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Research article

Effects of High-Intensity Endurance Exercise on Epidermal Barriers against Microbial Invasion

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Abstract
For athletes, preventing infectious disease on skin is important. Examination measurement of epidermal barriers could provide valuable information on the risk of skin infections. The aim of this study was to determine the effects of high-intensity endurance exercise on epidermal barriers. Six healthy adult males (age; 22.3 ± 1.6 years) performed bicycle exercise at 75%HRmax for 60 min from 18:30 to 19:30. Skin surface samples were measured 18:30 (pre), 19:30 (post), 20:30 (60 min), and 21:30 (120 min). Secretory immunoglobulin A (SIgA) and human β-defensin 2 (HBD-2) concentrations were measured using an enzyme-linked immunosorbent assay (ELISA). SIgA concentration at pre was significantly higher than at post, 60 min and 120 min ($p < 0.05$). HBD-2 concentration at post and 120 min was significantly higher than at pre ($p < 0.05$). Moisture content of the stratum corneum was significantly higher at post than at pre, 60 min, and 120 min ($p < 0.05$). On the chest, moisture content of the stratum corneum was significantly lower at 120 min than at pre ($p < 0.05$). The number of staphylococci was significantly higher at post than at pre ($p < 0.05$), and tended to be higher at 60 min than at pre on the chest ($p = 0.08$). High-intensity endurance exercise might depress the immune barrier and physical barrier and enhance the risk of skin infection. On the other hand, the biochemical barrier increases after exercise, and our findings suggest that this barrier might supplement the compromised function of other skin barriers.

Key words: Staphylococcus, skin infections, athletes, secretory immunoglobulin a, beta-defensins.

Introduction

The peak condition of an athlete can be seriously hampered by injuries and infections that occur before a competitive event. Infections are particularly common among athletes because of chronic immunosuppression in response to intensive physical training and major competitions (Shephard et al., 1991). An athlete's skin is often macerated from sweating, a condition which provides a fertile breeding environment for microorganisms, while trauma inherent to sporting activities can damage the cutaneous barrier allowing microorganisms to infiltrate deeper layers (Adams, 2008). It is therefore possible that skin infections in susceptible athletes might negatively influence physical condition and performance.

Staphylococcal skin infections occur in various contact sports such as football, basketball, rugby, hockey, and wrestling (Adams, 2002). Staphylococci are classified as *Staphylococcus aureus* (*S. aureus*) or coagulase-negative *Staphylococcus*. In a study of 110 football players and 8 athlete trainers, 67 (57%) were positive for *S. aureus*, of which 8 (12%) developed staphylococcal skin infections (Fontanilla et al., 2010). Previous studies on football players (Bartlett et al., 1982) and basketball players (Sosin et al., 1989) have reported an increase in *S. aureus* on the skin after both training sessions and games. Skin to skin contact between athletes and contact with sports equipment was considered a major cause of increasing the spread of *S. aureus* (Pollard, 1966). *Staphylococcus epidermidis* (*S. epidermidis*) is the most frequently isolated bacterium of coagulase-negative *Staphylococcus* and normally inhabits the skin as non-pathogenic resident (Vuong and Otto, 2002). Moreover, *S. epidermidis* might have a beneficial role on the skin surface by producing its own antimicrobial peptides (Cogen et al., 2010) and enhancing the expression of human antimicrobial peptides from keratinocytes (Dinulos et al., 2003). On the other hand, *S. epidermidis* attaches more tightly to the surface of epithelial cells than *S. aureus* (Kume and Fukushima, 1994) and forms biofilms which is considered to be the main virulence factor (Raad et al., 1998). In clinical practice, *S. epidermidis* can cause skin infections, such as folliculitis and sycosis vulgaris, in compromised hosts and patients with the damaged cutaneous barrier (Katayama et al., 2009). Intensive exercise-induced immunosuppression might promote infectious skin disease, therefore, athletes are habitually at risk for skin infections, which are important causes of temporary disability (Pecci et al., 2009). Moreover, it is possible that skin infections negatively affect athlete performance. Therefore, countermeasures against infectious disease on the skin are significant for maintaining conditions in athletes.

