Irritant contact dermatitis

A strategy for prevention in Dutch health care workers

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Irritant Contact Dermatitis
A strategy for prevention in Dutch healthcare workers

Maryam Soltanipoor
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Colofon

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Irritant Contact Dermatitis
A strategy for prevention in Dutch Healthcare workers

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# Table of Contents

Chapter 1  General Introduction  7

## PART I  Intervention study in healthcare workers

Chapter 2A  The Effectiveness of a skin care program for the prevention of contact dermatitis in healthcare workers (the Healthy Hands Project): study protocol for a cluster randomized controlled trial  37

Chapter 2B  Statistical analysis plan for the Healthy Hands Project; single centre cluster randomized clinical trial of a skin care program for the prevention of contact dermatitis in health care workers  53

Chapter 3  Effectiveness of a skin care program for the prevention of contact dermatitis in healthcare workers (the Healthy Hands Project): a single centre cluster randomized controlled trial  67

Chapter 4  Evaluating the effect of electronic monitoring and feedback on the hand cream use in health care workers: Healthy Hands Project  89

## PART II  Experimental studies on the *in vivo* effects of skin irritants on the skin barrier and inflammatory response

Chapter 5  Specific barrier response profiles after experimentally-induced skin irritation *in vivo*  113

Chapter 6  Barrier damaging effects of n-propanol in occlusion-modified tandem repeated irritation test: modulation by exposure factors and atopic skin disease  131

Chapter 7  GENERAL DISCUSSION  151

Chapter 8  SUMMARY / SAMENVATTING  171

ADDENDUM  187
General Introduction
(Occupational) Contact Dermatitis

In the workplace skin is often exposed to various irritants and contact allergens that can result in development of contact dermatitis (CD). CD, also referred to as contact eczema, is an inflammatory skin disease that accounts for almost all occupational skin diseases (OSD) and is one of the most common registered work-related disorders. (1, 2) OSD are recognized as a significant public health problem (European Agency for Safety and Health at Work, EU-OSHA, EU-25 report, 2008), representing up to 40% of all reported occupational diseases (3, 4). CD is characterized by acute symptoms like erythema and vesicles and in the chronic form by xerosis, lichenification and fissures. (4, 5) The morphological variety is wide and largely non-specific. CD can roughly be classified in irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). (6) Although ICD and ACD have similar clinical features and morphology, their pathophysiology is different. (Fig. 1) ACD is a delayed-type IV reaction mediated by T cells, while ICD does not involve the adaptive immune system. (7) In the workplace, ICD is more prevalent and is responsible for 50 to 80% of all occupational CD cases. (8) The most common causes of ICD are detergents, organic solvents, acids and alkalis, metal working fluids, water and disinfectants. (8, 9) Next to chemical irritants, ICD can be caused by damage of the skin barrier due to physical and mechanical factors and climate conditions (dry air and low temperature). (10) High risk occupations for ICD include hairdressing, health care, food handling, construction industry, cleaning and the metal industry. (9)

In the health sector, the hands are the most affected part of the body. The 1-year prevalence of hand dermatitis (HD) among health care workers (HCW) in the Netherlands is reported by van der Meer, et al., (2013) to be 12%, although in the same study, a higher prevalence of symptoms related to HD was reported (47%). (11) In a prospective cohort study among apprentice nurses of Visser et al., (2014) a one-year prevalence was reported to be 21% in the first year, 25% in the second year and 31% in the third year of the traineeship. (12) Hand dermatitis was here defined as the presence of at least fissures and redness, fissures and itch, fissures and scaling or vesicles, or papules, with a duration of > 3 days or recurrence. (12) Compared to other countries, the estimated prevalence in the Netherlands seems low, however the prevalence rates of occupational HD vary across studies. (13-17) Due to difficulties in diagnosis but also the fact that many workers experience their skin problems as a ‘part of work’, figures on HD are underreported and likely do not represent the true prevalence. (4)
The main risk factors in the health care sector include hand washing with soap, disinfection and use of disposable gloves referred to as “wet work” activities (12, 18, 19). Due to more strict infection control programs and hand hygiene campaigns (e.g. the ‘World Health Organization (WHO), SAVE LIVES, Clean Your Hands Campaign (20), ‘wet work’ load in health care sector is increasing and leads to an increase in chronic ICD in HCW.(21, 22) When disease becomes chronic, this can lead to high rates of disability, sick leave, impaired quality of life and unemployment.(23, 24) The economic burden of OSD in the EU exceeds €5 billion spent on treatment, compensation, and loss of productivity every year.(25)

**Etiology**

ICD develops primarily upon exposure to chemical or physical irritants, which damage the skin barrier.(26, 27) Changes in the skin barrier itself may lead to the release of a preformed pool of IL-1α in the stratum corneum (SC), triggering release of other pro-inflammatory cytokines and eventually leading to inflammation of the skin.(26, 27) Some irritants like soaps can also penetrate through the SC into viable epidermis and exert toxic effects on the keratinocytes itself and initiate this inflammatory cascade as well.(28-30) (Fig.1A) ICD-associated inflammatory processes occur only within the exposure site and are manifested as topical red patches on the skin with desquamation and itching. (29, 31)
Although skin barrier damage directly resulting from contact with irritants are a prerequisite for development of ICD, the risk may be modified by intrinsic factors, such as atopic dermatitis (AD) or presence of loss-of-function mutations in the filagrin gene (FLG). (33-35) Epidemiologic studies reported up to three-fold higher risk for developing occupational ICD in individuals with a history of AD. (36, 37) Individuals with both FLG mutations and AD have more than a five-fold increased risk for ICD, while FLG mutations in absence of AD seem to play a minor role. (36) The exact mechanisms by which AD and FLG mutations predispose for ICD have not been fully clarified. An impaired skin barrier associated with AD or filagrin deficiency can at least partly be responsible, as it will facilitate ingress of skin irritants. Evidence that AD is associated with an impaired skin barrier has been accumulating in the last years, in particular after identification of FLG loss of function mutations as a strong risk factor for AD. (38) Filagrin is a multifunctional protein and contributes to skin hydration, mechanical strength of the skin and inflammatory status. (39, 40) In addition to AD, female gender is also suggested as a susceptibility factor, likely caused by more frequent domestic exposure. (41) Furthermore, although some studies have reported a positive relation between HD and smoking (42-45), a recent review
concluded that no definitive statement on the relationship between hand eczema and smoking can be made.(46-48)

**Diagnostics of ICD and severity assessment**

The diagnostics of HD is based on etiology when possible, and morphology when necessary. (49, 50) It remains a constant challenge to distinguish the etiologically different subtypes, as ICD, ACD and AD share morphological characteristics.(51-53) Classification of HD is further complicated by the fact that morphology may change significantly over time, and in many patients HD has more than one cause.(49, 52-54) Diagnostics of ICD is based on exclusion of ACD by patch testing and exposure assessment, both occupational and recreational (including smoking, medication and use of harmful substances). In the workplace, however, skin is exposed to both irritants and allergens. A detailed anamnesis, including history of previous or current AD may be helpful with regard to atopic hand dermatitis (AHD). There is still a group of HD cases that cannot be explained from exposures to irritants and allergens or from previous or contemporary skin disease involving the skin barrier function. Often, in HD cases where no etiology could be identified, HD is classified according to morphology.(52, 53) Adequate and early diagnosis of HD is after all, crucial for appropriate prevention and management.

In the clinical practice, there are several classification and scoring systems developed for the assessment of the disease severity of HD, mostly including both morphological and etiological parameters.(55) The most used are the “Hand eczema severity index score” (HECSI) (56-59), the Osnabrueck Severity index (OHSI)(60, 61), and the photographic guide.(62)

The HECSI was developed specifically for contact dermatitis of the hands. The HECSI scores the severity of six morphological symptoms (scale 0-3), that is erythema, vesicles, fissures, scaling, infiltration and oedema in combination with the area affected (scale 0-4) per location of the hand (fingertips, fingers, palms, back of hands, wrists). The total score is calculated by multiplying severity with extent, and ranges from 0 (no eczema) to 360 (extremely severe eczema). Validation by experts demonstrated excellent agreement for both inter- and intra-observer reliability.(57)

With the OHSI-score clinical severity is assessed on the basis of the extension or clinical characteristics of six morphological characteristics (erythema, scaling, papules, vesicles, infiltration and fissures).(60, 61) The OHSI provides
relatively fast and easy evaluation of HD by occupational health professionals. The inter-observer reliability showed to be good. Comparative studies showed a strong correlation (r =0.84, P <0.001) between OHSI and HECSI.(63)

Coenraads et al. designed a more simplified approach based on photo guide which illustrate various degrees of severity of hand dermatitis based on morphology.(62, 64) The guide is comprised of five severity levels and for each severity level four photographs are presented. Additionally, use of explicit verbal criteria is recommended to assure that all relevant signs (e.g. itch, oedema, etc.) are included in the assessment. Evaluation proved high reliability and good reproducibility.(62)

Prognosis and Therapy

The long term prognosis for occupational CD is often poor.(65, 66) In studies with varying follow-up periods, patients with occupational CD were reported to recover in 21-72% of all cases.(44, 66) This condition is reported to even persist in some patients years after the exposure was permanently eliminated.(67) Prognosis can negatively be affected by various factors such as atopy, contact allergies (44), older age, and the severity and duration of the dermatitis (24, 66)

Immediate treatment is recommended once diagnosis is established. This should be tailored to the severity of disease and broadly corresponds with management of AD. A complete emollients regiment and avoidance of irritants and allergens are the first step in the treatment of mild and moderate forms. (68) Educating patients on the importance of regular skin care and avoidance of exposure to skin irritants is crucial. Topical corticosteroids are the standard treatment for moderate to severe HD. They are very effective in the short term, but may inhibit repair of the stratum corneum causing adverse effects like skin atrophy in the long-term, and may interfere with recovery.(65, 69) If corticosteroids provoke contact allergies or don’t provide sufficient effect despite good adherence, topical calcineurin inhibitors (tacrolimus, pimecrolimus) may be considered for long-term treatment, although there is moderate evidence for their efficacy.(51, 70-72) When topic treatment fails to control the HD, phototherapy should be considered, with the condition that frequent clinic visits and the time it requires is acceptable for patients. Recently, home phototherapy devices are available that are less demanding for patients.(71) Phototherapy is irradiation with ultraviolet light (UV), administered via one of several modalities: UVA (400–320 nm), UVA-1 (400–340 nm), PUVA (psoralen plus UVA), UVB
(320–270 nm), narrowband UVB (313–308 nm), or monochromatic excimer light (308 nm).(71) UVA and the various UVB modalities of therapy may offer comparable clinical improvements to those of PUVA therapy.(71) Several trials have shown that UVB therapy may improve vesicular and chronic fissured HE over a period of ten weeks.(73) Another important second-line treatment, next to phototherapy, is systemic treatment. This is considered only after initial diagnosis and adherence are reconsidered, also the adverse events, benefits and costs should be emphasized regarding patients. Alitretinoin has been suggested as a consensus-based recommendation in the guidelines (69) and seems to be the only registered systemic treatment option for all types of severe chronic hand eczema in the Netherlands.(74) In a trial of patients with chronic HD Alitretinoin showed clear or almost clear responses in 48%, compared to 17% with a placebo.(75) Systemic corticosteroids are considered effective for acute HD or flares in chronic disease. Chronic use is discouraged due to severe side-effects. Cyclosporine is considered to have moderate evidence for its efficacy. (69) Several studies have shown cyclosporine to have positive effects on HD. Granlund et al., reported improvement for 50% of the 41 chronic HD patients.(76) The 1-year success rate for chronic hand eczema was 74% in a different studies performed in 27 patients treated with oral cyclosporine 3 mg/kg/day for 6 weeks. (77) In case of vesicular HD, cyclosporine is preferred and showed improvement of at least 50% in more than half of the study population.(78) The effect of cyclosporine is usually evaluated after 6 weeks, and when effective, treatment is continued for 3-6 months, during which blood pressure and creatinine are monitored. If alitretinoin, acitretin and cyclosporine are unsatisfactory, there are a number of other drugs available.(74) For azathioprine and methotrexate there is very limited evidence for efficacy in the treatment of HD, however both are used (off-label) over the years in clinical practice. (69) Methotrexate (10-15 mg/week) with folic acid is found to be useful in hyperkeratotic hand eczema, while azathioprine (50-150 mg/day) can be satisfactory in recurrent vesicular hand eczema and chronic hand eczema.(74)

Skin Barrier

Structure and composition
The skin consists of two primary layers, the dermis (inner layer) and epidermis (outer layer). The predominant cell type in the epidermis are keratinocytes which are continuously formed and divided in the basal layer migrating up to
the stratum corneum (SC), where they finally shed off during a process called desquamation. Dependent on body location, the SC consists of 10-20 layers of corneocytes, which are nucleus-free flattened keratinocytes. The epidermis undergoes complete turnover in approximately four weeks.

