

Association of dietary fat, vegetables and antioxidant micronutrients with skin ageing in Japanese women

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Daily diet may have implications for skin ageing. However, data on the relationship between diet and the parameters of skin conditions are scarce. The present study aimed to examine the associations of biophysical properties of the skin of women with intakes of fats and antioxidant micronutrients as well as food groups as sources of these nutrients. In a cross-sectional study, we measured the hydration, surface lipids and elasticity of the skin of 716 Japanese women using non-invasive techniques. The extent of facial wrinkles in the crow's-foot area was determined by observation using the Daniell scale. Each subject's usual diet was determined with the use of a validated FFQ. After controlling for covariates including age, smoking status, BMI and lifetime sun exposure, the results showed that higher intakes of total fat, saturated fat and monounsaturated fat were significantly associated with increased skin elasticity. A higher intake of green and yellow vegetables was significantly associated with a decreased Daniell wrinkling score. Intake of saturated fat was significantly inversely associated with the Daniell wrinkling score after additional adjustment for green and yellow vegetable intake. Further studies with more accurate measurement methods are needed to investigate the role of daily diet in skin ageing.

Dietary fats: Skin elasticity: Facial wrinkling: Vegetables

Interest in the impact of ageing on the function and appearance of the skin has been increasing⁽¹⁾. Aged skin has several typical characteristics, including fine wrinkles, dryness, sallowness and loss of elasticity⁽²⁾. These characteristics are thought to result from a complex process controlled by both environmental and genetic factors⁽³⁾. Among environmental factors, sun exposure and cigarette smoking have long been investigated as risk factors for premature skin ageing^(4–6). Large amounts of micronutrients such as those with antioxidant capacity are present in the skin and are suggested to contribute to the maintenance of skin health⁽⁷⁾. *n*-3 PUFA, especially EPA and DHA, are known to have anti-inflammatory effects and thus are thought to have a protective effect against inflammatory skin disorders⁽⁸⁾. The effects of the antioxidant vitamins and carotenoids or fish oil on conditions of the skin have been examined in some intervention studies⁽⁹⁾. However, these studies are limited by the use of supplements. Little is known about the effects of the long-term consumption of vitamins, carotenoids, or fatty acids at nutritional amounts in the daily diet.

To our knowledge, only three studies have examined associations between daily diet and skin conditions^(10–12). Purba *et al.*⁽¹⁰⁾ assessed actinic skin wrinkling of the back of the hand based on the grading of cutaneous microtopographs of 453 men and women living in Europe and Australia. Using

a non-invasive technique, Boelsma *et al.*⁽¹¹⁾ measured the hydration and surface pH of the skin of the right arm and the sebum content of the forehead of 302 Dutch men and women. Cosgrove *et al.*⁽¹²⁾ assessed the appearance of wrinkles, senile dryness and skin atrophy, which were classified as present or absent based on observations by dermatologists, among 4025 American women. These studies revealed that certain types of fats or antioxidant micronutrients were associated with some measured skin properties. However, considering that the sites of the skin measured, the parameters of skin conditions, and the skin assessment methods varied among these studies, cumulated data are still scarce and far from conclusive. In the present study of Japanese women, we examined cross-sectional relationships between dietary intakes of fats and antioxidant micronutrients as well as food groups as sources of these nutrients and biophysical properties of the skin, including hydration, sebum content, elasticity and wrinkle appearance.

Materials and methods

Study population

The present study was part of a larger study designed to assess the relationships among lifestyle, environmental factors and

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women's health⁽¹³⁾. Study subjects were participants in a medical health check-up programme provided by a general hospital in Gifu, Japan, between October 2003 and March 2006. A total of 2073 individuals, including return visitors to the programme during the study period, were invited to join the study, and 1545 agreed to participate (response proportion: 74.5%). When the response proportion was calculated for only new visitors to the programme during the study period, it was 83.2% (1103 out of 1325 individuals). The details are described elsewhere⁽¹³⁾. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethical board of the Gifu University Graduate School of Medicine. Written informed consent was obtained from all subjects.