The epidermis barrier functions as an immunological, biochemical, and physical barrier against pathogen invasion. Secretory immunoglobulin A (SIgA), one type of immune barrier, is found on the skin in sweat from eccrine glands (Okada et al., 1988). Mucosal SIgA works as the first line of defense for the human body against pathogenic microbial invasion (Lamm et al., 1995). Previous studies have shown that *S. aureus* opsonized with mucosal SIgA is significantly ingested by polymorphonuclear leucocytes (Gorter et al., 1987) and that the number of *S. aureus* is lower around sweat ducts (Goto et al., 1995). Therefore, it is possible that skin-SIgA works similarly as the first line of defense against pathogenic microbial invasion on the skin surface. A previous study of patients in Brazil with IgA deficiency reported that mucosal infections of the skin and the respiratory and

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gastrointestinal tracts occurred in 80 of 126 (63.5%) patients (Jacob et al., 2008). Therefore, skin-SIgA might be a useful indicator for estimating athlete skin condition. The previous study on salivary SIgA secretion has shown a transient decrease following high-intensity endurance exercise (Mackinnon et al., 1993). However, no study has investigated the effects of exercise on skin-SIgA.

Defensin, which is an antimicrobial peptide, provides a biochemical barrier and exhibits anti-pathogenic activity (Ganz, 1999). Defensin exerts an antimicrobial effect by breaking the hydrophobic core of the lipid bilayer on microbes (Oren et al., 1999; Schaller et al., 2000). Human β-defensin 2 (HBD-2) expression is induced by the stimulation of bacterial bodies such as staphylococci, *Candida*, and *Pseudomonas aeruginosa* in keratinocytes (Dinulos et al., 2003), and tumor necrosis factor (TNF)-α and interleukin (IL)-1 in keratinocytes (Liu et al., 2002) and respiratory epithelial cells (Harder et al., 2000). In a previous study on salivary HBD-2, high-intensity endurance exercise increased HBD-2 expression transiently (Usui et al., 2011).

The stratum corneum serves as a physical barrier against percutaneous penetration of chemicals and microbes (Madison, 2003). It is known that the moisture content of the stratum corneum is a useful measure of barrier function (Proksch et al., 2008). The previous study reported that the low water content of the stratum corneum in patients with atopic dermatitis is related to a clinical condition (Lodén et al., 1992).

We have previously investigated skin SIgA concentration and the number of staphylococci before and after exercise and reported a skin SIgA decrease and staphylococci increase (submitted). However, our previous study only determined the alteration of skin SIgA and staphylococci before and after exercise, and no study has investigated the time course of epidermal barriers after exercise. In this study, we examine skin SIgA, staphylococci, HBD-2, and moisture content of the stratum corneum after 60 min and 120 min of exercise. Elucidating this relationship will contribute to the development of conditioning programs which include prevention of skin infection for athletes.

The aim of this study therefore, was to determine the effect of high-intensity endurance exercise on epidermal barriers, primarily skin SIgA, HBD-2, and moisture content of the stratum corneum. We hypothesized that high-intensity exercise may decrease both skin SIgA secretion and moisture content of the stratum corneum while increasing skin HBD-2 expression.

Methods

Participants

Six healthy adult males (age, 22.3 ± 1.6 years; height, 1.77 ± 0.05 m; body mass, 69.4 ± 4.9 kg; body fat percentage, 17.4 ± 4.8%; and body mass index, 22.3 ± 2.0 $kg \cdot m^{-2}$) participated in the study. Participants were given a detailed explanation of risks, stress, and the potential benefits of the study before they signed an informed consent form. All participants had passed a complete medical examination within the preceding year. No participants had been treated with any drugs that are known to affect immune function. No participants reported allergies such as atopic dermatitis or recent infections (prior 3 months) in the determination. The study was approved by the Ethics Committee on Human Research of Waseda University. Experiments were conducted in accordance with the Declaration of Helsinki.

Protocol

Participants performed a bicycle-ergometer exercise test for 60 min from 18:30 to 19:30 in a laboratory at Waseda University. They then rested for the remainder of the assessment. Measurements were carried out in a climate-controlled room at 25 °C and 35% relative humidity at 18:30 (Before exercise; pre), 19:30 (After exercise; post), 20:30 (After 60 min of exercise; 60 min), and 21:30 (After 120 min of exercise; 120 min).

Exercise

Previously, power at 75%HR_{max} (PWC75%HR_{max}) was determined using a bicycle ergometer (75XLIII; Combi Wellness, Tokyo, Japan). After resting for 60 s, loading motion was progressively increased by 15 $W \cdot min^{-1}$ until reaching 75%HR_{max}. During exercise, the pedalling rate was maintained at 50 rpm. From 18:30 to 19:30 on the measurement day, participants pedalled a bike for 1 min at 50%HR_{max} and for 59 min at 75%HR_{max} in the climate-controlled room. We asked participants not to touch the ergometer with their forearms or to wipe sweat from their chest or forearms with a towel during exercise.

