# Relationship Between NMF (Lactate and Potassium) Content and the Physical Properties of the Stratum Corneum in Healthy Subjects

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**Natural moisturizing factor (NMF) of the stratum corneum (SC) has been established to play important roles in the physical properties of the SC. Few studies, however, have investigated the specific influences of NMF components other than the amino acids. In this study, therefore, we focus on the relationship between the ion content and physical properties of the SC in 40 healthy subjects. Changes in the physical properties of the SC induced by the extraction of NMF were equivalent to the changes that took place from summer to winter, demonstrating the important role of NMF in the physical properties of the SC in healthy subjects. The seasonal changes in the physical properties of the SC from summer to winter were accompanied by significant decreases in the levels of lactate, potassium, sodium, and chloride in the SC. Lactate and potassium were the only components found to correlate significantly with the state of hydration, stiffness, and pH in the SC. Interestingly, the levels of lactate and potassium in the SC were also significantly correlated. Moreover, potassium lactate restored the SC hydration state decreased by extraction of NMF. These results suggest that lactate and potassium may play roles in maintaining the physical properties of the SC in healthy subjects.**

Key words: lactate/physical properties/potassium/stratum corneum
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While water is well known to play important roles in maintaining the mechanical properties of the healthy stratum corneum (SC) (Takahashi *et al*, 1981), overhydration has been shown to be harmful to the SC. Water increases skin permeability (Schueplein, 1978), for example, and overhydration might disrupt the SC intracellular space by degrading the desmosomes (Warner *et al*, 2003). Thus, the health of the SC is thought to depend on the maintenance of the SC water content to an optimal level, a function predominantly handled by natural moisturizing factor (NMF).

NMF of the SC plays important roles in maintaining the physical properties of the SC (Middlenton, 1968). NMF is made up chiefly of amino acids and metabolites of amino acids such as pyroglutamic acid, and many reports have suggested the important role of amino acids in influencing the state of hydration in the SC (Horii *et al*, 1989; Watanabe *et al*, 1991; Hara *et al*, 1993). Another group found that NMF components such as basic amino acids were responsible for maintaining the flexibility of the SC (Jokura *et al*, 1995). These results, however, were obtained from quantitative studies of SC from subjects with atopic xerosis, ichthyosis vulgaris, senile xerosis (Horii *et al*, 1989; Watanabe *et al*, 1991; Hara *et al*, 1993), or artificially induced dry skin (Koyama *et al*, 1984).

Abbreviations: NMF, natural moisturizing factor; SC, stratum corneum

Besides amino acids, NMF contains lactate and inorganic ions such as potassium, sodium, and calcium. In spite of the high amino acid content in the NMF (approaching 48%), the lactate content was also high, reaching a level similar to that of pyroglutamic acid (about 10% in NMF) (Jokura *et al*, 1995). The inorganic ion content in NMF (about 5%) was just under the urea content (Jokura *et al*, 1995). In clinical experiments, topical treatment with lactate effectively alleviated the symptom of dry skin in xerosis (Dahl *et al*, 1983; Van Scott *et al*, 1984), and a treatment of lactate or lactate salts softened the SC (Takahashi *et al*, 1985). These reports, however, on the treatment effects of lactate have not been followed up by quantitative studies on the relationship between the lactate content in the SC and the physical properties of the SC.

A good number of reports have covered the roles of inorganic ions in the epidermis. The calcium ion gradient in the epidermis is generally important for terminal differentiation, and after barrier perturbation it disappears altogether (Menon *et al*, 1992). Denda *et al* (1999) reported that magnesium ion in the SC accelerates skin barrier recovery. In other studies, potassium ion was reported to play important roles in terminal differentiation (Hennings *et al*, 1983; Lee *et al*, 1992), and the potassium ion gradient in the epidermis was abolished, together with calcium, after barrier perturbation (Mauro *et al*, 1998). A number of other stimuli have been shown to change the potassium content in the epidermis. In studies by Lindberg *et al* (1983, 1989,

1992), for example, the potassium content in the epidermis fell as epidermal cell injury increased and rose as epidermal hyperplasia grew more severe. In another study, Bunse *et al* (1991) showed lower levels of potassium in aged epidermis than in young epidermis. Further, Warner *et al* (1995) reported that alteration in the composition of potassium and chloride in the inner SC might have a role in maturation of the SC. While the distribution of inorganic ions in the epidermis is known to play important roles in the formation of the SC, none of the earlier studies have quantitatively evaluated the relationship between the levels of inorganic ions in the SC and the physical properties of the SC.

In this report, we examine the relationship between NMF, particularly its lactate and inorganic ion components, and the physical properties of the SC in healthy subjects.

## Results

**Comparison of ionic compositions between the SC and sweat** At first, we confirmed that the ionic composition of extract obtained from the stripped SC by soaking in water for 24 h (Fig 1*b*) was similar to the ionic composition of the SC reported by others (Grundin *et al*, 1985; Zglinicki *et al*, 1993; Forslind *et al*, 1995), thereby confirming that the ionic content measured by our method was identical to that of the SC. Next, we examined whether the ionic composition of the SC was different from that of sweat. The ratios of both lactate and potassium to sodium were significantly lower in the sweat than in the SC (Fig 1), suggesting that the lactate and potassium in the SC were mainly derived from sources other than sweat.

