interleukin-6. The relationship between inflammatory markers and mood has been well documented (28). Although it is difficult to prove that ALA caused lower scores of negative mood from the present results alone, it is possible that negative mood was reduced through the control of inflammatory reactions by ALA.

We originally believed that *n*–3 and *n*–6 fatty acids exerted favorable and unfavorable effects on skin conditions, respectively. This idea depended on the relationship between essential fatty acids and inflammation (4) and the indirect relationship with various favorable effects of *n*–3 fatty acids on mood (5–7,9). Although we found only two significant unfavorable correlations between skin conditions and AA, there were no significant correlations between skin conditions and EPA, DHA, or ALA. The effects of *n*–3 fatty acids on skin conditions might be considerably small in participants who regularly consume a large amount of fish (17). *In vitro* experiments using cultured murine melanoma cells showed that ALA inhibited melanin production by more than 80% at 25 μmol/l (29). We could not find any correlations between RBC ALA concentrations and pigmentation probably because of very low concentrations of ALA at the tissue level (there was only 0.2% of ALA in RBC PL fraction; Table I).

In all participants, both depression-dejection and fatigue (POMS) were significantly correlated with the total length of wrinkles (Table II). Grimacing as a result of a negative mood might increase wrinkles.

There are a few intervention studies reporting the effects of fish oils on POMS scores. Antypa *et al.* (30) performed a placebo-controlled double-blind study of 54 healthy university

students who randomly received either fish oil (1.74 g EPA and 0.25 g DHA) or a placebo for 4 weeks. They found that fatigue scores in POMS were significantly decreased in the fish oil group. Fontani *et al.* (31) performed a randomized control study of 33 healthy participants administered fish oil (1.6 g EPA, 0.8 g DHA, and 0.4 g of other *n*–3 fatty acids) and 16 participants administered a placebo. They found that all mood states including fatigue were significantly improved when assessed within the fish oil group alone. Our finding showing the favorable correlation between DHA and fatigue is in line with those reports (30,31).

## CONCLUSIONS

Pigmentation characteristics were dependent on the area of the face. A negative mood and AA were unfavorably correlated with skin conditions; however, no meaningful correlations were found between *n*–3 fatty acids and skin conditions. ALA was favorably correlated with mood.

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