Skin surface SIgA and HBD-2 collection

Skin surface samples were collected from the middle chest (Ch) and the medial side of the forearm (Fa). First, the open end of a polypropylene tube (Centrifuge Tubes; AGC Techno Glass, Chiba) was placed on the skin surface. Second, 1 mL of extraction liquid was poured into the tube. Finally, the liquid was stirred at 9,000 rpm with a microtube homogenizer (23M; As One, Osaka, Japan) for 60 s and collected (Figure 1a). Collection sites were Ch1 and Fa1 at pre, Ch2 and Fa2 at post, Ch3 and Fa3 at 60 min, and Ch4 and Fa4 at 120 min (Figure 1b). The extraction liquids were prepared from 1 mM ethylenediaminetetraacetic acid disodium salt dehydrate (Sigma, St Louis, MO), phosphate buffered saline containing 1 % bovine serum albumin (Sigma, St Louis, MO), and 0.05% Tween 20 (Promega, Madison, WI). Skin surface samples were frozen at −50 °C and stored until the end of the study.

SIgA determination

Skin surface SIgA concentrations were measured using an enzyme-linked immunosorbent assay (ELISA). Briefly, a 96-well microtitre plate (Immulon II; Dynex Technologies, Chantilly, VI) was coated at 4 °C with 100 µL of anti-secretory component Ab-2 mouse monoclonal antibody (Thermo Fisher Scientific, Fremont, CA) which was diluted (1:500) with coating buffer containing sodium carbonate (Kanto Chemical, Tokyo, Japan) and sodium hydrogen carbonate (Kanto Chemical, Tokyo, Japan). After the addition of 250 µL of phosphate buffered saline

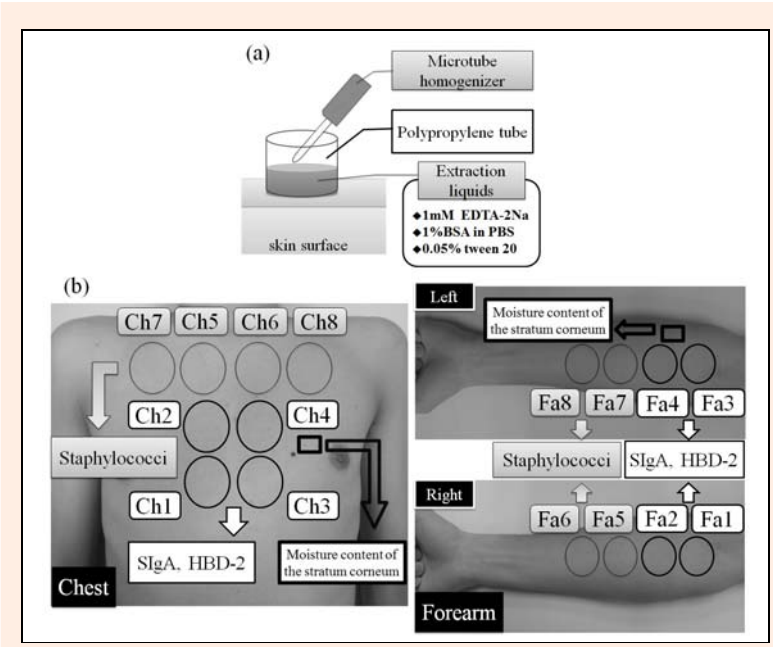

Figure 1. Skin surface SIgA and HBD-2 collection (a). First, the open end of a polypropylene tube was placed on the skin surface. Second, 1 mL of extraction liquid was poured into the tube. Finally, the liquid was stirred at 9,000 rpm with a microtube homogenizer for 60 s and collected. Skin-SIgA, HBD-2, staphylococci and moisture content of the stratum corneum measurement sites (b). Skin-SIgA and HBD-2 were collected from Ch1 and Fa1 at 18:30 (Before exercise; pre), Ch2 and Fa2 at 19:30 (After exercise; post), Ch3 and Fa3 at 20:30 (After 60 min of exercise; 60 min), and Ch4 and Fa4 at 21:30 (After 120 min of exercise; 120 min). Staphylococci were harvested from Ch5 and Fa5 at 18:30 (pre), Ch6 and Fa6 at 19:30 (post), Ch7 and Fa7 at 20:30 (60 min), and Ch8 and Fa8 at 21:30 (120 min).