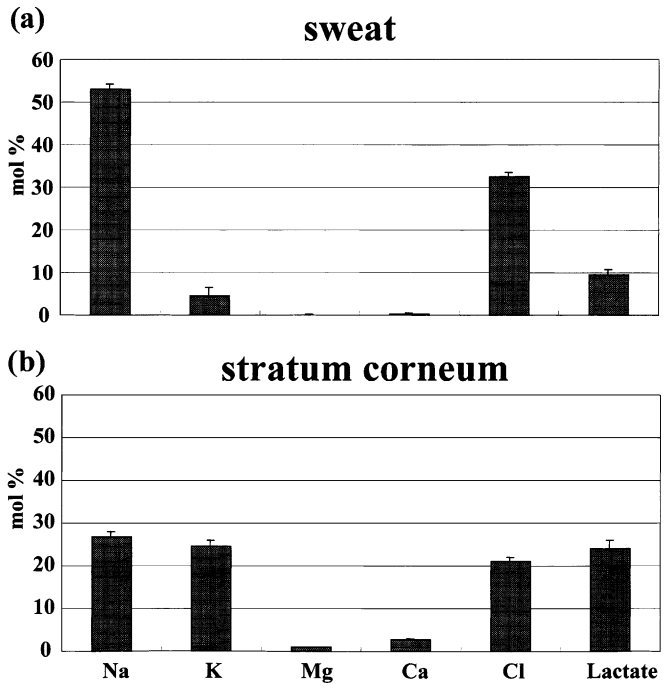


**Figure 1**
**Compositions of lactate and potassium in the SC were different from those in sweat.** Sweat was collected from the surface of the face. Pieces of forearm SC were collected in summer by a tape-strip method. Each substance was extracted and measured as described in the *Materials and Methods* section. (*a*) Composition of ions in sweat and (*b*) composition of ions in the SC. Values represent means with SEM from four subjects for sweat and 40 subjects for the SC per column. The differences in ionic ratios between sweat and the SC were significant ($p < 0.01$, ANOVA).

**Changes of the physical properties of the SC by extraction of NMF** To confirm that NMF plays important roles in the physical properties of the SC in healthy subjects, we examined whether the physical properties of the SC changed after the extraction of NMF. As it turned out, the state of SC hydration was significantly decreased after the extraction of NMF by water treatment (Fig 2*a*). Moreover, both the SC stiffness and pH were significantly increased after extraction (Fig 2*a*). These changes were equivalent to the normal changes of the SC from summer to winter (Fig 2*b*), thereby confirming that NMF plays important roles in the physical properties of the SC in healthy subjects.

**Seasonal changes in the physical properties of the SC and NMF content in the SC** Noting the significant changes in the physical properties of the SC between winter and summer (Fig 2*b*), we attempted to identify which of the NMF components in the SC (lactate, potassium, sodium, calcium, magnesium, chloride, and amino acids) underwent changes coinciding with the seasonal changes in the physical properties. The levels of potassium, lactate, sodium, and chloride were all significantly lower in the winter than in the summer (Fig 3). The ratio of potassium to total cation was also significantly lower in the winter than in the summer (0.27 *vs* 0.47; $p < 0.01$), although the ratio of sodium to total cation was not decreased (data not shown). In contrast, the amino acid content in the SC was significantly higher in the winter than in the summer (Fig 3).

**Correlation between the physical properties of the SC and NMF content** Noting that the seasonal decreases in lactate, potassium, sodium, and chloride accompanied the seasonal changes in the physical properties of the SC, we attempted to identify which NMF components were correlated with the physical properties.

During the winter months, we identified a significant positive correlation between the potassium content and the state of SC hydration ($r = 0.589$, $p < 0.01$, Fig 4), a significant negative correlation between the potassium content and SC stiffness ($r = -0.373$, $p < 0.05$, Fig 5), and a significant negative correlation between the potassium content and SC pH ($r = -0.737$, $p < 0.01$, Fig 6). All three of these correlations were also noted in the SC samples taken during the summer (Table I). On the other hand, the sodium content was not significantly correlated with the SC hydration state or stiffness in the winter ($r = 0.148$, Fig 4; $r = -0.023$, Fig 5) or summer (Table I). The chloride content was only negatively correlated with pH in the winter and summer (Table I).

The lactate content exhibited a pattern similar to that of the potassium content in winter, correlating positively with the SC hydration state ($r = 0.492$, $p < 0.01$, Fig 4), and negatively with the SC stiffness ($r = -0.444$, $p < 0.01$, Fig 5) and pH ($r = -0.787$, $p < 0.01$, Fig 6). The lactate content was also significantly correlated with physical properties other than the SC hydration in the summer (Table I).

In the NMF extraction experiment (Fig 2*a*), the amounts of potassium and lactate directly extracted from the SC of