Dietary measurement

The participants responded to a self-administered questionnaire that included questions on demographic characteristics, smoking and drinking habits, diet, exercise, sun exposure, and medical and reproductive histories. Intakes of foods and nutrients were estimated based on a validated 169-item semi-quantitative FFQ using the Japanese Standard Tables of Food Composition, 4th and 5th editions, and fatty acid composition data published by Sasaki *et al.*⁽¹⁴⁾. Intake of long-chain *n*-3 PUFA was calculated as the sum of EPA and DHA. Detailed information on the questionnaire, including its validity and reproducibility examined in other samples, has been described elsewhere⁽¹⁵⁾. The Spearman correlation coefficients between the questionnaire and twelve daily diet records kept over a 1-year period for major and micro-nutrients ranged from 0.29 for carotene (α - and β -carotenes plus cryptoxanthin; expressed as β -carotene equivalents) to 0.73 for Ca. Those for intakes of total fat, saturated fats, monounsaturated fat, polyunsaturated fat, and green and yellow vegetables were greater than 0.50. Our questionnaire was designed to measure an individual's relative intake of foods and nutrients rather than absolute values. The means estimated from the FFQ were generally higher than those estimated from twelve daily diet records. Although we presented the mean values of dietary intake, some of them may have been overestimated by our questionnaire.

Assessment of other exposure variables

Data on lifetime sun exposure were collected through face-to-face interviews. Subjects identified periods in their lives during which they had had stable patterns of outdoor activity. For each of these periods, the subjects were asked about the amount of time they spent outdoors per d during a typical week. Weekday and weekend exposures were recorded separately. We estimated the total time spent outdoors from age 12 years until the day of the interview. Current use of sunscreens during summer was also recorded. Exercise was assessed by asking the average time spent (h per week) performing various kinds of activities during the past 1 year. The details, including validity, are described elsewhere⁽¹⁶⁾.

Assessment of skin ageing

Skin properties were measured by two investigators. The subjects rested for about 15 min in a room for skin measurements. The cosmetics on the selected sites were removed by the investigators. All the measurements were performed in triplicate.

Skin hydration was measured with a corneometer (CM825; Courage and Khazaka, Electronic GmbH, Cologne, Germany). The probe was applied to the skin of the inner side of the right upper arm for 1 s at a pressure of 3.5 N/cm². The degree of epidermal humidity is indicated in system-specific units.

Skin surface lipids composed of sebum and corneal lipids were measured with a Sebumeter (SM810; Courage and Khazaka, Electronic GmbH). This device, which works on the principle of photometry, has a special plastic strip that becomes transparent when it absorbs fat. The measuring head equipped with this strip was pressed on the skin of the forehead with a constant pressure of 10 N/64 mm² for 30 s. The value displayed corresponded to the amount of sebum on the skin surface in $\mu\text{g}/\text{cm}^2$.

Skin elasticity of the inner side of the right upper arm was measured with a cutometer (SEM575; Courage and Khazaka, Electronic GmbH). The measurements were performed with a negative pressure of 350 mbar, a suction time of 2 s and a relaxation phase of 2 s. The assessment parameters consisted of the Ur/Uf coefficient, which represents the ability of the skin to regain its initial position after deformation.

The extent of facial wrinkles in the crow's-foot area was determined by the Daniell scale⁽⁴⁾ ranging from grade I (essentially no wrinkling) to grade VI (profound wrinkling extending over most of the face).

Data analyses

A total of 1086 women participated in the skin measurements. Women who had cancer (*n* 24) or collagen diseases (*n* 11) such as rheumatism and systemic lupus erythematosus were excluded from the present analyses. Additionally, 319 women who had not responded to the dietary questionnaire (*n* 259) or those with incomplete or unreliable responses to the dietary questionnaire (criteria shown elsewhere⁽¹⁷⁾; *n* 60) and sixteen women who did not complete the interview of lifetime sun exposure were excluded from the present analyses. Thus, 716 women aged 20–74 years comprised the study population. Skin elasticity data were unavailable for 124 women as the device was out of order during their check-ups. Women who did not respond to the dietary questionnaire were more likely to be older than those who did. After controlling for age, the means of skin property variables of the non-responders were similar to those who responded to the dietary frequency questionnaire except for a lower mean value for skin surface lipids.

For statistical analysis, dietary intakes were adjusted for total energy after log-transformation by using the residual method proposed by Willett⁽¹⁸⁾. Quintiles of the dietary variables were derived based on their distribution in the total population. The means of skin properties for each quintile were provided using analysis of covariate models. A linear trend was assessed using continuous values. Since skin hydration was correlated with room temperature and humidity

Table 1. Characteristics of study subjects and their correlations with skin parameters (*n* 716) (Mean values and standard deviations, percentages and correlations)