containing 1% bovine serum albumin, wells were blocked for 2 h. Skin surface samples were thawed, centrifuged at 5,000 rpm for 10 min, and 100 µL of each was added and the mixture was incubated for 1 h. Using purified human SIgA (MP Biomedicals, Illkirch, France), known concentrations of SIgA were plated to establish standard values. After the plate had been washed with phosphate buffered saline/Tween, 100 µL of biotinylated anti-human IgA (α-chain specific; Vector Laboratories, Burlingame, CA), which was diluted (1:250) with phosphate buffered saline containing 1% bovine serum albumin, was added to the plate and incubated for 1 h. After washing, 100 µL of high sensitivity streptavidin HRP (Thermo Fisher Scientific, Fremont, CA), which was diluted (1:500) with phosphate buffered saline containing 1% bovine serum albumin, was added to the plate and the mixture was incubated for 1 h. After washing, 100 µL of substrate solution containing citric acid monohydrate (Kanto Chemical, Tokyo, Japan), disodium hydrogenphosphate 12-water (Kanto Chemical, Tokyo, Japan), hydrogen peroxide (Kanto Chemical, Tokyo, Japan), and o-phenylenediamine dihydrochloride (Wako Pure Chemical Industries, Osaka, Japan) was added. After 10 min, 50 µL of sulfuric acid (Kanto Chemical, Tokyo, Japan) was added to stop the chromogenic reaction and the color intensity was measured by a microplate reader (MTP-800; Corona Electric, Hitachinaka, Japan) at 492 nm. All samples were assayed in duplicate, and the average absorbance value was used as the representative value. Regression analysis using the relation of standard SIgA concentrations and absorbance (nm) was used to interpolate the concentration of SIgA in the samples.

HBD-2 determination

HBD-2 concentration on the skin surface was measured using an ELISA kit (Phoenix Pharmaceuticals, Burlingame, CA). A 96-well microtitre plate was washed with 300 µL of assay buffer and blocked for 5 min. Skin surface samples were thawed and centrifuged at 5,000 rpm for 10 min, and 100 µL of each sample, which was diluted (1:11) with assay buffer, was added to the mixture which was then incubated for 2 h. Using HBD-2 standard solutions, known concentrations of HBD-2 were plated to establish standard values. After the plate had been washed with assay buffer, biotinylated anti-human HBD-2 detection antibody, which was diluted (1:150) with assay buffer, was added to the plate and incubated for 2 h. After washing, streptavidin-horseradish peroxidase (SA-HRP) solution, which was diluted (1:2000) with assay buffer, was added to the plate and the mixture was incubated for 2 h. After washing again, substrate solution (TMB) was added, and the color intensity produced after 10 min was measured using a microplate reader (MTP-800; Corona Electric, Hitachinaka, Japan) at 450 nm. All samples were assayed in duplicate, and average absorbance was used as the representative value. Regression analysis using the relation of standard HBD-2 concentration to absorbance (nm) was used to interpolate the concentration of HBD-2 in the samples.

Moisture content of the stratum corneum

Moisture content of the stratum corneum was measured by pressing a moisture checker (MY-808S; SCALAR, Tokyo, Japan) against the skin surface on the middle chest and the medial side of the forearm (Figure 1b). Measure-

ments were carried out in a climate-controlled room at 25 °C and 35% relative humidity at pre, post, 60 min, and 120 min. Moisture content of the stratum corneum was measured three times at each time point, and the mean value was estimated (Lee et al., 2006).

Staphylococci

Staphylococci were harvested by pressing agar-based media composed of tellurite-glycine-salt-egg yolk (Food Stamp; Nissui Pharmaceutical, Tokyo, Japan) against the skin surface on the middle chest and the medial side of the forearm. Collection sites were Ch5 and Fa5 at pre, Ch6 and Fa6 at post, Ch7 and Fa7 at 60 min, and Ch8 and Fa8 at 120 min (Figure 1b). Samples were cultured in a CO_2 incubator (B111-9; Sanyo Electric, Osaka, Japan) at 37 °C for three days. We counted black colonies surrounding the media which were clouds of *S. aureus* and coagulase-negative *Staphylococcus* sp. In this study, we measured only a small amount of *S. aureus*; therefore, the total number was expressed as number of staphylococci colony forming units (CFU).

