[Objectives]
The causes of skin aging can be roughly divided into endogenous aging and photo-aging. Folds and spots formed on the skin are said to be attributable to exposure to ultraviolet ray in 80% of all cases. We previously reported that Chlorella inhibited the activity of matrix metalloproteinase 1 (MMP-1), an enzyme involved in the formation of folds during photo-aging. The present study was conducted to evaluate the effects of cream containing hot water extracts of Chlorella in improving the skin quality (primarily folds).

[Methods]
The skin of 12 healthy women aged 40-56 (mean: 49.6) was evaluated before, during and after use of this cream. Each subject applied the cream containing hot water extracts of Chlorella twice daily to the entire face in an appropriate amount (approximately 0.5 g) each time after use of a basic cosmetic. The test lasted for 8 weeks. Each subject was instructed to visit the clinic at the start and 2, 4, 6 and 8 weeks after the start of cream application to receive skin quality evaluation, skin sample collection at the corner of eye and a questionnaire survey on subjective symptoms.

[Results]
(1) Visual evaluation by the physician (grade of folds at the corner of eye)
   Improvement was seen 2 weeks or later after the start of the study, with the response rate (percentage of subjects rated as slightly improved, improved or much improved) was 93% at 8 weeks (the end of the study).

(2) Skin quality
   Relative moisture content in the horny layer significantly increased at 2 weeks from the start of the study. Relative skin elasticity increased significantly at 8 weeks from the start of the study. Relative percutaneous moisture evaporation tended to decrease in Week 4 and afterwards, although this tendency was not significant (Fig. 1).

(3) Skin replica analysis
   Relative maximum width of folds decreased significantly at 8 weeks from the start of the study. Fig. 2 shows a case of marked improvement.

(4) Questionnaire survey on subjective symptoms
   A questionnaire survey involving 17 items about skin condition was carried out. Many items showed subjective improvement in Week 2 through Week 8, with the improvement of 9 items being statistically significant in Week 8.
These results suggest that the cream containing hot water extracts of Chlorella improves the skin quality primarily through moisturizing effects soon after the start of application (Week 2) and exerts barrier function-improving and anti-fold effects thereafter.

![Time course of relative skin quality (%)](image)

**Fig. 1.** Skin quality evaluation after cream containing hot water extracts of Chlorella application. Time course of relative values (%) & (horny moisture content, percutaneous moisture evaporation, skin elasticity)

N-12, Bar: Standard Error

- ●: Relative horny moisture content (%)
- ▲: Relative skin elasticity (%)
- ■: Relative percutaneous moisture evaporation (%)
- &: Relative value (%) = [(each measured value) / (value measured at the start)] x 100 (%)

*: Significant vs the start of application (p<0.05, Wilcoxon signed rank sum test)

**: Significant vs the start of application (p<0.01, Wilcoxon signed rank sum test)

¥: Significant vs Week 6 of application (p<0.05, Wilcoxon signed rank sum test)
Fig. 2. Changes in right eye corner skin replica after cream containing hot water extracts of Chlorella application in 2 cases showing marked improvement.

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<table>
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<tr>
<th>Journal</th>
<th>Medicine and Biology Vol.157 No.6 (2013) 1251-1262</th>
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</thead>
<tbody>
<tr>
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