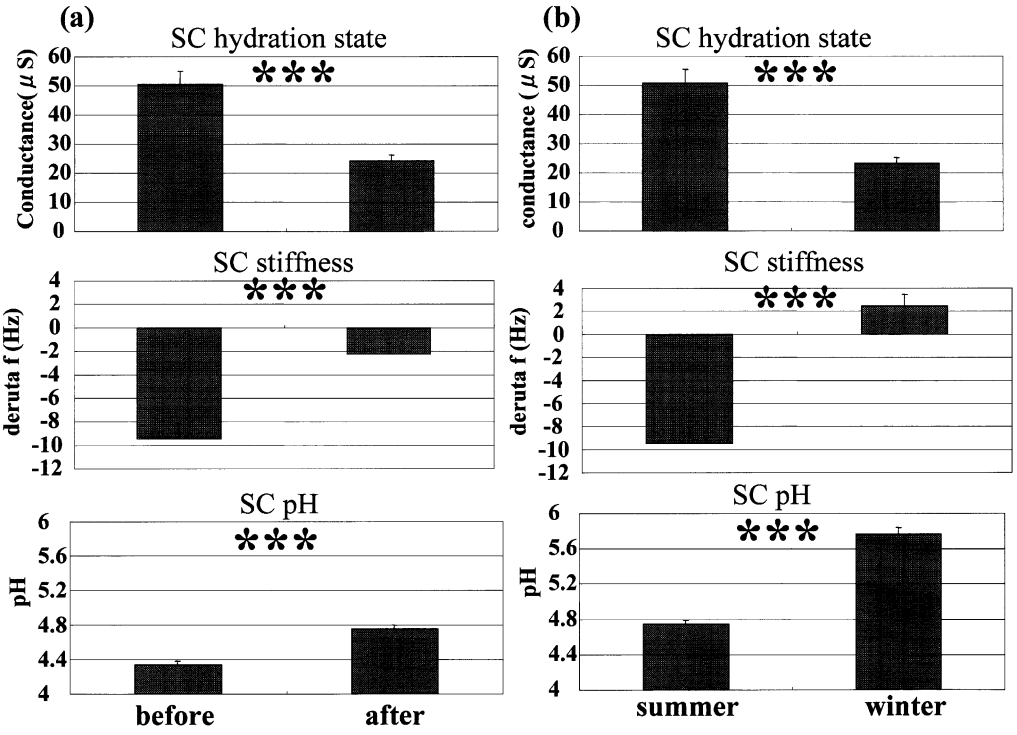


**Figure 2**
**NMF extraction induced changes in the physical properties of the SC similar to the changes observed between summer and winter.** The physical properties were measured before and after NMF extraction in summer (*a*), and without NMF extraction in winter and summer (*b*), as described in the *Materials and Methods* section. Values represent means with SEM from 40 subjects per column. A probability of $p < 0.05$ was considered significant: ***$p < 0.001$. SC represents the stratum corneum.

forearm were also correlated with the changes in SC hydration and stiffness after the NMF extraction (Fig 7).

The amino acid content was not significantly correlated with any of these physical properties in winter (Figs 4–6) or summer (Table I).

**Correlation between potassium ion and lactate content** As the levels of both lactate and potassium were significantly correlated with the physical properties, we next examined whether they were correlated with each other.

A significant positive correlation was found between the potassium content and lactate content in both winter ($r = 0.814$, $p < 0.01$, Fig 8) and summer ($r = 0.397$, $p < 0.05$).

**Restoration of the SC hydration state with topical application of NMF components** Lastly, we examined whether potassium lactate could restore the SC hydration of the forearm after the NMF had been extracted by water. As it turned out, the potassium lactate restored the SC hydration, exhibiting a significantly higher restorative effect than sodium lactate, potassium chloride, and water (Fig 9).

## Discussion

This study using healthy subjects is the first to show that the levels of lactate and potassium in the SC correlated with the physical properties of the SC, as well as with each other. Moreover, the topical application of potassium lactate was found to restore the SC hydration state of the forearm after NMF had been extracted by water.

The ions in the SC can conceivably originate from three organs, namely, the sweat glands, sebaceous glands, and epidermis. The decreases in the levels of inorganic ions and lactate from the outer SC to inner SC suggest that these ions and lactate originate mainly from the sweat glands (Warner *et al*, 1988; Caspers *et al*, 2001). Our study, however, showed that the potassium and lactate composition of the SC was significantly different from that of sweat, as previously reported by Patterson *et al* (2000). It appears, therefore, that the lactate and potassium in the SC do not originate mainly from the sweat gland. With regard to the epidermis, Zglinicki *et al* (1993) suggested that the sodium, chloride, and potassium were in equilibrium in the skin. Given that the intracellular potassium content exceeds the extracellular potassium content, we can speculate that the potassium and lactate in the epidermal cells are directly transferred from the keratinocyte layer to the SC in the process of differentiation. As the sebaceous glands are holocrine, the sebum from sebaceous glands may contain various sebocyte constituents other than lipids. Though it has so far proven difficult to determine the origin of inorganic ions and lactate, we speculate that they partially originate from the sebaceous glands.

In previous *in vitro* studies, treatments with ether and water significantly reduced the water binding of the SC of guinea-pig footpad (Middleton, 1968), as well as the elasticity of the SC of the back skin of pig (Jokura *et al*, 1995). In our study on healthy subjects, we confirmed that the extraction of NMF from the SC decreased the SC hydration and increased the SC stiffness and pH. These physical changes were equivalent to those that naturally occur in the SC from summer to winter, suggesting that NMF plays important roles in maintaining the physical properties of the SC.

NMF contains various substances such as amino acids, organic acids, and inorganic ions. Our results confirmed significant decreases in the lactate and potassium content from summer to winter, as well as correlations of both