Body water loss rate

Body water loss rate was calculated by dividing the amount of body water loss by time. The amount of body water loss was obtained by adding weight loss and water intake (Moriya and Ohira, 2009). After micturition, subjects were weighed in their underwear (DP-7100PW; Yamato Scale, Hyogo, Japan). Participants were free to drink mineral water during exercises and while at rest. Water intake was assessed by measuring the change in mass of mineral water by using a digital cooking scale (KD-321; TANITA, Tokyo, Japan).

Data analysis and statistics

Descriptive data are presented as mean ± SD values. The Friedman test was used to assess differences throughout the day. Post hoc analysis (Wilcoxon's signed rank sum test) was used to compare specific differences when significance was found. For all analyses, $p < 0.05$ was set for statistical significance.

Results

SIgA concentration on the middle chest was 8.24 ± 4.23 (pre), 2.97 ± 2.23 (post), 3.01 ± 2.23 (60 min), and 3.45 ± 2.78 ng·mL^{-1} (120 min). On the chest, SIgA concentration at pre was significantly higher than at post (60 min) and 120 min (Figure 2a; $p < 0.05$). SIgA concentration on the medial side of the forearm was 6.88 ± 4.21 (pre), 4.42 ± 2.50 (post), 2.55 ± 1.52 (60 min), and 4.25 ± 2.59 ng·mL^{-1} (120 min). On the forearm, SIgA concentration was significantly higher at pre than at 60 min and 120 min ($p < 0.05$) and was significantly lower at 120 min than at 60 min (Figure 2b; $p < 0.05$).

HBD-2 concentration on the middle chest was 0.56 ± 0.25 (pre), 1.25 ± 0.72 (post), 0.84 ± 1.07 (60 min), and 0.94 ± 0.32 ng·mL^{-1} (120 min). On the chest, HBD-2 concentration at post and 120 min was significantly higher than at pre (Figure 2c; $p < 0.05$). HBD-2 concentration on the medial side of the forearm was 0.22 ± 0.08 (pre), 0.36 ± 0.16 (post), 0.18 ± 0.14 (60 min), and 0.23 ± 0.08 ng·mL^{-1} (120 min). On the forearm, HBD-2 concentration at post was significantly higher than at pre, 60 min, and 120 min (Figure 2d; $p < 0.05$).

Moisture content of the stratum corneum on the middle chest was 34.48 ± 2.47 (pre), 40.20 ± 5.33 (post), 33.65 ± 3.04 (60 min), and 32.97 ± 2.61% (120 min). On the chest, moisture content of the stratum corneum was significantly higher at post than at pre, 60 min, and 120

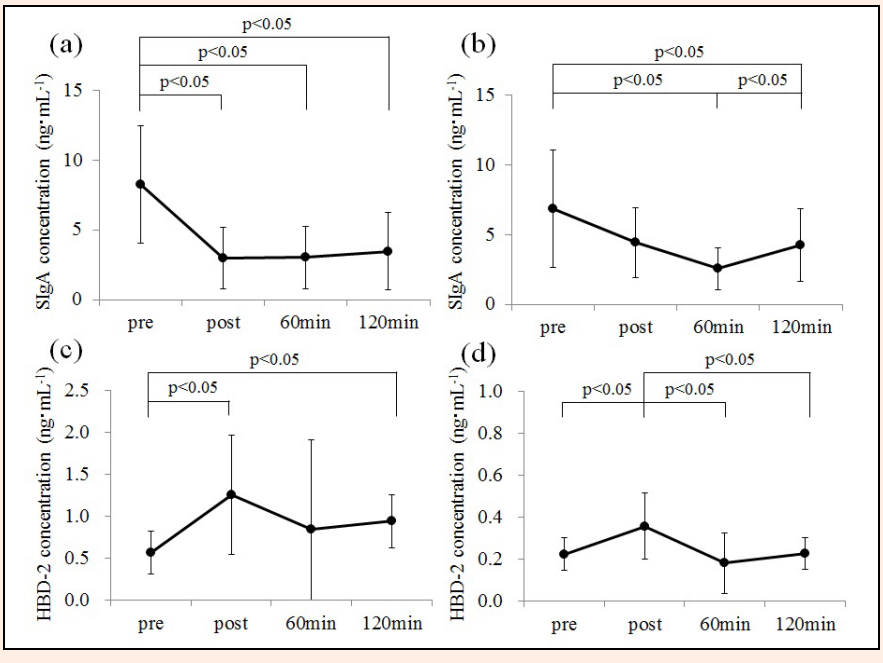

Figure 2. Skin-SIgA concentration on the chest (a) and forearm (b), and HBD-2 concentration on the chest (c) and forearm (d). Values are expressed as means ± SD. pre = Before exercise (18:30), post = After exercise (19:30), 60 min = After 60 min of exercise (20:30), 120 min = After 120 min of exercise (21:30).