	Characteristics		Correlations†			
	Mean	SD	Hydration	Surface lipids	Elasticity	Facial wrinkles
Age (years)	43.3	8.2	0.04	-0.20**	-0.32**	0.70**
BMI (kg/m ²)	21.3	2.9	0.12	0.02	0.13**	0.03
Education (years)	13.7	2.0	-0.14	0.005	-0.02	0.02
Exercise (METs h/week)	26.2	33.0	-0.0003	-0.03	-0.06	-0.07
Sun exposure (cumulative h)	17 430	10 264	0.01	0.02	-0.01	0.17**
Alcohol intake (ml/d)	6.6	16.0	0.03	0.05	-0.03	0.05
Not married (%)	18.7		-0.04	0.001	-0.07	-0.03
Postmenopausal (%)	19.1		-0.03	-0.05	-0.03	0.04
Current smokers (%)	6.0		-0.01	0.07	-0.03	0.10**
Ex-smokers (%)	3.9		0.02	-0.01	0.005	0.03
Current HRT use (%)	3.4		0.03	0.05	0.01	-0.05
Current OC use (%)	0.8		0.01	0.002	0.005	-0.03

METs, metabolic equivalents, HRT, hormone replacement therapy; OC, oral contraceptives.

** $P < 0.01$.

† Spearman correlation coefficients were adjusted for age, except for age. Dummy variables were given for categorical variables.

(Spearman's r were -0.19 and 0.62 , respectively), the results under restricted ranges (temperature, $20-25^{\circ}\text{C}$; humidity, $40-60\%$; n 209) are shown. Room temperature and humidity were dealt with as covariates because they were strongly correlated with skin hydration (r 0.09 for temperature and r 0.50 for humidity). Although the correlation coefficients of room temperature and humidity with other skin variables were less than 0.08 , they were also included as covariates. The known or suspected risk factors for skin conditions, such as age, BMI, smoking status and sunlight exposure⁽¹⁹⁾, were included in the models as covariates in addition to room temperature and humidity. The indicator for raters was also included as a covariate. All the statistical analyses were performed using SAS programs (SAS Institute Inc., Cary, NC, USA). Significance was defined as two-sided $P < 0.05$.

Results

Table 1 shows the characteristics of the study subjects and their correlations with skin parameters. Age, BMI, smoking status and sunlight exposure were significantly correlated with one or more parameters. Table 2 shows the intercorrelations among the skin parameters.

Table 3 shows the means for each skin parameter according to the quintile of the selected nutrient or food intake after controlling for the covariates. Surface lipids were significantly positively associated with fresh and processed meats. Skin elasticity was significantly positively associated with total fat, saturated fat and monounsaturated fat, although the differences in the mean elasticity between the highest and the lowest quintiles of these types of fats were less than 2% . The Daniell wrinkling score was significantly inversely associated with green and yellow vegetable intake. Saturated fat was marginally significantly associated with Daniell's wrinkling score, but this association obtained significance after additional adjustment for green and yellow vegetable intake ($P = 0.049$). The association between the Daniell wrinkling score and green and yellow vegetable intake remained statistically significant after additional adjustment for saturated fat

intake ($P = 0.04$). Vitamins C and E and Zn were unrelated to any parameter of the skin measurements.

Discussion

Skin elasticity on the forearm has been reported to decrease with chronological age^(20,21). In the present study, skin elasticity was significantly inversely correlated with facial wrinkle score, suggesting the importance of this parameter in skin ageing. We observed that higher intakes of total fat, saturated fat and monounsaturated fat were moderately but significantly associated with increased elasticity. A higher intake of long-chain $n-3$ fatty acids was marginally significantly associated with increased elasticity. The associations with elasticity did not differ greatly by type of fat, although polyunsaturated fat was not significantly associated with skin elasticity. Fats provide building blocks for many components of epidermal and dermal tissues, and they are sources of energy in cell proliferation, maturation and homeostasis⁽²²⁾. Fats are sensitive to the oxidation process⁽²²⁾. However, maintenance of collagen and elastic fibres may require adequate amount of fat. Higher saturated fat intake was also significantly associated with a decreased facial wrinkling, suggesting a favourable effect of fat. None of the previous cross-sectional studies on diet and skin conditions⁽¹⁰⁻¹²⁾ included the measurement of skin elasticity but addressed the association of fat intake with other parameters of skin conditions. Purba *et al.*⁽¹⁰⁾ found that higher intakes of total fat and monounsaturated fat were significantly associated with decreased wrinkling on

Table 2. Intercorrelations among skin parameters (Spearman correlation coefficients)

	Hydration	Surface lipids	Elasticity	Facial wrinkles
Hydration	1.00	0.02	0.03	0.12
Surface lipids	-	1.00	0.13**	-0.11**
Elasticity	-	-	1.00	-0.30**
Facial wrinkles	-	-	-	1.00

** $P < 0.01$.

Table 3. Adjusted* means of skin parameters according to quintiles (Q) of selected dietary variables