The skin barrier is located mainly, although not exclusively, in the SC. The barrier protects the skin from penetration of irritants and allergens and prevents loss of water. The ‘brick and mortar’ model is often used to visualize the structure of the SC. In this model, the corneocytes are the bricks, surrounded by mortar representing the intercellular lipid bilayers. The rigid organization of the intercellular lipid matrix plays a key role in maintaining barrier properties of the SC. The main components of the SC lipids are ceramides (CER), free fatty acids (FFA), and cholesterol (CHOL), which in human SC present in approximately equimolar ratios. The corneocytes are encapsulated by the cornified envelope (CE) and connected together by corneodesmosomes, optimizing the stability of the brick wall. Inside the corneocytes keratins are aggregated by filaggrin monomers into microfibrils, cross-linked to the CE. Next to its role in providing mechanical strength of the corneocyte, keratin may also play a role in binding water molecules in the SC contributing to SC hydration. Hydration of the SC is furthermore regulated by highly organized intracellular lipids and a mixture of small hygroscopic molecules referred to as natural moisturizing factors (NMF). The NMF are located mainly within the corneocytes where they are formed by degradation of filaggrin protein. Low content of water, caused e.g. by low levels of NMF or impaired composition of SC lipids may lead to dry and cracky skin which will facilitate entrance of skin irritants and allergens.

Effects of irritants on the skin barrier

Basically all components of the ‘brick and mortar’ structure can be affected by skin irritating compounds. The nature and degree of the skin barrier damage is dependent on the intrinsic properties of the irritant, exposure pattern and dose penetrating into the skin. Two of the most frequent causes of irritation in health care sector are water and detergents. Water acts by causing excessive hydration leading to corneocyte swelling, which on its turn will affect a rigid organisation of lipid intercellular matrix and increase permeability to other irritants. Dependent on their physico-chemical properties detergents can interact with both, lipids and proteins of the SC. Regarding the interaction of detergents with the lipid structure of the SC, they are known to solubilize the fatty acids in the lipid bilayers or incorporate into the lipid layers resulting in loss of water from
the SC, eventually leading to dry skin, cracking, and erythema.(29) With regard to interaction with SC proteins, detergents are known to cause protein denaturation in the SC(89) and disruption of the corneodesmosomes. (26) Most research on the impact of detergents on skin is focused on sodium lauryl sulphate (SLS), an alkaline detergent with strong irritant properties. SLS is known to induce skin barrier damage and irritation through various mechanisms. Experimental studies reported that SLS causes damage to the corneocyte by affecting CE and keratin, which introduces new water binding sites and consequently hyperhydration of the SC.(26) SLS has also been shown to downregulate expression of structural proteins involucrin and profilaggrin and furthermore of lipid-metabolizing enzymes, transglutaminase and serine proteases kallikreins.(26, 90) Next to its effect on lipids and SC proteins, SLS particularly demonstrated to strongly decrease the levels of NMF.(91, 92)

Other common irritants in the health care sector are organic solvents, such as ethanol, iso- and n-propanol, which are used in hand sanitizers. Alcohols, for example n-propanol, are shown to denaturate the SC proteins and exert various irritant-effects by changing activity of proteases involved in homeostasis of the SC, pro-filaggrin processing or desquamation.(93) In addition, recent studies showed that n-propanol decreases NMF levels.(94)

Assessment of skin irritation

Bioengineering parameters: erythema, TEWL and capacitance

Various non-invasive skin bioengineering methods are used in studying skin irritation. Classic techniques involve measurements of transepidermal water loss (TEWL) and erythema (skin redness).(95) The TEWL reflects the skin barrier function and increases upon barrier damage.(96) Erythema, an indicator of vasodilatation, is traditionally scored visually (97), but can also be measured by a spectrophotometer(98) or by Doppler Flowmetry.(95) Another commonly used bioengineering parameter is the SC surface hydration which can be determined by measuring electrical capacitance. (99)

Biochemical parameters: NMF and cytokines

Various studies have assessed irritant skin response by measuring the levels of the biomarkers in the stratum corneum collected by a minimally invasive procedure of tape stripping. With this method consecutive layers of the SC are removed by adhesive tapes. Among most frequently determined biochemical
parameters are pro-inflammatory cytokines (mainly IL-1 (33, 91) and TNF-alfa (100) and NMF. (82, 92)

**Morphological parameters: corneocyte surface morphology**

Recently, atomic force microscopy demonstrated to be a robust method to study changes in the corneocyte surface topography in tape strips collected from skin exposed to irritants and allergens.(91) Alterations in the skin barrier are manifested as abundant presence of circular nano-size objects protruding approximately 300 nm above the corneocyte surface.(100) The density of these nano-size objects can be quantified with image analysis and is expressed as the Dermal Texture Index (DTI).(101) A marked increase in DTI has been shown in skin exposed to SLS, and to allergens with irritating properties. Elevated DTI has also been found in AD patients, carriers of FLG mutations.(102) Interestingly, DTI showed strong inverse relationship with NMF levels. Furthermore, the presence of protrusions was associated with impaired corneodesmosome degradation, however this process is not completely clarified yet.

**Restoration of the skin barrier**

**Dry skin**

Excessive loss of water from the skin caused by skin barrier impairment will lead to development of dry skin, one of the first signs after exposure to skin irritants. (Fig. 2) The amount of water in the SC is crucial for the softness and flexibility of the SC. Dry skin becomes rough and scaly and often presents with cracks and even fissures.(103) The amount of water in the SC is dependent on the composition and organization of the lipid layers and furthermore on the levels of small hygroscopic molecules, called natural moisturizing factors (NMF), which binds water.(26, 82, 83, 104) The main constituents of NMF are the breakdown products of the epidermal protein filaggrin, inorganic salts, sugars, as well as lactic acid and urea.(105) Patients with atopic dermatitis, in particular carriers of FLG loss-of-function mutations, have up to 80% reduced levels of NMF (39) dependently on FLG genotype and inflammation.(82, 83) In atopic patients decreased NMF levels can at least partly explain the presence of dry skin in AD. Dryness of the skin is also common in the elderly and in subjects with diabetes. (106, 107) Next to pathological skin conditions, dry skin can be aggravated by exposure to water, surfactants, alcohols and even contact allergens which have irritating properties.(91, 103, 104)
Management of dry skin: moisturizers
Based on the underlying mechanism, there are several classes of formulations used to prevent and treat dry skin.\(^{(82, 108, 109)}\)

**Occlusives.** The most straightforward way to keep the skin hydrated is to form an occlusive layer on the surface of the skin. The most common occlusive emollients contain long-chain hydrocarbons (mineral oils, petrolatum, paraffin), fatty acids, fatty alcohols and waxes that provide an occlusive layer over the SC and block epidermal water loss. (Fig.3)\(^{(110)}\) Thereby they prevent the water from evaporating and allow it to pass back into the corneocytes resulting in a more resilient skin barrier.\(^{(82, 83)}\) For example, petrolatum in a concentration of at least 5% reduces TEWL by more than 98% followed by lanolin, mineral oil, and silicones, which only reduce TEWL by 20-30%.\(^{(111)}\) However, occlusive moisturizers are least acceptable for users due to the greasy feel.

**Humectants** contain hygroscopic compounds that attract water from the deeper epidermis and dermis and from the surrounding atmosphere. The key endogenous humectant for hydration of the SC \(^{(110)}\) is a mixture of small hygroscopic compounds collectively called ‘Natural Moisturizing Factors’ (NMF) (Fig. 4), composed of lactic acid, pyrrolidone carboxylic acid, amino acids and urea. Some constituents of NMF, such as pyrrolidone carboxylic acid, urea and lactate are used in a number of moisturizing formulations. \(^{(105)}\) Over the years many skin care products are developed with novel components, such as aquaporin 3 modulators (AQP-3).\(^{(112)}\) The most common humectants found in moisturizers are glycerol and urea.\(^{(27)}\) Glycerol is used as the most effective moisturizer and humectant in cosmetic products critical in skin hydration, cutaneous elasticity and epidermal barrier repair.\(^{(27, 113)}\) Urea works as a humectant at low concentrations (10%) and has been shown to reduce SLS-induced skin irritation. Although urea is known for its beneficial hygroscopic properties, some studies showed that it also acts as a penetration enhancer.\(^{(110)}\)

In terms of user acceptance, humectants are preferred above ointments as they feel less greasy.\(^{(82, 83)}\)
Fig. 2. How the eczema affects the skin barrier. Source: Cork et al., British Journal of Nursing 2009 (82)

Fig. 3. The effect of occlusive emollients on the skin barrier. Source: Cork et al., British Journal of Nursing 2009 (82)

Fig. 4. The effects of moisturizers affecting NMF. Source: Cork et al., British Journal of Nursing 2009 (82)
Preventive strategies for (occupational) hand dermatitis

Interventions are traditionally considered to operate at three different levels of prevention: primary, secondary and tertiary. Primary prevention includes the traditional hierarchy of controls including elimination or substitution, engineering controls, administrative controls, personal protective equipment and education. (68, 114) Secondary prevention is mainly focused on the detection of early stages of disease, prevention of progression, inducing behavioural change and training workers to protect their skin. Although tertiary prevention resembles methods used at the secondary prevention level, it is particularly implemented in rehabilitation centers where expert management is provided. (115)

Hierarchically, elimination or substitution of a hazard is the very first step in the primary prevention. In many European countries legal regulations enforce elimination or substitution of harmful exposures by technical and organisational measures. However, in practice this is often impossible, e.g. the in health care sector use of soaps and disinfectants are strictly regulated to comply with hygienic requirements. Therefore, to limit the risk of hand dermatitis in high risk occupations often personal protection measures e.g. gloves, skin barrier creams and moisturizers are recommended to minimize exposures to skin hazards and damage of the skin barrier (116). Here, lack of knowledge, misconceptions, lack of incentives (e.g. financial or regulatory) and low compliance hamper effectiveness of these measures. Therefore, most of prevention programs for OSD in health care workers focus on raising awareness and early dermatological interventions. (11, 13, 25, 117-119). In the following sections, a brief overview of different strategies aiming at prevention of occupational hand dermatitis will be given.