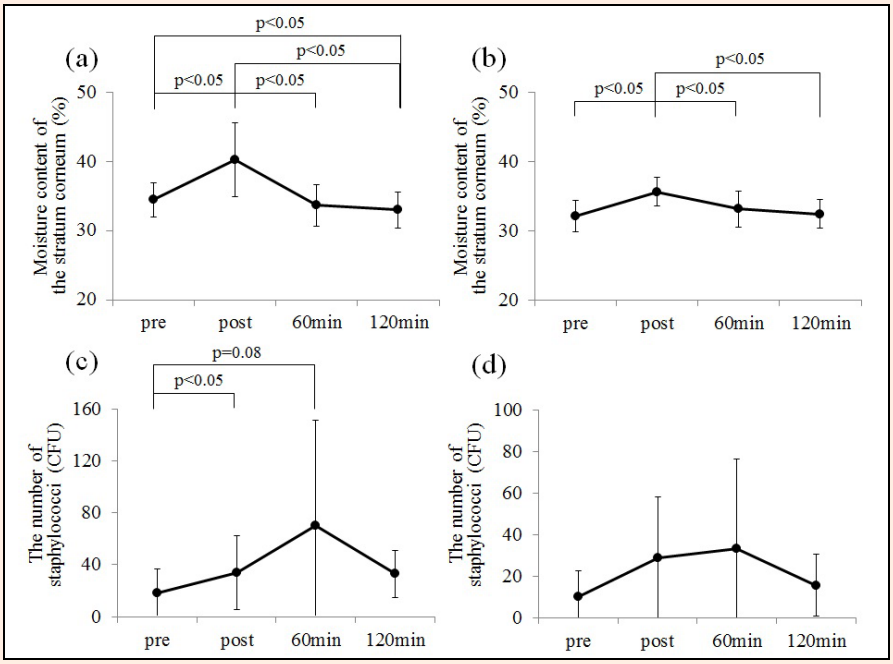

Figure 3. Moisture content of the stratum corneum on the chest (a) and forearm (b), and the number of staphylococci on the chest (c) and forearm (d). Values are expressed as means ± SD. pre = Before exercise (18:30), post = After exercise (19:30), 60 min = After 60 min of exercise (20:30), 120 min = After 120 min of exercise (21:30).

min ($p < 0.05$) and was significantly lower at 120 min than at pre (Figure 3a; $p < 0.05$). Moisture content of the stratum corneum on the medial side of the forearm was 32.11 ± 2.23 (pre), 35.62 ± 2.12 (post), 33.13 ± 2.59 (60 min), and 32.42 ± 2.11% (120 min). On the forearm, moisture content of the stratum corneum at post was significantly higher than at pre, 60 min, and 120 min (Figure 3b; $p < 0.05$).

The number of staphylococci on the middle chest was 18.33 ± 18.71 (pre), 33.83 ± 28.26 (post), 70.50 ± 80.66 (60 min), and 32.83 ± 18.37 CFU (120 min). On the chest, the number of staphylococci was significantly higher at post than at pre ($p < 0.05$), and tended to be higher at 60 min than at pre (Figure 3c; $p = 0.08$). The number of staphylococci on the medial side of the forearm was 10.00 ± 12.52 (pre), 28.67 ± 29.53 (post), 33.17 ± 43.00 (60 min), and 15.50 ± 14.98 CFU (120 min). The number of staphylococci showed no significant difference on the forearm (Figure 3d).

Body water loss rate was 1096.67 ± 218.26 (pre-post), 143.27 ± 75.16 (post-60 min), and 71.33 ± 53.66 $g \cdot h^{-1}$ (60-120 min). Body water loss rate between pre and post was significantly higher than between post and 60 min and between 60 min and 120 min (Figure 4; $p < 0.05$).