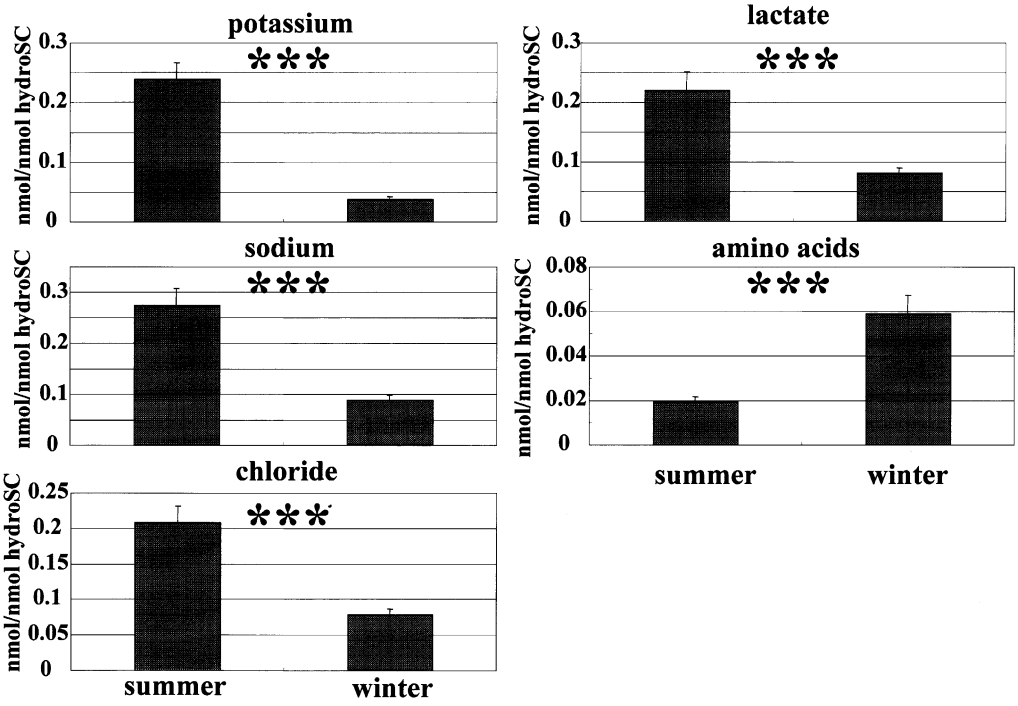


**Figure 3**
**Levels of some NMF components changed between winter and summer.** Pieces of forearm SC were collected in winter and summer by a tape-strip method. Each NMF component was extracted and measured by the method described in the *Materials and Methods* section. Values represent means with SEM from 40 subjects per column. A probability of $p<0.05$ was considered significant: $^{***}p< 0.001$.

lactate and potassium levels with three physical properties of the SC. Further, potassium lactate restored the hydration of forearm SC after NMF had been extracted. Takahashi *et al* (1981, 1985) demonstrated that the topical application of lactate plasticized the SC in the same manner as water via a reduction of the interaction between the polar groups of the keratin chain. This suggests that the lactate in the SC influences the physical properties of the SC. The pH of the SC was found to influence the integrity and cohesion of the SC (Fluhr *et al*, 2001), and a more recent study suggested that products from the degradation of desmosomes might reside within the intercellular domains (Warner *et al*, 2003). According to findings on the SC by Behne *et al* (2002), the intercellular domains were more acidic than the intracellular domains. Lactate is generally secreted from the intracellular to intercellular domains in living cells. Based on the findings summarized above, we speculate that lactate may remain both in the interspaces and inner spaces of the corneocytes, in the former to regulate the SC pH and/or degradation of desmosomes, and in the latter to maintain the physical properties of the SC.

In our study, potassium lactate turned out to restore SC hydration more effectively than sodium lactate, suggesting that the potassium ion itself may play certain roles in maintaining the physical properties of the SC. The potassium ion is known to be structure-destructive, while the sodium ion is known to be structure-constructive (Henry *et al*, 1957; Manfred, 1957). Thus, the structure-destructive property of the potassium ion in the SC may influence the physical properties of the SC by breaking the hydrogen bond of water and/or keratin fiber.

In our study on healthy subjects, the amino acid content was significantly higher in winter than in summer and did not correlate with the physical properties of the SC. Horii *et al* (1989) previously demonstrated a correlation between amino acids and hydration, but their investigations focused on skins afflicted by either ichthyosis vulgaris or senile xerosis. Most data suggestive of important influences of amino acids on the physical properties of the SC were derived from quantitative studies of the SC of dry skin under conditions such as atopic xerosis or senile xerosis (Watanabe *et al*, 1991; Hara *et al*, 1993), or the SC of dry skin induced artificially (Koyama *et al*, 1984; Jokura *et al*, 1995). Amino acids may play more important roles in maintaining the homeostasis of the SC in diseased conditions than in healthy normal ones. Our findings illustrate the important roles of lactate and potassium in maintaining the physical properties of the SC in healthy subjects. Further studies will be needed to reveal how lactate and potassium contribute to the physical properties of the SC in dry skin conditions such as atopic xerosis and senile xerosis.

In a recent study using asebia mice, Fluhr *et al* (2003) reported that sebaceous-gland-derived glycerol may contribute to the SC hydration state. Yoneya *et al* (1979), on the other hand, demonstrated that the glycerol content on the surface of human forearm skin surface was 2.1 nmol per $cm^2$. Using the same method described by Yoneya, we confirmed that the levels of lactate and potassium of the forearm during the summer months were 209 and 136 nmol per $cm^2$, respectively. Thus, it seems that lactate and potassium may play a more significant role than glycerol in hydrating the SC of the healthy forearm. On the other hand, there are many components of NMF in the SC. It may be that the compositions of NMF vary from site to site in the body. More studies should be conducted in the future to examine how other NMF components such as urea and PCA contribute to SC hydration.

Our results confirmed that lactate and potassium levels were correlated with each other. Lactate is an end-product of glycolysis, and glycolysis is known to be the main energy (ATP) source in the epidermis (Cruickshank *et al*, 1957). On