Organizational Measures: reducing harmful exposures

Elimination and Substitution

The principles of prevention strategies in high risk occupations are unambiguous and focus on workplace hazard control, e.g. by removing current hazardous substances. (120-123) Good examples are the introduction of powder-free gloves which limit the amount of leachable protein in latex gloves, chromate-free cement, removal of para-Phenylenediamine (PPD) in hair dyes and elimination of aldehyde disinfectants. (124, 125) Specifically for health care workers, when hands are not visibly soiled, use of alcohol based disinfectants instead
of hand washing are recommended in current guidelines on the prevention of hand dermatitis. The harmful effects of detergents have been demonstrated in multiple studies. Visser et al. found that washing of hands >10 times per shift doubles the risk for HD, whereas frequent exposure to alcohol based disinfectant are generally well tolerated. (12)

**Personal protection**

If elimination or substitution are not feasible, a set of preventive measures are recommended aiming at maintenance and enforcement of the skin barrier. (123) The most studied personal protective measures are skin care products (moisturizers), skin barrier creams and gloves. (69) Recently, a Cochrane review focused on interventions for primary prevention of occupational HD. (123) In that review, six studies were included which investigated the effects of moisturizers or barrier creams or both combined. (123) Meta-analyses showed inconsistent effect of the interventions and a moderate quality of evidence. (123) Based on three studies included in the meta-analysis, the relative risk for developing HD was 0.71, (95% CI 0.46-1.09). (123, 126, 127) Goh (1994) et al. demonstrated the effectiveness of creams in terms of the reduction of number of workers with occupational HD at follow up. (126) Küttting et al. showed a beneficial effect of hand creams regarding decrease in period prevalence, however only in combination with barrier creams. (127) Smedley et al. concluded that there is consistent evidence that good hand care, including educational programs and use of conditioning creams, improves skin condition in employees with dermatitis, however this conclusion was based on a small body of evidence. (128-130)

Moisturizers are also studied as a part of successful multi-component intervention studies targeted at secondary prevention. (13, 131) The impact of each individual component in these type of intervention are however not clear, as several measures like moisturizers, educational training and dermatologic consultation are combined. (118)

Although the evidence for the effectiveness of hand creams in prevention of occupational HD is still lacking, (123) regular use of hand creams are recommended in current guidelines for the prevention of OIHD. (9, 69) These guidelines do not specify the type of hand creams, as the evidence body for specific cream recommendations is too small. (69)

Gloves are the most used control measure in the work place, although their effectiveness in prevention of HD is inconclusive. (69) Due to occlusion of the
skin, wearing of gloves itself can be regarded as “wet work”. In the health care sector use of hand gloves are indispensable to protect workers from chemical, biological or physical hazards, however in the case of long wearing times it is recommended that thin cotton gloves are worn under the outer gloves to limit the moisture.(132-134)

**Educational programs**

Raising awareness on risk factors and personal protection are important in current prevention programs.(11, 25, 118, 119, 135, 136) A universal set of rules, regardless of level of prevention, but relevant to wet work occupations have been introduced by Agner and Held (Table 1).(137)

Although workers education and application of skin protective measures are regarded as crucial, evidence on their effectiveness at workplaces is still limited. (138, 139) A recent review concluded that there is uncertainty whether in primary prevention skin protection education is effective. (15, 123, 136, 140) The quality of evidence was considered very low, as the results varied substantially across the trials, the effect was imprecise, and the pooled risk reduction was not large enough to be clinically important.(123)

The effect of education as a secondary prevention measure in patients with HD has only been studied in four RCTs.(13, 58, 131, 141) Two of them have indicated a positive effect of individual skin care education on severity of disease.(123)

**Table 1. Evidence-based recommendations on skin protection for prevention of occupational HD. Source: Agner et al., Contact Dermatitis 2002. (137)**

1. Wash your hands in lukewarm water. Rinse and dry your hands thoroughly after washing
2. Use protective gloves when starting wet-work tasks
3. Protective gloves should be used when necessary but for as short a time as possible
4. Protective gloves be intact and clean and dry inside
5. When protective gloves are used for more than 10 min. cotton gloves should be worn underneath
6. Do not wear rings at work
7. Disinfectant should be used according to the recommendation for the workplace
8. Apply moisturizers on your hands during the working day or after work. Select a lipid-rich moisturizer free from fragrances and with preservatives having the lowest allergen potential
9. Moisturizers should be applied all over the hands including the fingerwebs, fingertips and back of the hand
10. Take care also when doing housework, use protective gloves, for dishwashing and warm gloves when going outside in winter
Monitoring hand care practices

Whereas regular use of hand creams is widely regarded as beneficial in prevention of OSD in healthcare, there is a lack of practical strategies to comply with the guidelines on skin care, and the consumption of creams at workplaces remains low. (123, 141) The presented research was designed to find a new approach for monitoring and ultimately improving skin care compliance to prevent occupational HD, following successful hygiene strategies.

Hand hygiene studies describing interventions that include behavioural change methods have shown improvement in compliance. (142, 143) These interventions share a set of characteristics, like long period of follow up, engaging staff as well as onsite leaders, not disturbing the workflow and being non-intrusive and non-punitive. (142, 144, 145)

For monitoring hand hygiene compliance direct observation was traditionally considered to be the gold standard, but many limitations of this method, including the Hawthorne effect, have been addressed over the past few years. (146, 147) As critical components to new approaches for measuring and ultimately improving compliance, education combined with audit and feedback are recognized. (148) Unless staff is engaged through education, and information about compliance rates gathered by monitoring are provided to staff, it seems unlikely that improvement of ingrained practices will be reached. (144) To be effective, feedback to staff must be meaningful and motivational. Positive feedback has been associated with improved practice. (144, 149) Also, feedback must be relevant and delivered in a way that is readily accessible, so it’s easy to interpret and act upon. (148)

New technologies are developed to monitor compliance, like automated monitoring systems using electronic counting devices placed inside dispensers that record every time the dispenser is accessed. (150) These systems support behaviour change by enabling detailed feedback on frequency and pattern of cream use, thought to drive improvement when regularly communicated to nurses. (148)

The gains of these systems allow for minimal resources once installed, no disturbance to the workflow, potentially less Hawthorne effect and provision of large data sets. (148) As with current measures for monitoring, like direct observation and self-reporting, electronic monitoring also has its limitations in terms of practicality and accuracy. Nonetheless, it has shown to improve compliance for hand hygiene among healthcare workers. (151, 152) Thus, we hypothesized that a similar approach would have potential to improve hand
care among HCW as well. To that end, we developed an intervention based on provision of hand creams, continuous electronic monitoring and repeated feedback of cream use to healthcare workers and assessed its effectiveness in a RCT was performed among HCW.

Aims of this thesis

This thesis is structured in two parts: an intervention study in health care workers and an experimental part that focuses on the effects of skin irritants on skin barrier and inflammation. The main objective in the first part was to assess the effectiveness of an intervention program. In the second part, we aimed to investigate in vivo the effects of commonly used skin irritants on the skin barrier and the inflammatory response.

These objectives will be outlined in the following chapters:

**Part I Intervention study in healthcare workers**
In the first part of this work (Chapter 2, 3 and 4) we aim to assess the effectiveness of an intervention program aimed at reducing HD symptoms by increasing skincare use among HCWs. Chapter 3 focuses on two questions: Is a prevention program focused on skincare monitoring and feedback effective in reducing the severity of hand dermatitis in HCWs? Does a prevention program focused on skincare monitoring and feedback improve NMF levels in the skin? Chapter 4 discusses whether a new technology in the form of electronic monitoring combined with feedback increases skincare use among HCWs?

**PART II Investigating the in vivo effects of commonly used irritants on the skin barrier and inflammatory response**
In these last two chapters (chapter 5 and 6) the following questions are answered: 5 What are the effects of various skin irritants on the skin barrier and inflammation? 6 What is the effect of the concentration, occlusion and of atopic dermatitis on the barrier function and inflammatory response after experimental exposure to n-propanol? Finally, in chapter 7 the main findings are discussed, including our recommendations for future research and practice.
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PART I
Intervention study in health care workers
The effectiveness of a skin care program for the prevention of contact dermatitis in healthcare workers (the Healthy Hands Project): study protocol for a cluster randomized controlled trial

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Abstract

Background
Health care workers (HCW) are at high risk for developing occupational hand dermatitis (HD) due to frequent exposure to ‘wet work’. Amongst HCWs, nurses are at highest risk, with an estimated point prevalence of HD ranging between 12 and 30%. The burden of disease is high with chronicity, sick leave, risk of unemployment and impaired quality of life. Despite evidence from the medical literature on the risk factors and the importance of skin care in the prevention of HD, in practice, compliance to skin care protocols are below 30%. New preventive strategies are obviously needed.

Methods/design
This is a cluster randomized controlled trial, focusing on nurses performing wet work. In total, 20 wards are recruited to include 504 participating nurses in the study at baseline. The wards will be randomized to an intervention or a control group and followed up for 18 months. The intervention consists of the facilitation of creams being available at the wards combined with the continuous electronic monitoring of their consumption with regular feedback on skin care performance in teams of HCWs. Both the intervention and the control group receive basic education on skin protection (as ‘care as usual’). Every 6 months, participants of both groups will fill in the questionnaires regarding exposure to wet work and skin protective behavior. Furthermore, skin condition will be assessed and samples of the stratum corneum collected. The effect of the intervention will be measured by comparing the change in Hand Eczema Severity Index (HECSI score) from baseline to 12 months. The Natural Moisturizing Factor (NMF) levels, measured in the stratum corneum as an early biomarker of skin barrier damage, and the total consumption of creams per ward will be assessed as a secondary outcome.

Discussion
This trial will assess the clinical effectiveness of an intervention program to prevent hand dermatitis among health care workers.
Background

Healthcare workers (HCW) are at high risk for developing occupational hand dermatitis (HD) due to frequent exposure to ‘wet work’. (1) ‘Wet work’ defined as unprotected exposure to humid environments/water; high frequencies of hand washing procedures or prolonged glove occlusion, is believed to cause irritant contact dermatitis in a variety of occupations.(2) Despite no clear scientific definition for ‘wet work’ in terms of exposure frequency, duration or intensity most study groups refer to regulatory guidelines. For example, the German guidelines, in which ‘wet work’ is defined as having wet hands for more than 2h per regular work day (per shift), hand cleansing more than 20 times per day or wearing of occlusive gloves for 2h per day. (3) Amongst HCWs, nurses are particularly at high risk of HD, with an estimated point prevalence of 12-30% (4,5). Almost 60% of HCWs are reported to have eczema-related sick leave during the first year after notification of disease (6). This makes the burden of disease high for affected individuals as well as in the socio-economic context. The total annual costs for occupational skin diseases for medical care, absenteeism and disability pensions are estimated to be €98 million in the Netherlands (7).

Recently, infection prevention policy in the Netherlands has become stricter, with emphasis on the frequent use of hand alcohol and hand washing with soap to prevent transmission of infections. This has led to an increase in exposure to irritants and a higher risk of skin barrier damage.