Discussion

The major finding of this study was that the bicycle-ergometer exercise test at 75%HR_{max} for 60 min was associated with a significant decrease in SIgA concentration and an increase in HBD-2 concentration, moisture content of the stratum corneum, and number of staphylococci on the skin surface. These results suggested that high-intensity endurance exercise might depress immunological and physical barrier and enhance biochemical barrier.

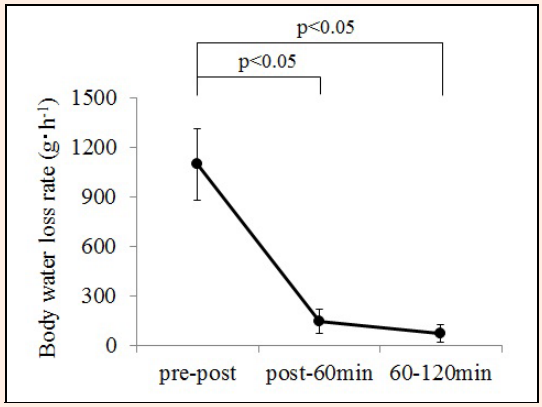

Figure 4. Body water loss rate. Values are expressed as means ± SD. pre-post = Before exercise (18:30) - After exercise (19:30), post-60 min = After exercise (19:30) - After 60 min of exercise (20:30), 60 min-120 min = After 60 min of exercise (20:30) - After 120 min of exercise (21:30).

SIgA plays an important role in local immunity on the skin surface and mucosa by inactivating and agglutinating pathogenic microorganisms and by inhibiting their attachment to a mucosal surface (Kobayashi, 1986). SIgA exists in sweat secreted by eccrine glands and plays a crucial role in cutaneous immunity (Okada et al., 1988). A previous study showed that skin-SIgA concentration in atopic dermatitis patients was lower than that in healthy people (Imayama et al., 1994), and that *S. aureus* habitually colonizes the skin surface of atopic dermatitis patients (Hauser et al., 1985). In this study, SIgA concentration on the middle chest was significantly lower after

exercise (post), after 60 min of exercise (60 min), and after 120 min of exercise (120 min) than that before exercise (pre), while that on the medial side of the forearm was significantly lower after 60 min of exercise (60 min) and after 120 min of exercise (120 min) than before exercise (pre). The collection of skin surface samples was carried out by stirring the liquid on the skin surface, so there was possible that SIgA concentration at the same site reduced after sampling. Therefore, we used the contiguous sites for sampling and there was no possibility of SIgA decreases by sampling. Participants carried out high-intensity endurance exercise for 60 min between pre and post, and body water loss rate between pre and post was significantly higher than between post and 60 min and between 60 min and 120 min. It is therefore possible that SIgA on the skin surface is flushed out by sweating. Moreover, high-intensity endurance exercise has the potential to reduce SIgA secretion on the skin surface. With regard to salivary SIgA, high-intensity endurance exercise reduces salivary SIgA secretion (Mackinnon et al., 1993). IgA is produced by plasma cells in the lamina propria mucosa. SIgA is secreted by epithelial cells and constitutes dimeric IgA bound to polymeric immunoglobulin receptor (pIgR) (Mostov et al., 1984). It has been reported that exhaustive exercise decreases submandibular pIgR expression and salivary SIgA concentration in rats (Kimura et al., 2008). On the skin surface, SIgA is secreted by mucous cells of eccrine sweat glands via the same secretory mechanism as that mediating salivary SIgA (Goto et al., 1995; Okada et al., 1988). Therefore, high-intensity exercise might decrease SIgA secretion on the skin surface, leading to susceptibility to skin infection.