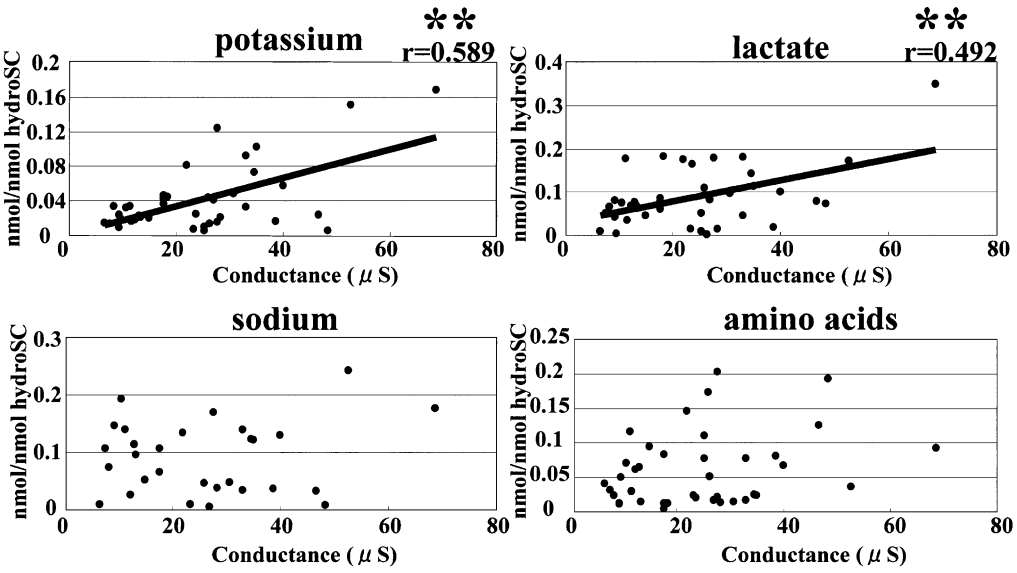


**Figure 4**
**Potassium and lactate levels both correlated with the SC hydration state in winter.** The SC hydration state was measured in winter, as described in the *Materials and Methods* section. Pieces of forearm SC were collected in winter by a tape-strip method. Each NMF component was extracted and measured by the method described in the *Materials and Methods* section. A probability of $p<0.05$ was considered significant: $^{**}p<0.01$; r = correlation coefficient.

the other hand, the intra- and intercellular gradients of sodium and potassium are known to be maintained by a Na/K pump, a mechanism that consumes most of the ATP produced in the animal cells. In fact, James *et al* (1996) have shown a reduction of lactate production by ouabain (an inhibitor of the Na/K pump) in skeletal muscle. In an *in vitro* study by Shen *et al* (2001), insulin stimulation induced keratinocyte proliferation, and this action was confirmed to involve the activation of the Na/K pump. Moreover, in a comparison between aged and young epidermis in human, the aged epidermis was found to have a markedly reduced potassium content together with decreased epidermal cell proliferation (Bunse *et al*, 1991). Though the relationship between epidermal cell proliferation and lactate remains obscure, lactate may be closely connected with potassium through the Na/K pump in the epidermis. The physical properties of the SC may be regulated not only by the lactate and potassium composition of the SC, but also by that of the epidermis under the SC and/or skin appendages.

In conclusion, this study is the first to suggest that lactate and potassium in the SC may play roles in maintaining the physical properties of the SC in healthy subjects.

## Materials and Methods

**Subjects** The experiments in this report were approved by the ethics committee at Kanebo, and informed written consent was obtained from each subject. A number of techniques were used to evaluate the physical properties of forearm skin in 40 healthy male subjects aged from 27 to 56 y (mean 38.7 y; eight in their 20s, 16 in their 30s, 14 in their 40s, and two in their 50s). The temperature during measurements was maintained at 22°C, and the relative humidity was maintained at 50%. The measurements were taken in July 2001 and January 2002 at the same location in Japan.

**Measurements of the hydration state, stiffness, and pH of the SC** The state of SC hydration was determined by measuring the high-frequency conductance of the SC using an impedance meter (Skicon-200, IBS, Hamamatsu, Japan). Results were obtained from an average of five measurements (Tagami *et al*, 1980).

In most cases, a Cutometer SEM (Courage + Khazaka, Cologne, Germany) was used to measure the elasticity of the skin, as this device has proven effective in revealing reductions in skin elasticity in association with age (Escoffier *et al*, 1989; Cua *et al*, 1990) and diabetes (Yoon *et al*, 2002). The reports suggest that the Cutometer is an effective method available for measuring the elasticity of the dermis.

The SC stiffness in this experiment was determined by measuring resonant frequency (Δf) values using a Venustron tactile sensor (Axiom, Fukushima, Japan). This device determines changes in the Δf that occur when a vibrating probe (resonant frequency: 50 Hz) comes into contact with an object. When the probe contacts softer objects, a lower Δf value is obtained. Lindahl *et al* (1998) found Δf suitable for evaluating skin stiffness, since Δf is correlated to spring constant k. More recently, Sakai *et al* (2000) reported that the Δf value obtained with the Venustron tactile sensor at a pressure of 2 g was significantly correlated with the SC hydration state. The Venustron tactile sensor, however, has been confirmed to measure the stiffness of not only the SC but also the dermis in the face (Sakai *et al,* 2000). Thus, we decided to restrict our evaluation solely to the forearms of healthy subjects after confirming that the viscoelastic/elastic ratio (Uv/Ue) and elasticity (Ur/Uf) measured by the Cutometer were both uncorrelated with Δf (data not shown). Measurements of the skin were performed three times at the same site, and Δf values (mean) at the pressure of 2 g were determined.

Two methods can be used to measure the pH of the SC. The first employs a flat glass electrode connected to a pH meter (Ohman *et al*, 1998; Krien *et al*, 2000), and the second is a method of fluorescence lifetime imaging microscopy (Behne *et al*, 2002, 2003). The former has the advantage of non-invasiveness, but it cannot detect the distribution of the SC pH gradient in good detail. The latter is invasive, but serves very well in detecting not only precise pH differences between the inter- and intra-corneocytes, but also the pH gradient from the surface SC to the deeper SC (Behne *et al*, 2002). In this experiment, we chose the non-invasive method. The measurements were performed using a flat glass electrode connected to a pH meter (Horiba, Kyoto, Japan), and all the results were obtained from one measurement.