	Median†	Hydration (arbitrary units)	Surface lipids ($\mu\text{g}/\text{cm}^2$)	Elasticity (%)	Facial wrinkles (Daniell score)
Total fat (g/d)					
Q1	50.8	41.4	68.6	81.8	2.48
Q2	57.3	41.6	69.8	82.4	2.48
Q3	62.4	40.2	75.9	83.1	2.57
Q4	67.2	40.2	73.3	82.8	2.41
Q5	73.8	40.0	72.3	83.6	2.38
<i>P</i> for trend		0.28	0.26	0.007	0.17
Saturated fat (g/d)					
Q1	13.6	41.3	68.2	82.0	2.51
Q2	16.2	41.5	69.5	82.8	2.54
Q3	18.2	39.3	72.8	82.7	2.43
Q4	19.8	41.5	77.0	82.9	2.45
Q5	23.4	40.4	72.3	83.3	2.39
<i>P</i> for trend		0.64	0.12	0.03	0.06
Monounsaturated fat (g/d)					
Q1	16.3	41.3	68.2	81.7	2.54
Q2	19.2	42.0	70.9	82.2	2.46
Q3	21.2	40.5	72.8	83.2	2.44
Q4	23.2	39.4	74.4	83.0	2.52
Q5	26.1	40.0	73.5	83.6	2.36
<i>P</i> for trend		0.18	0.07	0.007	0.34
Polyunsaturated fat (g/d)					
Q1	12.1	42.8	74.5	82.5	2.43
Q2	14.0	39.8	72.0	82.3	2.51
Q3	15.3	40.3	70.1	82.6	2.44
Q4	16.6	40.7	70.9	83.0	2.45
Q5	18.9	39.8	72.3	83.2	2.48
<i>P</i> for trend		0.27	0.72	0.19	0.11
Long-chain <i>n</i>-3 fatty acids (mg/d)					
Q1	366	42.0	70.1	81.9	2.46
Q2	508	41.4	71.2	82.6	2.49
Q3	630	40.1	79.0	83.5	2.44
Q4	817	39.2	70.7	82.7	2.52
Q5	1121	40.7	68.7	83.0	2.41
<i>P</i> for trend		0.21	0.87	0.09	0.95
Carotene (mg/d)					
Q1	2973	41.6	69.7	82.6	2.54
Q2	4014	40.0	77.3	83.4	2.57
Q3	4848	39.7	67.4	82.5	2.45
Q4	6188	40.6	73.9	82.6	2.39
Q5	8820	41.2	71.5	82.6	2.36
<i>P</i> for trend		0.69	0.53	0.56	0.08
Vitamin C (mg/d)					
Q1	90.9	41.4	73.4	82.9	2.48
Q2	115.6	38.7	63.7	82.5	2.43
Q3	141.8	41.8	74.8	82.3	2.51
Q4	170.2	40.3	72.8	82.7	2.35
Q5	235.3	41.0	75.1	83.1	2.55
<i>P</i> for trend		0.95	0.47	0.76	0.78
Vitamin E (mg/d)					
Q1	8.4	40.6	74.8	82.3	2.51
Q2	9.6	41.2	70.9	82.8	2.54
Q3	10.4	40.3	73.9	82.9	2.52
Q4	11.5	40.0	70.4	82.8	2.30
Q5	13.2	41.3	69.8	82.9	2.45
<i>P</i> for trend		0.88	0.54	0.49	0.27
Zn (mg/d)					
Q1	9.4	41.2	69.1	82.5	2.56
Q2	10.2	39.0	72.1	82.9	2.47
Q3	10.9	40.9	71.5	82.5	2.41
Q4	11.5	41.5	72.0	82.5	2.47
Q5	12.6	40.4	75.2	83.3	2.40
<i>P</i> for trend		0.53	0.36	0.18	0.36
Fresh and processed meats (g/d)					
Q1	37.9	40.9	64.0	82.6	2.38
Q2	58.3	42.1	72.1	82.6	2.46
Q3	72.4	41.9	71.2	82.4	2.50
Q4	90.6	38.3	73.2	82.8	2.59
Q5	123.0	39.5	79.5	83.3	2.39
<i>P</i> for trend		0.25	0.01	0.37	0.65