In the Netherlands, the Dutch Society of Occupational Medicine (NvAB) established guidelines for the prevention of OHD in 2006 (8). The guideline stresses the importance of the maintenance of uncompromised skin barrier for the prevention of HD and recommends regular use of skin care products such as emollients and ointments. A more recent update of contact dermatitis guidelines from the Netherlands Society of Dermatology and Venereology (NVDV) (9) consistently recommended the use of emollients to help prevent irritant contact dermatitis, referring to three studies (10,11,12). The findings from one RCT showed that the use of creams decreased occupational hand dermatitis, compared to the control without any intervention (11). In another RCT, healthy volunteers washed their hands 15 times a day, after which they used cream. Compared with the control group, skin hydration was significantly higher in the group which used creams (10). In an experimental study of Kampf et al. (12), a test group comprising 25 subjects applied hand creams after every hand wash. The result was that the use of hydrating creams decreases the symptoms of HD including skin hydration and skin roughness. Several skin care
programmes have been effectively introduced in the healthcare setting to help prevent occupational diseases (13,14). The effectiveness of these programmes seemed to depend on three factors: 1) the effectiveness of protective measures (i.e. skin care products); 2) the adherence to these measures (i.e. frequency of application); and 3) the effectiveness of education on preventive behaviour (by raising awareness about the risk factors for HE and the importance of protective measures)(15). Moisturizers belong to the most widely used preparations to decrease dryness and improve skin barrier function. The effects of moisturizers on the skin barrier has mainly been investigated in several experimental irritation studies (16,17). A number of different mechanisms behind the barrier improving effects of various creams have been suggested, but still their mechanisms are not fully understood.

With respect to the effectiveness of education, several intervention studies have shown moderate evidence that education influences behaviour, which leads to a reduction in skin symptoms (18). Regarding the second factor, despite evidence from interventions which consider appropriate skin care effective, the adherence to these preventive measures in the workplace remains low (19).

Recently, an electronic monitoring system has been developed for the continuous registration of hand cream consumption and data recording. This system enables a detailed feedback to HCWs on the frequency with which the hand cream is used as well as when it is used. In hand hygiene studies, a similar monitoring system was earlier proven to improve compliance by 42 percent(20). Monitoring and feedback are widely used as a strategy to improve professional performance and patient outcomes (21). The effects are generally moderate and vary based on the way the intervention is designed and delivered (22). Feedback is suggested to be more effective to improve performance when: 1) baseline performance is low; 2) when the source is a supervisor; 3) when it is provided more than once; and 4) when it is provided both verbally and in written form (22). Group monitoring is widely recognized as being more effective than other monitoring systems that track individuals’ actions, which can be seen by staff as punitive or an invasion of their privacy (23). Providing group data to units has been shown to encourage group collaboration in a positive manner as staff work together to improve compliance, resulting in better and longer-sustained results.

In the present study, we aim to investigate the effectiveness of such a feedback-monitoring system combined with raising awareness in a randomized controlled trial. The intervention will comprise of the placing of the dispensers with hand cream on the wards, electronic monitoring of use, and repeated feedback to the HCWs on the wards. The efficacy will be assessed by measuring
skin conditions in HCWs before and after the intervention, compared with a control group.

Objectives
We will investigate whether an intervention programme, based on the provision of hand creams and regular feedback on cream consumption, can improve the skin condition in nurses engaged in wet work, when compared to ‘care as usual’.

Methods

Trial design
A two-arm, single-blinded randomized clustered controlled trial, based on hospital wards as the unit of randomization. The aim is to recruit wards, randomly allocate them to an intervention or control group after stratification on exposure to ‘wet work’. The follow-up is 18 months. The primary means of data collection will consist of two assessments (one clinical and the other biochemical), questionnaires and electronic consumption records.

Trial setting
The trial will take place at a large medical academic centre in the Netherlands, the VUmc (Free University Medical Center), with a total of 45 departments of which approximately 20 are clinical wards. The investigators are responsible for the protocol, the conducting of the trial, analysis of the data and all other aspects involved.

Recruitment of participants
The trial includes nurses engaged in ‘wet work’ activities (e.g. hand washing, use of hand disinfectants, wearing gloves) at clinical wards of the VUmc. The identification of the ‘high-risk wards’ will be based on the consumption of soap and disinfectants per ward (normalized for number of HCW/ward). Wards where nurses have an increased risk of HD due to the nature of their work are defined as “high risk”. For recruitment, the manager responsible for a ward will receive a letter from the investigators stating the purpose of the study, a short version of the study protocol, and a brief description of the expected burden for the participant during the intervention. On the wards which are willing to participate, the researcher will give a short presentation on HD in HCWs and the preventive measures as described in Dutch guidelines (8,9). During the presentation, the
researcher will also inform the participants about the study and provide leaflets with study outlines. Furthermore, the HCW will get an application form with contact details in which they can express their interest to participate in the study. The investigator will distribute the patient information letter on the wards and invite the interested participants for a visit. Nurses agree to participate by signing a consent form.

Products used in the Intervention group
In the electronic dispensers, placed at the intervention wards, Stokoderm® aqua sensitive was used; a white, perfume and silicone-free soft cream, with no particular pharmacological function. The main ingredients are glycerol and urea, which are known to prevent loss of water leading to dry skin.

Inclusion criteria
• Willing to give informed written consent
• Age 18 to 65 years
• Having daily exposure to ‘wet work’ activities during work
• Being employed as nurse or nutrition assistant on the participating wards

Exclusion criteria
Being employed at more than one ward.

Description of the study procedures and intervention
The flow chart of the study design is shown in (Fig. 1). After inclusion, the participants will fill in the baseline questionnaire and undergo baseline measurements (clinical scoring and SC collection). The baseline questionnaire includes information on participants’ characteristics (including number of working hours, and years of employment), history of atopic diseases, relevant medical history, skin condition and the risk factors/exposures (self-reported average frequency of hand washing, use of hand disinfectants, use of skin care products and gloves during a shift). Filling in the questionnaires will take approximately 3-5 minutes per participant.

Both the intervention and the control group will receive basic education on skin protection (as “care as usual”). These educational courses will be given on each ward every three months from baseline to the end of study. Our group education program (as described in Table 1) will comprise basic knowledge about the skin, the development of eczema and recommendation for skin protection, and care as proposed by the guidelines for contact dermatitis of the NVAB (8) (Table 2).
At baseline and 12 months after baseline, the participants in both groups will undergo clinical measurements (HECSI scoring), SC samples will be collected for NMF analysis and they will fill in the questionnaire.

Participants in the control group (just as in the intervention group) with severe eczema requiring medical treatment will be advised to consult the occupational physician or dermatologist.

Additional procedure in the intervention group:
After randomization into the intervention group, the hand cream (Stokoderma Aqua Sensitive) will be provided in electronic dispensers (with monitoring system) on the wards at places which are easily accessible. At least five dispensers per ward will be located at sinks, at the entrance and exit of each ward and other relevant places, like coffee rooms and toilets. The provided hand cream is commercially available and is widely used in the health care sector.

Each dispenser records continuously each application event, providing information on the timing and frequency of use of hand creams during the working shift. The system provides robust and easy to interpret web-based reports on cream use per dispenser. Data on use pattern (frequency, total consumption, moments of use) and trends will enable a structured feedback on hand cream use to the nurses and management to motivate and improve compliance. Feedback sessions on hand cream use will be done every six months after baseline during regular meetings of the nursing staff and performed by the head nurse.
Fig. 1 Flowchart for the Healthy Hands Project (HHP). The trial will run for 18 months in total, the primary and secondary outcomes will be assessed after 12 months, during the second and last visit.
Table 1. Educational Program HHP

| Methods                      |
|------------------------------|
| Educational courses every 3 months |
| Booklet on preventive measures |

| Program Topics               |
|------------------------------|
| What is hand eczema and what are the symptoms |
| Risk factors of hand eczema |
| Consequences of hand eczema |
| Preventive measures          |

Table 2 Main recommendations for the prevention of hand dermatitis (NVAB guideline, 2006) (8)

1. Use disinfectants instead of water and soap to disinfect the hands, when hands are not visibly dirty
2. Wear gloves when performing wet work
3. Wear cotton under-gloves when you wear gloves for longer than 10 min
4. Use a moisturizer on a daily basis to nurse the skin
5. Creams should be applied over the whole hand, including the webs fingertips and dorsal aspects

Outcome measures

Primary outcome
The change in disease severity as assessed by the Hand Eczema Severity Index (HECSI) score. This validated scoring system, the HECSI [24], grades the intensity of erythema, induration, papules, vesicles, fissures, scaling and oedema for five areas of each hand (fingertips, fingers, palms, back of hands, wrists) on a scale from 0 (not present) to 3 (severe). The extent of affected skin in each area is graded from 0 to 4. The total index score with a range from 0 to 360 is then found by multiplying the intensity with the extent [24]. The HECSI will be assessed at baseline and after 12 months in both the intervention and the control group.

Secondary outcomes
The change in levels of NMF in the skin as a marker of early signs of barrier damage. NMFs are mainly composed of the breakdown products of epidermal protein filaggrin. NMFs play an important role for the skin barrier function as they
contribute to skin hydration, the maintenance of the acidic pH of the skin, and the epidermal inflammatory response [25]. Recently, it has been shown that various skin irritants significantly reduce the levels of NMF [26]. One study showed that during cleansing (and more than 10-min water contact) large quantities of NMF can leach from the skin surface, leading to dry skin and, by repetitive exposures to skin barrier damage, to inflammation [27]. Therefore, the NMF levels might reflect early damage of the skin barrier.

The NMF levels will be determined in the uppermost layers of the skin, the stratum corneum (SC). The SC samples will be harvested by using adhesive tape strips, a method which is extensively used in experimental studies [28]. Briefly, round adhesive tape discs (3 × 8 cm2, DSquame; CuDerm, Dallas, TX, USA) will be attached to the skin of the right hand. Each tape is pressed onto the volar aspect of the hand for 10 s with standardized force, using a disc pressure applicator (CuDerm, Dallas, TX, USA). The first four successive strips from the same skin site will be discarded and for the NMF analysis the fifth to seventh tape strips will be collected. The tape strips are gently removed with tweezers and stored in a closed vial at −20 °C until analysis. For the analysis of NMF in tape strips, NMF constituents will be extracted from the tape strips using 40% ammonia and subsequently analysed by HPLC-UV [29]. To compensate for variable amount of SC harvested by a tape, the protein amount will be determined on each tape by measuring optical density (SquameScan, CuDerm, Dallas, TX, USA). NMF concentration on the tape will be normalized by the protein amount.

**Process outcomes**

Individual consumption of hand creams (application frequency/per shift) will be assessed by questionnaires at baseline and every 6 months from baseline. Total consumption of creams in the intervention group will be measured by real-time monitoring per ward as a secondary outcome and compared over time.

**Skin exposure to irritants**

Individual exposure will be assessed by questionnaires (estimated frequency of soap and alcohol use per shift), completed every 6 months. Furthermore, exposure per ward will be estimated from data on the purchase of soap and disinfectants.

**Sample size**

This trial is planned to include 544 individuals. The sample size is based on a previous study, in which the Osnabruck Hand Eczema Severity Index score after 12 months’ follow-up in the control group was 0.1 points with a standard
deviation of 1.2. A difference in the Osnabruck Hand Eczema Severity Index score of 0.4 points is clinically significant [30]. Using a two-sided t test with a significance level of 0.05, we calculated that a study with 17 clusters per treatment group with 16 individuals per cluster would have 81% power to detect a difference of 0.4 in group means when the standard deviation is 1.2 and the intra-cluster correlation is 0.05.

**Randomization**
The method of fixed-block randomization will be used to carry out randomization with block sizes of 2. The blocks (one high-exposure block and one low-exposure block) will be stratified by exposure to wet work, estimated from the purchase of soaps per ward. Two blocks will be randomized at a time to reduce bias and achieve balance in the allocation of participants to the intervention or control arm. Randomization will be performed prior to baseline collection.