**Extraction of NMF from the forearm** Open-ended, 3.6-cm-diameter cylinders filled with 5 mL of water were pressed onto the forearm with gentle pressure for 5 min. After air-drying the sites for 30 min, various techniques were used to evaluate the physical properties.

**Determination of inorganic ion levels of the SC and sweat** The ion levels in the SC were determined by stripping off sections of the

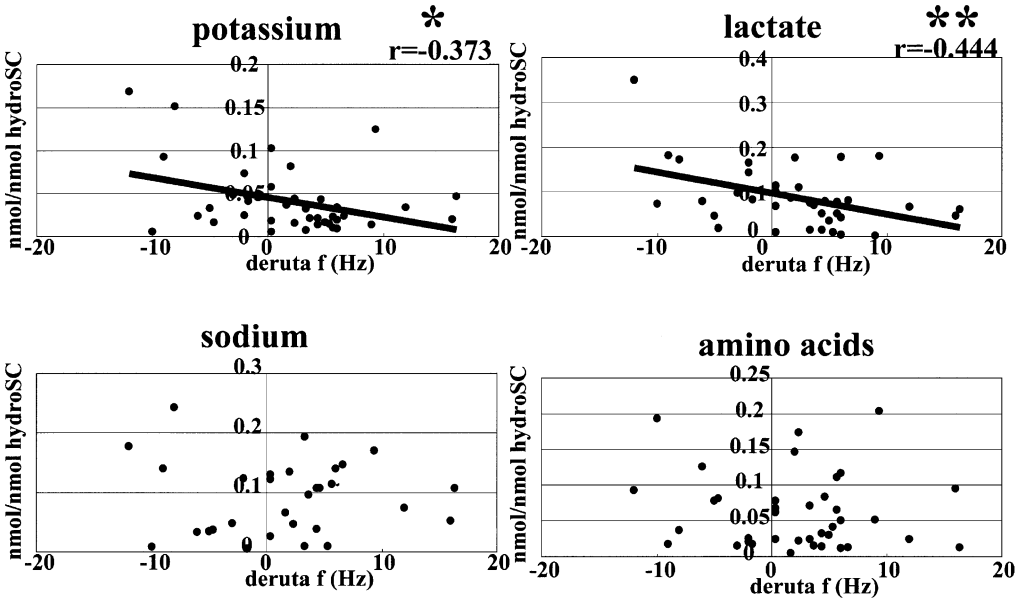


**Figure 5**
**Potassium and lactate levels both correlated with the SC stiffness in winter.** The SC stiffness was measured in winter as described in the *Materials and Methods* section. Pieces of forearm SC were collected in winter by a tape-strip method. Each NMF component was extracted and measured by the method described in the *Materials and Methods* section. A probability of $p<0.05$ was considered significant: $^{**}p<0.01$, $^{*}p<0.05$; r = correlation coefficient.

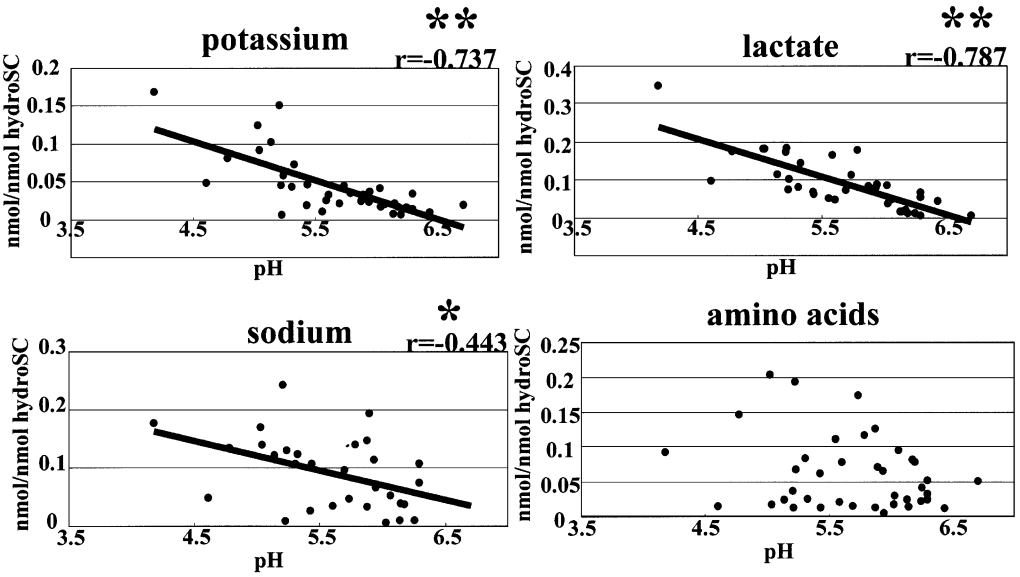


**Figure 6**
**Potassium and lactate levels both correlated with the SC pH in winter.** The SC pH was measured in winter as described in the *Materials and Methods* section. Pieces of forearm SC were collected in winter by a tape-strip method. Each NMF component was extracted and measured by the method described in the *Materials and Methods* section. A probability of $p<0.05$ was considered significant: $^{**}p<0.01$, $^{*}p<0.05$; r = correlation coefficient.

forearm SC with adhesive tape (one stripping by Celotape-405, Nichiban, Tokyo, Japan) and then extracting the NMF with water for 24 h. To quantify the ion levels in sweat, flowing sweat was collected from the faces of the subjects ($n=4$) after they had played sports for 15 min in the summer. The cation content was analyzed by an ion chromatography IC-8010 system (TOSOH, Tokyo, Japan) and a Shodex packed column HPLC YK-421 system (Showa Denko, Tokyo, Japan) using 3 mM phosphate buffer at 1 mL per min flow. The chloride content was analyzed by an ion chromatography IC-8010 system (TOSOH) and a Shodex packed column HPLC I-524A system (Showa Denko, Tokyo, Japan) using IC NY-5 solution (Showa Denko) at 1 mL per min flow.