Defensin has a broad antibacterial spectrum of activity against microorganisms such as bacteria, fungi, and viruses, and plays an important role in the innate immune system (Ganz, 1999). HBD-2 is produced by epidermal keratinocytes on the skin surface (Ganz, 1999). In the present study, HBD-2 concentration on the chest was significantly higher after exercise (post) than that before exercise (pre), and HBD-2 concentration on the forearm was significantly higher after exercise (post) than that before exercise (pre), after 60 min of exercise (60 min), and after 120 min of exercise (120 min). Usui et al. (2011) reported that endurance exercise at 75%VO_{2max} for 60 min increases HBD-2 expression transiently. In other previous studies, TNF-α and IL-1β induced expression of HBD-2 (Harder et al., 2000), and production of these cytokines was promoted by high-intensity exercise (Ostrowski et al., 1999). Therefore, it is thought that the HBD-2 increase after exercise in this study was induced by TNF-α and IL-1β. Moreover, on the chest, HBD-2 concentration after 120 min of exercise (120 min) was significantly higher than that before exercise (pre), and the number of staphylococci after 60 min of exercise (60 min) tended to be significantly higher than that before exercise (pre). Previous studies have reported that staphylococci might have a beneficial role on the skin surface by producing phenol-soluble modulins (Cogen et al., 2010) and enhancing the expression of HBD-2 from keratinocytes (Dinulos et al., 2003). It is therefore possible that the HBD-2 concentration increase at 120 min is the result of HBD-2 expression induced by the stimulation of staphylococci. However, in this study, there was no significant correlation between increases in staphylococci and HBD-2 because of a small number of participants. Further examination needs to increase participants and measure HBD-2 in the absence of staphylococci and estimate the relationship between staphylococci and HBD-2 after exercise.

The low water content in the stratum corneum in patients with atopic dermatitis is related to a clinical condition (Lodén et al., 1992). Moisture content of the stratum corneum is a useful measure of the physical barrier function of the skin surface (Sumikawa et al., 2007). Here, moisture content of the stratum corneum was significantly higher after exercise (post) than that before exercise (pre), after 60 min of exercise (60 min), and after 120 min of exercise (120 min) on the chest and forearm. Body water loss rate increased significantly during exercise due to sweating. Moisture content of the stratum corneum is regulated by lower water content near the skin surface (Warner et al., 1988), which inhibits the colonization of microorganisms (Aly et al., 1978). It is known that colonization of *S. aureus* and *Streptococcus* increases in response to increased moisture content of the stratum corneum, and in clinical practice, skin with too much water is susceptible to bacterial infection (Bibel et al., 1989). In the present study, the moisture content of the stratum corneum on the chest was significantly lower after 120 min of exercise (120 min) than that before exercise (pre). Shiohara (2009) reported that moisture content of the stratum corneum was lower after showering than before showering, suggesting that the stratum corneum prominently swells on exposure to water during showering, causing the outflow of ceramide and intercellular lipids such as natural moisturizing factor, which is hydrosoluble. Therefore, it is possible that the decrease in moisture content of the stratum corneum after 120 min of exercise (120 min) was caused by swelling. Moisture content of the stratum corneum is associated with atopic dermatitis and dry skin (Sumikawa et al., 2007), and we suggested that the lower water content of the stratum corneum enhanced the risk of skin infection. From a practical point of view, athletes should use moisturizers and maintain their skin surface in good condition (Tabata et al., 2000).

Skin infection by *S. aureus* is a serious problem for athletes and has an adverse effect on athlete condition and performance (Pecci et al., 2009). In this study, we collected *S. aureus* and coagulase-negative *Staphylococcus* sp. from the skin surface, and we determined total *Staphylococcus* number. On the middle chest, the number of staphylococci after exercise (post) was significantly higher than that before exercise (pre), and that after 60 min of exercise (60 min) also tended to be higher than that before exercise (pre). Staphylococci are resident bacteria on the skin surface that might exit the skin during exercise through sweat pores. Yamada et al. (1991) suggested that washing with a skin brush might scratch the skin surface and promote the appearance of staphylococci from sebaceous glands. Additionally, staphylococci multiply mitotically once every 28.8 min at 37 °C (Anthony

et al., 2010). Therefore, our findings suggest that sweating during exercise promotes the appearance of staphylococci and their subsequent increase on the skin surface. From a practical point of view, athletes should take a shower immediately after sports activities (Yamaguchi, 2007).

Conclusion

High-intensity endurance exercise might depress the immune barrier and physical barrier and enhance the risk of skin infection. On the other hand, the biochemical barrier increases after exercise. Our findings suggest that the biochemical barrier might be enhanced by the stimulation of staphylococci on the skin surface and supplement the compromised function of other skin barriers. From a practical point of view, we recommend that athletes maintain their skin surface in good condition, for example, by showering immediately after sports activities and using moisturizers.

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Key points

- The immune barrier and physical barrier might be depressed and the risk of skin infection might be enhanced by high-intensity endurance exercise.
- The biochemical barrier increases after high-intensity endurance exercise and might supplement the compromised function of other skin barriers.
- We recommend that athletes maintain their skin surface in good condition, for example, by showering immediately after sports activities and using moisturizers.

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