The investigator, assessing the clinical outcomes, will be blinded with respect to treatment allocation until after analysis.

**Statistical methods and data analysis**
We will use state-of-the-art methods to deal with missing data and statistical methods to analyse our data and publish a full statistical analysis plan before the researchers are unblinded. When reporting the results of this study, we will adhere to the Consolidated Standards of Reporting Trials (CONSORT) Statement [31] and its extensions on the reporting of patient-reported outcomes in randomized trials [32] and on cluster randomized trials [33].

**Blinding**
The investigator, assessing the clinical outcome, will be blinded with respect to treatment allocation. It is not possible to blind the participants.

**Participants’ withdrawal**
Subjects are free to leave the study at any time for any reason if they wish to do so, without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

**Finance and insurance**
The trial is (partly) financed by an unrestricted grant from the company DEB, which covers all expenses related to the study. The participants in the study are covered by their work insurance.
Discussion

The overall purpose of the Healthy Hands Project is to change the behavior of HCWs towards hand care and make skin care part of the work culture. We look at HD among nurses, who are known to have an increased risk of occupational hand eczema due to wet work. We focus on the facilitation of cream availability combined with the continuous monitoring of its consumption with regular feedback on skin care performance in HCWs, aimed at improving their preventive behavior. In addition, this trial will provide information on the actual degree of exposure to wet work in HCWs in The Netherlands, which is important for focused prevention.

The intervention is general, straightforward and, therefore, easy to implement in health care institutions. The results of this study will help to gain insight into the effectiveness of our intervention program on skin condition and, secondarily, on the consumption of hand creams. We will provide relevant data on the current use of moisturizers in practice in health care work environments and the efficacy of an intervention which is relatively easy to implement.

Currently, a multicenter intervention RCT, which investigates a range of interventions aimed at the reduction of hand dermatitis among nurses, is running in the UK (the SCIN trial) [34]. In contrast to our study, the SCIN trial focuses on behavioral change to improve hand care, based on the theory of planned behavior and implementation intentions. The impact of the interventions will be assessed via questionnaires and standardized photographs of hands/wrists, and the primary outcome parameter will be the change in point prevalence of visible hand dermatitis from baseline to 12 months after the intervention. In the present study, the changes in the skin condition between control and intervention groups are used as the primary outcome.

The skin condition will be assessed by clinical scoring (HECSI score) performed by a trained physician. In addition, we include as a secondary outcome the NMF levels, which might be a more sensitive parameter of the skin damage than clinical scoring.

With an RCT design, the risks of selection bias will be reduced, and random and design errors limited [30]. The risk of bias will be further reduced by conducting a central randomization stratified for exposure to soaps as an important prognostic factor. We blinded the outcome assessors to minimize the risk of detection bias. In order to limit contamination, the control group will not receive information about the existence of an intervention group or the purpose of the study [35]. It must be acknowledged, however, that a risk of contamination bias cannot entirely be avoided because the participants work in the same medical center and could find out about the intervention group.
Strong points: real-time monitoring of consumption of creams, objective assessment of the skin condition, facilitation of creams, feedback on performance, and determining NMF levels to detect early signs of skin barrier damage.

Drawbacks: self-reported hand cream use to assess individual use of skin care in both the control group and the intervention group. The objective data on cream use in the intervention group are ward-based. Exposure estimates are ward-based and self-reported at the individual level.

Trial status
Recruitment into this trial started in June 2016 and is taking place in the VU Medical Center in Amsterdam. Patient recruitment has not been completed at the time of submission.

Acknowledgements
The HHP trial is sponsored by DEB Group Ltd. The sponsor has delegated the study design, management of trial data and writing reports to the researchers.

| TIMEPOINT | Enrolment | Allocation | Post-allocation |
|-----------|-----------|------------|-----------------|
| **-t₁**   | 0         | T₀m        | T₃m T₆m T₁₂m T₁₈m |
| **ENROLMENT:** |           |            |                 |
| Eligibility screen | X |          |                |
| Informed consent     | X |          |                |
| [Standard safety monitoring] | X |          |                |
| Allocation           | X |          |                |
| **INTERVENTION**     |           |            |                 |
| [providing cream and monitoring] | |          |                |
| [educational lessons] | |          |                |
| [feedback on wards]  | |          |                |
| **ASSESSMENTS**      |           |            |                 |
| [Individual characteristics] | X |          |                |
| [Primary and secondary outcome] | X |          |                |
| [Consumption data]   | X | X         | X               |

Fig. 2. The Healthy Hands Project (HHP) trial assessments performed at different time points
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Statistical analysis plan for the Healthy Hands Project; single centre cluster randomized clinical trial of a skin care program for the prevention of contact dermatitis in healthcare workers

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Abstract

Background
The Healthy hands Project (HHP) is a randomised clinical trial, aiming to determine the effectiveness of an intervention program in prevention of hand dermatitis in healthcare workers (HCWs). The intervention comprised of placing dispensers with hand creams at wards combined with continuous electronic monitoring of cream consumption and regular feedback to HCWs. The clinical severity (HECSI-score) was used as the primary outcome and the natural moisturizing factor (NMF) as the secondary outcome. The study protocol for the cluster randomised controlled trial of HHP was published in Trials in 2017. This article describes the detailed statistical analysis plan for the HHP trial.

Methods
The Healthy hands project was a single center, cluster-randomized controlled trial with two parallel groups and blinded outcome assessment. This update article presents (1) the descriptive statistics of the primary and secondary outcomes, (2) the statistical models used for the analysis of the main outcomes (3) sensitivity analyses on the effect of observed exposure to wet work (4) handling of missing data including sensitivity analysis (5) an updated power calculation. This statistical analysis plan was written prior to unblinding of the study.

Discussion
This paper presents a comprehensive statistical analysis plan for the data resulting from the HHP trial. It supports transparency in reporting by clarifying differences between the previously published protocol and the proposed actual statistical analyses.
Background

Health care workers (HCW) have increased risk to develop occupational hand dermatitis (HD) due to frequent exposure to ‘wet work’ [1, 21]. Wet work, defined as unprotected exposure to humid environments and water; high frequency of hand-washing procedures or prolonged glove occlusion, is believed to cause irritant contact dermatitis in a variety of occupations [2, 21].

Amongst HCWs, nurses run the highest risk of HD, with an estimated point prevalence of 12 to 30% [3, 4, 21]. Almost 60% of sick leave reported during the first year after notification of the disease is related to HD [5]. Hence, HD represents a significant burden for affected individuals as well as for the society. The annual costs due to occupational skin diseases spent for medical care, absenteeism and disability pensions are estimated to €98 million in The Netherlands [6, 21].

In The Netherlands, the Dutch Society of Occupational Medicine (NvAB) established a guideline for the prevention of occupational hand dermatitis (OHD) in 2006 [7, 21]. This guideline emphasizes the importance of the skin barrier for the prevention of HD and recommends regular use of skin care products such as emollients and ointments. Consistently, in a more recent update of contact dermatitis guidelines issued by The Netherlands Society of Dermatology and Venereology (NVDV) [8], regular use of emollients to help prevent irritant contact dermatitis has been recommended [9-11].

Several skin care programs have been introduced in the health care setting to help prevent occupational skin diseases [12, 13, 21]. The effectiveness of these programs seemed to depend on several factors: (1) the effectiveness of protective measures (i.e. use of skin care products), (2) the adherence to these measures (i.e. frequency of application) and (3) the effectiveness of education on preventive behaviour (by raising awareness about the risk factors for HE and the importance of protective measures) [14, 21]. To avoid skin dryness and improve skin barrier function a variety of moisturizers and emollients have widely been used. The effect of skin care products on the skin barrier has mainly been investigated in experimental irritation studies [15, 16], and randomized controlled studies in HCW are scarce. With respect to the effectiveness of education, several intervention studies have shown moderate evidence that education influences behaviour, leading to a reduction in skin symptoms [17]. Regarding adherence, despite evidence from intervention studies that skin care is effective in prevention of HD, the adherence to these preventive measure in the workplace remains low [18].
Monitoring and feedback is a widely used behavioral strategy to improve adherence [19, 21]. The effects depend largely on the way that the intervention is designed and delivered [20]. Recently, an electronic monitoring system has been developed for the continuous registration of hand cream consumption. This system enables a detailed feedback to HCWs on the frequency and moments when the cream is used. In hand hygiene studies, a similar monitoring system was shown to improve compliance by 42% [20, 21].

As previously described in the published protocol [21], the Healthy Hands Project single centre cluster-randomized clinical trial investigate whether an intervention program, based on the provision of hand creams and regular feedback on cream consumption, can improve the skin condition of the hands of nurses engaged in wet work, when compared to a ‘care as usual’ control group. The intervention comprised of the provision of hand cream dispensers on the wards, electronic monitoring of their use, and repeated feedback to the HCW. Randomization to the intervention program or ‘care as usual’ was at the ward level. The effectiveness of the program will be assessed by measuring hand skin condition in workers before and after the study period. This trial has been registered in the Netherlands Trial Register with identification number NTR5564.

In this paper, we will provide an update on the status of the trial and present a detailed description of the proposed data analysis. This statistical analysis plan was written and submitted before the researcher analysing the data (MS) was un-blinded to the treatment allocation.

**Primary and secondary objectives**

The primary objective of the Health Hands project trial was to examine whether the provision of a skin care program for the prevention of HD improves the hand skin condition of health care workers engaged in wet work. Hand skin condition was assessed using the Hand Eczema Severity Index (HECSI) score [22]. The HECSI score grades the intensity of erythema, induration, papules, vesicles, fissures, scaling and oedema for five areas of each hand (fingertips, fingers, palms, back of hands, wrists) on a scale from 0 (not present) to 3 (severe). The extent of affected skin in each area is graded from 0 to 4. The total score is obtained by multiplying the intensity with the extent and ranges from 0 to 360 points. The primary outcome focusses on the change in HECSI score between baseline and after 12 months.
The secondary outcome is the change in levels of natural moisturizing factors in the uppermost layers of the skin, as a marker of early signs of barrier damage [23].

**Trial design**

The Healthy Hands Project trial was a single centre, cluster-randomized, open, blinded endpoint parallel group trial conducted on the wards of one University Medical Center in The Netherlands. This academic hospital has a total of 45 departments of which approximately 20 are clinical wards. Between 2 May and 14 June 2016, a total of 504 nurses on 20 wards were enrolled. The participants in this trial were randomized to the intervention or ‘care as usual’ group at the ward level. Wards were randomized two at a time in a 1:1 ratio stratified according to wet work exposure (high or low) using fixed blocks of size two. Wet work exposure was estimated at the ward level from internal purchases of soap in the period January 2016 to May 2016. Nurses and some investigators (SK) were not blinded to the allocated treatment group, but the primary outcome at 12 months was assessed by an investigator (MS) blinded to treatment outcome.

This Statistical Analysis plan (SAP) focuses on the analysis of primary and secondary outcomes measured at baseline and 12 months. The extended follow-up period of six months (i.e. 12-18 months) stated in the flow diagram (fig. 1) will be used to assess process outcomes, and will be out of the scope of the SAP described in the present article.