**Determination of lactate content of the SC** NMF was extracted from tape-stripped sections of SC (one stripping by Celotape-405, Nichiban) with water. The lactate content was quantified with a commercial kit (F-kit L-lactic acid, J.K. International, Tokyo, Japan) using lactate dehydrogenase according to the manufacturer's protocol.

**Determination of amino acid content of the SC** NMF was extracted from tape-stripped sections of SC (one stripping by Celotape-405, Nichiban) with 10 mM HCl. The amino acid analyzer is a useful device, but it takes a great deal of time to quantify amino acid levels. As an alternative, we developed a faster method to quantify amino acids with *o*-phthalaldehyde (OPA), a compound generally used as a fluorescent probe for the amino acid analyzer. The amino acid content was measured using the OPA method. Briefly, 150 μL of fluoraldehyde OPA reagent solution (Pierce, Rockford, Illinois) was added to 15 μL of NMF fractions or the amino acid standard according to the microassay protocol attached to the solution. After 1 min, the fluorescent intensity was measured by a SPECTRA MAX GEMINI XS system (Molecular Devices, Sunnyvale, California) with excitation set at 365 nm and emission set at 450 nm. Amino acid standard H (Pierce) was used as the standard. We confirmed that the amino acid content determined by the OPA method correlated well with that determined by the amino acid analyzer ($r=0.996$, $y=1.0429x-94.872$).

**Determination of the amount of tape-stripped SC** The amount of amino acid within the SC was measured by the method described by Schreiner *et al* (2000). To quantify the total amount of tape-stripped SC, the samples stripped from the forearm were directly hydrolyzed in 6 M KOH for 24 h at 95°C. After cooling, the hydrolysate was neutralized with 6 M HCl, and the amount of total amino acid was measured by the OPA method described above.

**Table I. Correlation coefficients between NMF content and physical properties**

| | Winter | | | Summer | | |
|---|---|---|---|---|---|---|
| | Conductance | Δf | pH | Conductance | Δf | pH |
| K | 0.589 | −0.373 | −0.737 | 0.471 | −0.509 | −0.524 |
| | (p = 0.000) | (p = 0.021) | (p = 0.000) | (p = 0.002) | (p = 0.001) | (p = 0.001) |
| Lactate | 0.492 | −0.444 | −0.787 | 0.311 | −0.490 | −0.395 |
| | (p = 0.002) | (p = 0.006) | (p = 0.000) | (p = 0.051) | (p = 0.001) | (p = 0.012) |
| Na | 0.148 | −0.023 | −0.443 | 0.079 | −0.172 | −0.240 |
| | (p = 0.435) | (p = 0.906) | (p = 0.014) | (p = 0.628) | (p = 0.290) | (p = 0.136) |
| Mg | −0.145 | 0.171 | 0.095 | 0.348 | −0.412 | −0.160 |
| | (p = 0.379) | (p = 0.304) | (p = 0.566) | (p = 0.035) | (p = 0.011) | (p = 0.345) |
| Ca | −0.127 | 0.083 | 0.005 | 0.223 | −0.281 | −0.300 |
| | (p = 0.441) | (p = 0.621) | (p = 0.976) | (p = 0.167) | (p = 0.079) | (p = 0.056) |
| Cl | 0.268 | −0.116 | −0.552 | −0.024 | −0.147 | −0.374 |
| | (p = 0.114) | (p = 0.508) | (p = 0.000) | (p = 0.884) | (p = 0.365) | (p = 0.018) |
| AA | 0.288 | −0.108 | −0.266 | −0.044 | −0.107 | −0.137 |
| | (p = 0.075) | (p = 0.519) | (p = 0.101) | (p = 0.791) | (p = 0.516) | (p = 0.407) |