Table 3. Continued

	Median†	Hydration (arbitrary units)	Surface lipids ($\mu\text{g}/\text{cm}^2$)	Elasticity (%)	Facial wrinkles (Daniell score)
Fish and shell fish (g/d)					
Q1	40.5	41.9	77.6	82.9	2.35
Q2	58.0	38.8	67.0	82.0	2.46
Q3	71.4	40.9	74.2	83.2	2.56
Q4	86.0	40.8	70.2	82.8	2.50
Q5	114.4	41.0	70.6	82.7	2.46
<i>P</i> for trend		0.29	0.37	0.56	0.42
Green and yellow vegetables (g/d)					
Q1	62.9	40.3	71.6	82.5	2.51
Q2	91.7	41.4	75.9	83.6	2.58
Q3	120.1	42.1	66.7	82.4	2.46
Q4	156.9	38.2	70.1	82.9	2.40
Q5	250.2	41.6	75.5	82.3	2.37
<i>P</i> for trend		0.82	0.36	0.91	0.04
Other vegetables (g/d)					
Q1	121.4	41.1	72.1	82.8	2.48
Q2	179.5	39.1	79.8	83.2	2.49
Q3	206.3	41.2	70.6	82.6	2.41
Q4	247.1	40.7	66.8	82.4	2.41
Q5	335.6	41.4	70.5	82.7	2.52
<i>P</i> for trend		0.45	0.57	0.32	0.73
Fruits (g/d)					
Q1	34.6	40.2	77.6	82.5	2.56
Q2	61.3	39.8	66.3	83.0	2.43
Q3	89.4	40.8	68.9	82.1	2.42
Q4	140.3	41.6	71.9	82.8	2.47
Q5	229.0	40.9	75.0	83.3	2.43
<i>P</i> for trend		0.55	0.69	0.12	0.24

* Adjusted for total energy, age, BMI, smoking status, cumulative sun exposure, rater, and room temperature and humidity.

† Adjusted for total energy.

the back of the hand; this result does not contradict the present results. However, a higher intake of butter or margarine was associated with increased wrinkling. Boelsma *et al.*⁽¹¹⁾ observed that higher intakes of total fat, saturated fat and monounsaturated fat were significantly associated with decreased skin hydration of the skin on the right arm. In the study reported by Cosgrave *et al.*⁽¹²⁾, a higher intake of fat was associated with wrinkled appearance and senile dryness, but a higher intake of linoleic acid was associated with decreased senile dryness. The results of these studies suggested an unfavourable role of certain types of fat in skin health. Fat consumption in the present study subjects, like that in other Japanese populations, was low as compared with those among Western populations, which may partially explain the discrepancies in the results.

Skin elasticity has been reported to be improved by hormone replacement therapy^(23,24). Higher fat intake has been associated with increased endogenous oestrogen concentrations of women in some studies^(25,26). The observed association of fat intake with skin elasticity may be explained by oestrogen profile. We expected that alcohol and phyto-oestrogens such as soya isoflavones might mimic the effects of oestrogen on the skin. However, neither alcohol nor dietary soya was associated with skin elasticity or the other parameters (data not shown).

Dietary supplementation with antioxidant vitamins or carotenoids has shown photoprotective effects on the skin in some studies^(27–29). In the present study, a higher intake of green and yellow vegetables was associated with decreased facial wrinkling. Carotene, which is abundant in green and

yellow vegetables, was marginally inversely associated with facial wrinkling. However, we cannot deny a possibility that the observed inverse association of green and yellow vegetable intake with facial wrinkling may be due to certain nutrients other than carotene. Purba *et al.*⁽¹⁰⁾ also observed that a higher intake of vegetables was associated with less actinic damage of the back of the hand.

The FFQ, like all methods of dietary assessment, is subject to measurement error. In addition, the measurements of skin properties are likely to be affected by environmental conditions, such as the temperature and humidity of the room for the measurements. A room humidity of between 40 and 60% is generally recommended for measurements of skin hydration⁽³⁰⁾. However, we noticed that the room humidity affected the skin hydration, even in such a restricted range. Nonetheless, the findings were not altered in subanalyses with narrow ranges for room temperature and humidity. It is unlikely that the measurement errors in the skin parameters are dependent on diet. Thus, the observed associations were modest but likely to be underestimated. The homogeneity of diet among the study subjects might have precluded us detecting a significant association.

Because of the cross-sectional nature of the data, no causal inferences can be drawn regarding any of the associations observed. The relatively narrow age range and the inclusion of women only are also limitations. Although fats and green and yellow vegetables were associated with some skin parameters of Japanese women, these modest associations may be by chance due to multiple testing. Given the small number of epidemiological studies on diet and skin health

and the potential for these studies to promote a healthy diet, further studies with more accurate measurement methods are needed.

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C. N. initiated and organised the study, and wrote the manuscript. K. N. and K. W. were involved in the analyses and interpretation of the study. S. O. helped to design the analytic strategy. M. H., N. T. and K. Y. helped to supervise the field activities and interpret the data.

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