The inclusion criteria were: willing to give informed written consent; aged 18 to 65 years at the start of the study; having daily exposure to wet work activities during work; and being employed as a nurse or nutrition assistant on a participating ward. The only exclusion criteria was being employed on more than one ward.
Fig. 1. Flow diagram Healthy Hands Project

Intervention

The intervention comprised of the provision of hand cream dispensers on the wards, electronic monitoring of their use and feedback to the health care workers. In the intervention group, the hand cream (Stokoderma® Aqua Sensitive) was provided in electronic dispensers with an electronic monitoring system on
the wards at places which are easily accessible. Data on patterns of usage (frequency, timing of use, total consumption) and trends enabled structured feedback on hand cream use to the nurses and management to motivate and improve compliance. Posters, designed to present the compliance data of the cream use, were placed at the wards to remind staff of their performance and motivate to use the creams. Both the intervention and the control group received education on skin care (as stated in Table I of our published protocol (21) on skin protection (as ‘care as usual’) every three months from baseline to the end of study.

**Table 1. Characteristics of participants in the intervention and control group**

| Characteristic                           | Control Group | Intervention Group |
|------------------------------------------|---------------|--------------------|
| **Type of ward** (n =)                   |               |                    |
| **Number of participants** (n =)         |               |                    |
| **Sex** (n (%)) female                   |               |                    |
| **Working years** (median (*IQR))        |               |                    |
| **Hours worked per week** (mean (*SD))   |               |                    |
| **History of atopic dermatitis** (n (%)) |               |                    |
| **Outcomes**                             |               |                    |
| **Median *HECSI-score** (*IQR)**         |               |                    |
| **Mean *NMF levels** (*SD)**             |               |                    |
| **Frequency of use of hand alcohol** (n (%)) |           |                    |
| **Frequency of hand washing** (n (%))    |               |                    |
| **Frequency of glove use** (n (%))       |               |                    |
| **Frequency of use of moisturizers** (n (%)) |           |                    |

*Characteristics of participants in the intervention and control group at enrolment.* *HECSI Hand Eczema Severity Index, NMF natural moisturising factor, IQR interquartile range, SD standard deviation*

**Data collection and outcomes**

The flowchart of the study design is shown in Fig. 1. After inclusion, the participants filled in the baseline questionnaire and underwent baseline measurements (skin condition assessment by using the Hand Eczema Severity Index (HECSI) and collection of SC samples for Natural Moisturizing Factor (NMF) analysis). The measurements were repeated at 12 months after baseline. The primary outcome is the difference in HECSI score between baseline and 12
months. The secondary outcome is the difference in levels of NMF in the skin between baseline and 12 months.

Statistical methods specified in the protocol

Sample size calculation
We planned to include a total of 34 wards with on average 16 employees on each ward (a total of 544 employees) [21]. The sample size calculation was based on the expected change in the Osnabruck Hand Eczema Severity Index score between T0 and T12. A difference in the Osnabruck Hand Eczema Severity Index score of 0.4 points is regarded as clinically significant and a previous study has shown that the standard deviation of this difference was 1.2 [24]. Using a two-sided t-test with a significance level of 0.05, degrees of freedom based on the number of wards and intra-cluster correlation of 0.05, we calculated that a study with 17 wards per treatment group and 16 employees per ward would have 81% power to detect a difference of 0.4 in group means. We based the sample size calculation on the Osnabruck Hand Eczema Severity Index rather than the HECSI score because in contrast to the former, no studies with the HECSI score in health care workers were available. This choice was supported by the fact that both scoring systems have similar content, and furthermore it has been shown that these two scores are well correlated (r=0.85; P<0.001)[25].

Proposed analyses
As stated in the published trial protocol a full statistical analysis plan will be published before the researchers are unblinded [21]. We specified that we would adhere to the Consolidated Standards of Reporting Trials (CONSORT) Statement [26] and its extensions on the reporting of patient reported outcomes in randomized trials [27] and on cluster randomized trials [28]. Due to the low risk nature of this study, no interim analyses or safety reporting were planned and no data safety monitoring board was installed.
Statistical analysis plan

Sample size calculation
In the protocol, we specified that we would include 34 wards with on average 16 employees on each ward (total of 544 employees) [21]. However, we actually included 20 wards, with an average of 25 participants per ward (total 504 participants). Consequently, assuming that all participants have provided baseline and 12-month data, the power would have fallen from 81% to 66%. If, in addition, HECSI data for both baseline and 12 months would have been only available from an average of 16 participants, the power would decrease to 56%. These power calculations were performed in PASS 15 (NCSS, LLC Kaysville, Utah, USA).

Overall principles
The data analysis will start after the 12-month follow-up data are available for all participants or it is clear that any participants, for whom no 12-month data are available, have dropped out of the study, and the study database has been cleaned and locked for this time point. All analyses will be performed by analysing participants in the trial arm, to which they were allocated in the ward level randomisation. The analyses will be first performed blinded to treatment allocation to allow the data and proposed analyses to be checked. Treatment allocation will only be unmasked when all data cleaning and analyses to be presented have been finalised.

We will present the characteristics of wards and participants using simple descriptive statistics. We will use the mean and standard deviation to describe normally distributed continuous variables and the median and upper and lower limits of the interquartile range to describe non-normally distributed continuous variables. We will assess the normality of continuous variables by visually inspecting histograms. We will use counts and percentages to present categorical variables. Two-sided p-values of less than 0.05 will be considered statistically significant and statistical uncertainty will be expressed using two-sided 95% confidence intervals. No formal statistical testing will be performed to examine differences in baseline characteristics between the trial arms. The analyses will be performed by one of the investigators (MS) supervised by the other investigators (SK, JS) and a statistician (RH). All statistical programming and analysis will be performed using IBM SPSS statistics version 24 (IBM Corp., Armonk. NY, USA).

Analysis populations and units
A true intention-to-treat population would include all participants randomized. However, due to substantial loss to follow-up in this study, we will perform the main
analyses on a modified intention-to-treat population. This population will consist of all the participants with a HECSI score at baseline and 12 months. The per protocol population will consist of all participants with a HECSI score at baseline and 12 months and who worked in the same ward for the whole duration of the study.

**Handling of missing data**

In our main analyses for the primary outcome, we will use a simple joint model approach to model the missings of the HECSI scores at 12 months and the observed difference between the HECSI scores at baseline and 12 months. We will perform three types of sensitivity analyses on the way we have dealt with missing data on the primary outcome. For participants with missing HECSI score data at 12 months, we will: 1) assume the best possible outcome (HECSI score of 0); 2) assume the worst possible outcome (highest observed HECSI score); and 3) perform multiple imputation for the difference between baseline and 12 month HECSI scores. We will use on baseline characteristics as independent variables in the multiple imputations.

**List of analyses**

*Recruitment and retention and baseline characteristics*

We will present the numbers of wards and employees assessed for eligibility, included, randomised to the intervention and control arms and lost to follow-up in a CONsolidated Standards of Reporting Trials (CONSORT) flow diagram (see figure 1). We will present the baseline characteristics of all randomized participants in each arm in a table, without performing formal statistical testing, including the type of ward, working years, working hours, sex, atopic tendency, self-reported hand dermatitis last month, NMF and HECSI.

*Deviations and violations from protocol*

No major deviation or violations from protocol occurred. The main difference from protocol is the number of wards included (20 wards), while the sample size calculation was based on 34 wards.

*Primary and secondary outcomes*

We will present crude means and 95% confidence intervals for the changes in HECSI score and levels of NMF between baseline and 12 months for the intervention and control groups. In addition, we will present crude proportions of participants with both baseline and 12 months HECSI scores for both groups.
We will obtain p-values for the difference between the intervention and control groups using generalised estimating equations with an exchangeable working correlations matrix to account for clustering within wards. We will use a linear model for the changes in HECSI score and levels of NMF and a binary model with a logit link function for the missing data. We will adjust the analysis of the primary outcome for the binary factor ward level exposure to wet work in the preceding year, used to stratify the wards in the randomization.

**Sensitivity analysis**

In addition to the three sensitivity analyses on the handling of missing data, we will perform a sensitivity analysis on the effect of exposure to wet work observed during the study. No subgroup analyses will be performed.

**Current trial status**

The Healthy Hands Project included 20 wards and 504 participants in The Netherlands from 2nd of May 2016 till approximately July 2016. The participants were followed up for 18 months. At the time of submission, the trial data are blinded to treatment allocation and the trial has not completed follow up of the last participant (end of study planned for January 2018). The data will be cleaned and checked for completeness and internal consistency, blinded to treatment allocation. The database will only be locked after this statistical analysis plan has been submitted for publication.

**Discussion**

The background to and methods for the Healthy Hands Project single centre cluster-randomized clinical trial have been previously described in the published protocol [21]. In this paper, we have provided an update on the status of the trial and presented a comprehensive statistical analysis plan for the resulting data. In addition, we have clarified differences between the previously published protocol and the proposed actual statistical analyses. The principal difference from the methods presented in the protocol is the actual inclusion of 20 rather than 34 wards and 504 rather than 544 participants. Consequently, the power has fallen substantially. This paper supports transparency in reporting in the Healthy Hands Project by clarifying differences between the previously published protocol and the proposed actual statistical analyses.
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Effectiveness of a skin care program for the prevention of contact dermatitis in healthcare workers (the Healthy Hands Project): a single centre cluster randomized controlled trial

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Contact Dermatitis. 2019 Jan 16 [Epub ahead of print]
Abstract

Background
Healthcare workers are at risk for developing hand dermatitis (HD). Guidelines recommend moisturizers to prevent HD, however in practice their effectiveness is poorly investigated.

Objectives
To assess whether an intervention aimed at improving skin care leads to reduction in HD severity.

Methods
In this 1-year RCT, 9 wards (285 HCW) were allocated to an intervention group (IG) and 10 wards (216 HCW) to the control group (CG). The intervention included provision of cream dispensers with electronic monitoring of use, regularly communicated to the HCW. The primary and secondary outcomes were change from baseline in the Hand Eczema Severity Index-score (ΔHECSI-score) and Natural Moisturizing Factor (ΔNMF) levels.

Results
At 12 months, lost-to-follow-up was 41% and 39% in IG and CG, respectively. The HECSI was reduced in the IG by -6.2 (95%CI -7.7,-4.7) and in the CG by -4.2 points (95%CI -6.0,-2.4). There was no difference in ΔHECSI or ΔNMF between groups. Relative improvement in HECSI was significantly higher in the IG than in CG (56% vs. 44%). In a subgroup of HCW with mild HD, IG showed larger HECSI decrease than CG (P<0.001)

Conclusion
Although there was no significant effect on the primary outcomes, the intervention showed overall positive effects on the HECSI.
Introduction

Occupational skin diseases (OSD), mainly comprised of irritant contact dermatitis (ICD), are common work-related diseases, mostly localized on the hands(1). OSD impact the quality of life of affected workers and pose a serious threat for workability as well as a high burden in the socio-economic context. In the Netherlands annual costs of medical care, absenteeism and disability pensions due to OSD in 2011 have been estimated at €98 million.(2) Continuous exposure to ‘wet work’ and its deleterious effect on the skin barrier has been identified as a major risk factor for OSD, in particular irritant contact dermatitis (ICD) on the hands. One of the most affected populations are healthcare workers (HCW), with an estimated point prevalence of 12-30%.(3, 4) The risk for occupational hand dermatitis (HD) in health sector is increasing, as infection hand hygiene has become more rigorous and use of hand sanitizers and hand washing has been intensified to prevent infections.(5) Next to the adverse effects on the quality of life of affected HCW, HD can negatively influence the safety of the patients. Recent studies reported that HCW with HD avoid use of hand disinfectants due to stinging sensation when using disinfectants on damaged skin and belief that disinfectants will further aggravate the symptoms. (6) Obviously, prevention of HD in health sector is absolutely critical for the sake of HCW as well for the patients. In several countries, national guidelines for the prevention of OSD have been established following common hierarchal structure in prevention of occupational diseases: first elimination of hazards or reduction of exposure by technical and organisational measures have to be applied, when this is not sufficient, also personal protection and behavioural measures. However, in the healthcare sector elimination or reduction of the hazards is difficult due to hygienic requirements.(7) Substitution of the main risk factor, hand washing, by hand disinfectants has been proposed, but hand washing can not completely be avoided as it is strictly prescribed by hygienic protocols. (8) As skin barrier damage is the etiological factor in ICD, several guidelines emphasize the importance of the maintenance of a competent skin barrier in its prevention. Thus, the guidelines of the Dutch Society of Occupational Medicine (NvAB) recommend use of emollients to maintain the competent skin barrier. (9) In recent guidelines of the European Society of Contact Dermatitis (ESCD), application of moisturizers on hands is recommended during the working day but especially after work and before bedtime.(10)