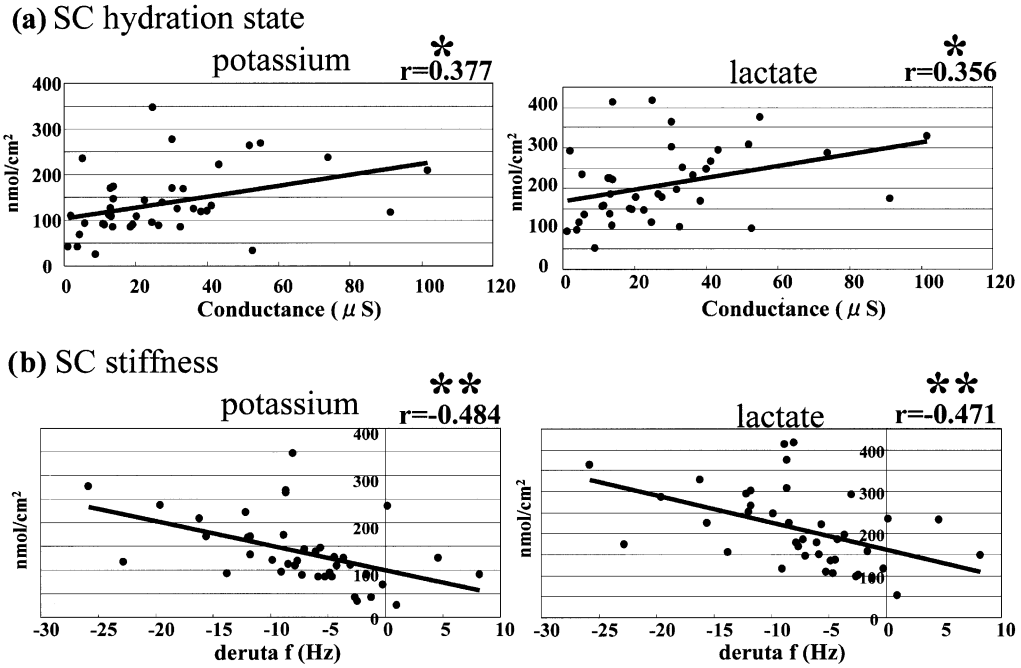


**Figure 7**
**Potassium and lactate levels directly extracted from forearm SC correlated with the changes in physical properties after NMF extraction.** NMF was directly extracted from the forearm SC by the method described in the *Materials and Methods* section. The SC hydration and stiffness were measured before and after NMF extraction with 30 min of subsequent air-drying, according to the method described in the *Materials and Methods* section. The levels of potassium and lactate in the extracted NMF were measured by the method described in the *Materials and Methods* section. Correlations were shown between differences of physical properties (*a*, SC hydration state; *b*, SC stiffness) before and after extraction, and the levels of potassium and lactate. A probability of $p < 0.05$ was considered significant: $^{**}p < 0.01$, $^{*}p < 0.05$; r = correlation coefficient.

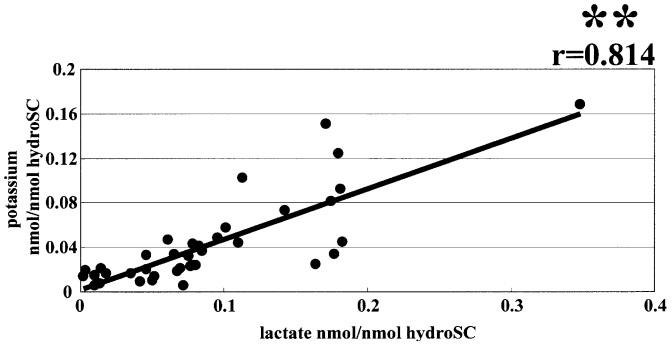


**Figure 8**
**Lactate and potassium levels were correlated with each other in winter.** Pieces of forearm SC were collected in winter by a tape-strip method. Each NMF component was extracted and measured by the method described in the *Materials and Methods* section. A probability of $p < 0.05$ was considered significant: $^{**}p < 0.01$; r = correlation coefficient.

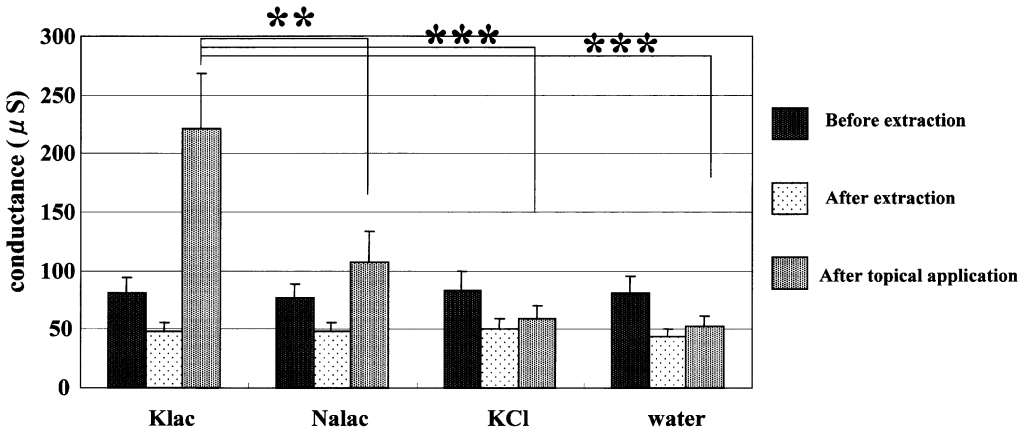


**Figure 9**
**Topical application of potassium lactate restored SC hydration after NMF extraction.** After extraction of NMF by water, each NMF component was applied to the SC by the method described in the *Materials and Methods* section. The SC hydration was measured by the method described in the *Materials and Methods* section. Values represent means with SEM from nine subjects per column. A probability of $p<0.05$ was considered significant: $^{***}p<0.001$, $^{**}p<0.01$. Klac, Nalac, and KCl represent potassium lactate, sodium lactate, and potassium chloride, respectively.

The amount of tape-stripped SC was represented as the total amount of amino acid of the hydrolyzed SC. All NMF quantities were normalized to nanomoles of the amount of tape-stripped SC (nmol NMF content per nmol hydroSC).

**Restoration of the SC hydration state with topical application of NMF components** Prior to water treatment, the SC hydration was measured at four sites of the forearm in nine healthy subjects using a Skicon-200 skin surface hygrometer. The measurements were taken after pressing an open-ended, 2-cm-diameter cylinder filled with 1.5 mL of water onto each site with gentle pressure for 5 min and then air-drying for 30 min. Next, the SC hydration measurements were repeated by the Skicon-200 after topically treating each site with 1 μL of each NMF component (500 mM potassium lactate, sodium lactate, potassium chloride in water, and water alone) per 1 $cm^2$ of skin (about 5-fold the level of NMF in the SC) and air-drying for 20 min.

**Statistics** Changes in the physical properties and NMF content were compared using the paired *t* test. All correlations were examined by Pearson's correlation coefficient analysis. The above analyses were performed using Microsoft Excel. The data in Fig 1 were compared by ANOVA and the data in Fig 9 were compared by ANOVA and the Tukey test, in both cases with the use of SAS software (SAS Institute Japan, Tokyo, Japan). A probability of $p<0.05$ was considered significant.

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