Although experimental studies have demonstrated skin barrier enhancing effects of emollients in ICD,(11) there are very few studies on their efficacy
in occupational setting and the effect of emollients has mainly been studied as a part of multifaceted interventions. (12, 13) The use of emollients in the workplace, however, remains low in general.(14)

This study focused therefore on improving hand cream use in HCW to prevent hand dermatitis symptoms. The intervention strategy was inspired by experiences from hand hygiene studies reporting positive effects of continuous monitoring and feedback to improve compliance.(15) Feedback is in particular suggested to be effective to improve performance when: 1) baseline performance is low; 2) when provided by a supervisor; 3) when it is provided more than once; and 4) when it is provided both verbally and in written form. (16) Group monitoring is widely recognized as being more effective than other monitoring systems that track individuals’ actions, which can be seen by staff as punitive or an invasion of their privacy.(17) Recently, we reported on an electronic monitoring system developed for the continuous registration of hand cream consumption with electronic dispensers at wards.(18) This system enables detailed feedback on the frequency and time-pattern of hand cream use on wards. In the present study we aimed to assess the effectiveness of this system in a randomized controlled trial (RCT). The intervention comprised of installing dispensers with hand cream on the wards, electronic monitoring of use, and repeated feedback. The effect of the intervention is measured as the change in clinical severity of HD between baseline and follow up at 12 months, compared with the control group. Furthermore, as a secondary outcome we determined the change in the stratum corneum levels of natural moisturizing factors (NMF) as a potential sensitive biomarker of skin barrier damage. Cluster randomisation was used to minimise the threat of ‘treatment contamination’. This paper focuses on the primary and secondary outcomes of the intervention. We will report the effects of the intervention on cream use behaviour in a separate paper.

**Patients and Methods**

The Healthy Hands Project (HHP) is a single centre, cluster-randomized, parallel group controlled trial with blinded outcome assessments. We described the design(18) and statistical methods(19) in depth previously.

The participants in this study were healthcare workers (HCW) clustered in wards of the Amsterdam University Medical Center, Amsterdam, Netherlands. They were enrolled between May 2nd and June 14th 2016. Only wards known for having substantial exposure to wet work were included. These wards were
categorized as low or high exposure wards based on the soap purchase in the previous year. The eligibility criteria were providing written informed consent, being employed as a HCW or nutrition assistant on one of the wards and being exposed to ‘wet work’.

**Intervention**

The intervention comprised of provision of dispensers with hand cream placed at accessible locations at wards, continuous electronic monitoring of cream use and feedback on the cream use at ward level. The feedback was provided by means of posters reflecting the compliance of HCW to the skin care recommendations they were given. The HCW were instructed to use hand cream at least two times per shift, preferably before starting a shift, following wet work activities or after finishing a shift. The hand cream in the electronic dispensers, Stokoderma® Aqua Sensitive from Stoko-Deb Group, was a perfume- and silicone-free soft cream, with no particular pharmacological function. The main ingredients were moisturizing compounds, like glycerol and urea.(18)

Both intervention and control groups received basic education on skin care and skin protective behaviour every three months from baseline to end of study. This education took the form of a short small-group lesson lasting five to 10 minutes.

The objective was to assess the effectiveness of the intervention in reducing the severity of hand dermatitis in HCW exposed to wet work. The primary outcome was the difference in the Hand Eczema Severity Index-score (HECSI-score) between baseline and 12 months follow-up (ΔHECSI). The secondary outcome was the difference in natural moisturizing factor (NMF) levels determined in the stratum corneum samples collected at baseline and follow-up (ΔNMF).

**Assessment**

At baseline, HCW completed a questionnaire on self-reported atopic dermatitis, here defined as a history of flexural dermatitis on the elbow or knees, a history of hand dermatitis, glove use, frequency of hand washing, and use of hand alcohol and hand creams at work. All outcomes were evaluated at the individual level.

The clinical assessment of the hands was performed by a trained physician (MS) using the HECSI-scoring system.(20) The HECSI grades the intensity (scale 0-3) of clinical symptoms including erythema, vesicles, fissures, scaling, papules and oedema in combination with the extent (affected area) of disease (scale 0-4) per area of the hand (fingertips, fingers, palms, back of hands, wrists). For the total score the extent per location was multiplied by the intensity of all clinical
symptoms and summed.(20) The HECSI-score ranges from 0 (no dermatitis) to 360 (extremely severe dermatitis). The severity of disease was classified as mild (0 to 11 HECSI points), moderate (12 to 27 points) and severe (28 or more points) dermatitis.(21) The minimal detectable change (MDC) in HECSI score has been defined according to Norman et al. (22) as 0.5 times the standard deviation of the mean value of ΔHECSI.(22) The MDC represents the smallest change that can be detected by a measurement instrument that corresponds to a true change rather than due to measurement error.

**NMF analysis**

The stratum corneum samples for the NMF analysis were collected by a tape stripping technique. Round, adhesive tapes (3.8 cm2, D-Squame; CuDerm, Dallas, TX, U.S.A.) were attached to dorsal part of the dominant hand and pressed on for five seconds with a standardized force (D500, D-Squame Pressure Instrument; CuDerm). The tape stripping from the same skin site was repeated with a new tape five consecutive times. For the NMF analysis the 5th consecutive tape was used. (18, 23) For the analysis, a slightly modified method of Dapic et al. has been applied. (24) Briefly, NMF was extracted from the tapes by two successive extractions with 0.5 ml water. The concentration of NMF in the extracts has been analysed by high performance liquid chromatography with UV-detection (HPLC-UV).(24) In contrast to the published protocol, the protein levels on the tape used to compensate for variable amount of the SC removed by a tape were not estimated from the values of optical density because visual inspection of the tapes revealed that the SC was not evenly distributed over the tape, which is the prerequisite for applying this method. Instead, the protein amount on the tape was determined in the extracts using a Pierce BSA assay.(24) The NMF levels were expressed as mmol g⁻¹ protein.

**Randomization and statistical analysis**

We based the sample size calculation on the Osnabruck Hand Eczema Severity Index score (OHSI)(25), as there were no studies available on primary prevention in which HECSI was used and the two scores are well correlated (r=0.85; P < 0.001). Using a two-sided t-test with a significance level of 0.05, we calculated that a study with 17 wards per treatment group with 16 healthcare workers per ward (a total of 544 healthcare workers) would have 81% power to detect a difference of 0.4 in group means(25), assuming a standard deviation of 1.2
points and an intra-cluster correlation of 0.05. (18) These power calculations were performed in PASS 15 (NCSS, LLC Kaysville, Utah, USA).

The HCW were randomized to the intervention or control groups at the ward level. Wards (as the unit of randomization) were randomized in fixed size blocks of two and stratified into “high” or “low” levels of exposure to “wet work”. Wet work exposure was estimated at the ward level from the quantity of soap purchased in the period January to May 2016. The first half of the wards with highest soap purchase were categorized as high-exposure, the lower half as low-exposure. The wards were randomized using a secure Internet-based service (www.sealedenvelope.com). The randomisation sequence was generated by the principal investigator (SK), who was not involved in enrolling HCW or assessing outcome measurements. The researcher (MS), who performed the outcome measurements and statistically analysis, was blinded for treatment allocation until all data had been collected and cleaned and a statistical analysis plan had been published. The HCW were not blinded to treatment allocation.

A statistical analysis plan has been described in depth previously. (19) Briefly, characteristics of wards, working years, working hours, sex, self-reported atopic dermatitis, self-reported hand dermatitis past year, and baseline values of NMF and HECSI are presented by using descriptive statistics and no formal statistical testing was carried out. We used counts and percentages to present categorical variables. For continuous variables the mean and standard deviation (normally distributed data) or the median and interquartile ranges (deviation from normal distribution) were used. The intraclass-correlation (ICC) was also calculated. Two-sided p-values of less than 0.05 were considered statistically significant and statistical uncertainty was expressed using two-sided 95% confidence intervals. The analyses were performed by an investigator (MS) supervised by the other investigators (SK, JS) and the Clinical Research Unit of the AMC. All statistical analysis was performed using IBM SPSS statistics version 24 (IBM Corp., Armonk. NY, USA). Writing this manuscript, we adhered to the CONSORT statement. (26, 27)

The main analyses of the primary and secondary outcomes was performed on a ‘modified intention-to-treat’ population, consisting of all participants with a HECSI score at baseline and 12 months. The per protocol population consisted of all participants with a HECSI score at baseline and 12 months and who worked in the same ward for the whole duration of the study. We present crude means and 95% confidence intervals for ΔHECSI and ΔNMF for the intervention and control groups. To model the missingness of ΔHECSI values when HECSI-scores at 12 months were not available, we used a simple joint model approach. We
used a linear model for ΔHECSI and ΔNMF and a binary model with a logit link function for the missing data.

We obtained $P$-values for the difference between the intervention and control groups using generalised estimating equations with an exchangeable working correlations matrix to account for clustering within wards. We adjusted the analysis of the primary outcome for the binary stratification factor ward level exposure to wet work in the preceding year.

We performed a sensitivity analysis using the observed (rather than predicted) ward level exposure to wet work and for missing data on the primary outcome at 12 months. Furthermore, sensitivity analysis has been carried out for participants with missing HECSI score data at 12 months. For this purpose we performed three types of sensitivity analysis: 1) assuming the best possible outcome (HECSI score of 0); 2) assuming the worst possible outcome (highest observed HECSI score); and 3) applying multiple imputation using baseline characteristics for the difference between baseline and 12 month HECSI scores.

As some intervention studies reported relative change in disease severity(12), we performed a post-hoc analysis of the relative change of HECSI from baseline. This was calculated by ($\Delta$HECSI /HECSI T0) * HECSI T0. Furthermore, we used the minimal detectable change (MDC) as proposed by Norman et al. to define meaningful improvement in HECSI in this trial. (22) The MDC has been calculated as $(0.5* SD) = 0.5*10.2 = 5.1.$

## Results

The study flowchart is shown in Figure 1. We included 501 HCW from 19 wards; on average 6 to 58 HCW per ward were included. Nine wards with a total of 285 HCW were allocated to the intervention group (IG) and 10 wards with a total of 216 HCW to the control group (CG). Nine wards with a total of 169 HCW in the intervention group and 10 wards with a total of 121 HCW in the control group had high exposure to wet work (Table 1). All other wards and HCW were classified as having low exposure to wet work.

At the 12 months follow-up, all randomized wards remained in the study. However, 118 (41%) HCW in the intervention group and 84 (39%) in the control group had been lost to follow-up. The proportion of HCW lost to follow-up was similar in both treatment groups ($p$-value = 0.653, ICC < 0.001). The per protocol population consisted of 299 participants (167 in IG and 132 in CG).
Fig. 1. Flow diagram of wards and employees included in the Healthy Hands Project, a randomized controlled trial

The baseline characteristics of the HCW are shown in Table 1. The proportion of males, number of hours worked per week, number of working years in a wet work occupation, proportion of healthcare workers with hand dermatitis and a history of atopic dermatitis, self-reported use of soap, hand alcohol and hand creams were similar in the intervention and control groups. The use of hand alcohol was high in both groups; 81% (405 HCW) used it at least 15 times per shift. Majority of nurses (57%, 286 nurses) reported to wash their hands at least 10 times per shift. High frequency glove use (category: more than 15 times a shift), was significantly higher in the intervention (59%, 167) than in the control (36%, 77) group. Moreover, the largest proportion of nurses (70%, 351 nurses) reported that they ‘never’ use creams before or during the shift.

At baseline, the median HECSI score was 8 (IQR 4-13) in the IG and 7 (IQR 4-12) in the CG. The numbers of HCW with no, mild, moderate or severe
The primary outcome: absolute change in HECSI from baseline

The mean decrease in HECSI scores between baseline and 12-month follow-up ($\Delta$ HECSI) was -6.2 points (95% CI -7.7 to -4.7) in the IG and -4.2 (95% CI -6.0 to -2.4) in the CG. The decrease in HECSI from baseline was significant in both groups ($p$-value < 0.0001). There was no significant difference in the change of the HD severity between the intervention and control groups; decrease in HECSI was for 2.6 points higher in the IG compared to the CG, but this difference was not significantly different between groups ($P$-value = 0.17, ICC = 0.12). The linear model revealed a significant effect ($P=0.04$) of the exposure to wet work.

The results from the sensitivity analysis using the best case ($p$-value= 0.20), worst case ($P$-value= 0.74) and multiple imputation ($p$-value =0.09) were similar to the main analysis, i.e. there was no difference in the $\Delta$ HECSI between IG and CG.

A sensitivity analysis using the current instead of historical data for wet work exposure (i.e. purchase of soap during the trial period rather than data preceding the trial) still showed a significant effect of exposure ($P=0.02$, the ICC was 0.107). Nevertheless, there was no significant difference in $\Delta$ HECSI between control and intervention group.
Table 1. Baseline characteristics of the health care workers included in the trial.

| HCW characteristics | Control group | Intervention group |
|---------------------|---------------|--------------------|
|                     | (10 wards, 216 HCWs) | (9 wards, 285 HCWs) |
| Type of wards       |               |                    |
|                     | 10 clinical wards | 8 clinical ward, 1 outpatient clinic |
| High risk wards*    | 5 wards, 121 HCW (56%) | 5 wards, 169 HCW (59%) |
| Sex n(% male)       | (33) 15% | 33 (12%) |
| Working years       | Median (IQR)* (n=498) | 11 (5-20) | 11 (5-27) |
| Hours worked per week | Mean (SD) ** (n= 499) | 31 (7) | 31 (6) |
| History of atopic dermatitis n (%) | 35 (16%) | 41 (14%) |
| History of hand dermatitis past year n (%) | 72 (33%) | 95 (33%) |
| Frequency of use of hand alcohol n (%) ; (n=499) | | |
| Less than 5 times a shift | 6 (3%) | 9 (3%) |
| 5 to 10 times a shift | 9 (4%) | 9 (3%) |
| 11 to 15 times a shift | 26 (12%) | 26 (9%) |
| More than 15 times a shift | 175 (81%) | 242 (85%) |
| Frequency of hand washing n (%) ; (n=499) | | |
| Less than 5 times a shift | 17 (8%) | 31 (11%) |
| 5 to 10 times a shift | 73 (34%) | 86 (30%) |
| 11 to 15 times a shift | 72 (33%) | 74 (26%) |
| More than 15 times a shift | 52 (24%) | 97 (34%) |
| Frequency of glove use n (%) ; (n=497) | | |
| Less than 5 times a shift | 30 (14%) | 20 (7%) |
| 5 to 10 times a shift | 48 (22%) | 34 (12%) |
| 11 to 15 times a shift | 56 (26%) | 60 (21%) |
| More than 15 times a shift | 82 (38%) | 171 (60%) |
| Frequency use of moisturising creams before shift n (%) ; (n=497) | | |
| Never              | 160 (74%) | 210 (74%) |
| About half of shifts | 26 (12%) | 23 (8%) |
| More than half of shifts | 9 (4%) | 16 (6%) |
| Almost always      | 22 (10%) | 34 (12%) |
| Frequency use of moisturising creams during shift n (%) ; (n=497) | | |
| Never              | 151 (70%) | 197 (69%) |
| About half of shifts | 43 (20%) | 48 (17%) |
| More than half of shifts | 9 (4%) | 9 (3%) |
| Almost always      | 15 (7%) | 13 (12%) |
Table 1. Continued.

| HCW characteristics | Control group (10 wards, 216 HCWs) | Intervention group (9 wards, 285 HCWs) |
|----------------------|------------------------------------|--------------------------------------|
| Type of wards        | 10 clinical wards                  | 8 clinical ward, 1 outpatient clinic |

**Frequency of use of moisturising creams after shift**

|                      | n (%) | \(n=497\) |
|----------------------|-------|-----------|
| Never                | 108 (50%) | 131 (46%) |
| About half of shifts | 26 (12%) | 22 (13%) |
| More than half of shifts | 24 (11%) | 29 (10%) |
| Almost always        | 58 (27%) | 88 (31%) |

**Median HECSI-score (IQR); \(n=501\)**

|                      |        |
|----------------------|--------|
|                         | 7 (4-12) | 8 (4-13) |

**Mean NMF levels (SD); \(n=497\)**

|                      |          |
|----------------------|----------|
|                         | 4.6 (1.4) | 4.3 (1.4) |

*Risk estimate (high or low) based on soap exposure year before trial, **IQR= Inter quartile range, **SD= Standard Deviation

HCW = Healthcare Worker, HECSI = Hand Eczema Severity Index, NMF = Natural Moisturizing Factor

Post hoc analysis of the primary outcome: relative decrease in HECSI and minimal detectable change

We performed three post-hoc analyses that were not defined in the statistical analysis plan(19): the relative change in HECSI, the minimal detectable change (MDC) for HECSI and subgroup analysis.

The relative change in HECSI from baseline to follow-up (% of the baseline value) is shown in Table 3. At follow-up the percentage change in HECSI- scores was 56% in the IG and 44% in the CG, which was significantly different between the two arms (\(P= 0.001; \text{ ICC } = 0.085\)). We calculated the minimal detectable change (MDC) to be 5.1 HECSI points, a value that has only been reached in the IG.

To explore whether HD severity plays a role in the effectiveness of the intervention, we performed a subgroup analysis based on the HECSI scores at baseline. To define the two subgroups we used a cut-off of HECSI of 11 points which was defined by Hald et al. (21) as mild dermatitis. The mean decrease in HECSI scores between baseline and 12-month follow-up (\(\Delta\) HECSI) was in the subgroup with mild HD (\(n=124\)) -3.0 points (95% CI -3.5 to -2.5) in the IG and -0.6 (95% CI -1.3 to 0.24) in the CG (\(n=95\)). In the subgroup with HECSI>11, the
Δ HECSI value was -15.6 points (95% CI -20.6 to -11) in the IG (n=43) and -13.4 (95% CI 18.8 to -8.6) in the CG (n=37). The results showed a significant effect of the intervention only in the subgroup with HECSI ≤11 matching early/mild HD (n=219).

Table 2. Classification of hand dermatitis severity in the intervention group (IG) and control group (CG) at baseline and follow-up

| Groups (n) | T0      | T12     | T0      | T12     |
|------------|---------|---------|---------|---------|
| No symptoms| IG(n=285)| IG(n=167)| CG(n=216)| CG(n=132)|
| Mild       | 199 (70%)| 129 (77%)| 151 (70%)| 104 (79%)|
| Moderate   | 63 (22%) | 10 (6%)  | 45 (21%) | 16 (12%) |
| Severe     | 19 (7%)  | 1 (1%)   | 13 (6%)  | 1 (1%)   |
| Median HECSI (IQR) | 8.00 (4.00-13.00) | 3.00 (1.00-4.00) | 7.00 (4.00-12.00) | 4.00 (2.00-6.00) |
| Median NMF (IQR)   | 4.28 (3.41-5.17)  | 3.27 (2.39-4.12) | 4.50 (3.57-5.26)  | 3.30 (2.48-4.19) |

Mild: HECSI 1-11, Moderate: HECSI 12-27, Severe: HECSI ≥ 28

Table 3. Changes in HECSI and NMF from baseline to follow up in the intervention group (IG) and control group (CG)

|            | IG       | CG       | IG vs. CG |
|------------|----------|----------|-----------|
| Δ HECSI    | -6.2 (-7.7 to -4.7) | -4.2 (-6.0 to -2.4) | ns        |
| Mean (95% CI) | 56%  | 44%      | p<0.001† |
| Relative change % |        |          |           |
| Δ NMF      | -1.0 (1.6) | -1.2 (1.6) | ns        |
| Mean (SD)  |          |          |           |

†Difference in the change of the outcome from baseline between IG and CG
(Δ HECSI, Δ NMF = respective changes of HECSI and NMF from baseline to follow-up)

Secondary outcomes

At baseline the NMF levels were similar between IG and CG. At follow up NMF levels decreased in both groups; -1.0 (SD 1.6) in the IG and -1.2 (SD 1.6) in the CG. There was no significant difference between the IG and CG.

The electronically measured cream use in the IG was on average 0.4 application events/HCW/shift during the trial.(28)
Discussion

With respect to the primary and secondary outcomes, respective changes in HECSI and NMF from baseline to follow-up, this RCT could not provide evidence of effectiveness of the intervention.

Primary outcome

Although in the present study we found no significant effect of the intervention on the main outcomes, the intervention did have overall positive effects on severity of HD symptoms, especially and significantly in specific subgroups. Decrease in HECSI-score was larger in the IG as compared to the CG (respectively 6.2 and 4.2 points). Furthermore, in contrast to CG, the decrease in the IG exceeded the estimated minimal detectable change (MDC) of 5.1 points.

As some intervention studies reported relative change in disease severity(12), we performed a post-hoc analysis, showing relative improvement in HECSI to be significantly larger in the IG as compared to CG (56% vs 44%; P<0.001). (12) To explore whether HD severity plays a role in the effectiveness of the intervention we performed a post-hoc subgroup analysis. The results revealed that the intervention did have a significant effect on HD severity in the subgroup of HCW with mild HD (HECSI≤11) (P<0.0001) while in the subgroup of HCW with moderate to severe there was no significant difference between IG and CG. This suggests that this intervention might be effective in averting progression of early skin barrier damage into clinical dermatitis, and may therefore be more suitable in primary prevention.

Intervention studies focussing on the effectiveness of skin care in high risk occupations are scarce, and often skin care is part of a multi-component prevention program.(12) The effect of moisturizers versus no treatment has previously been investigated in three studies focused on primary prevention of occupational HD, all suggesting a beneficial effect of moisturizers. (12, 13, 29) Based on these three studies, a recent Cochrane review concluded that indeed there may be a clinically important risk reduction in development of symptoms of HD (RR <0.75, 95% CI 0.46 to 1.09; 507 participants), but emphasized that the quality of evidence is low.(30)

This intervention aimed to reduce HD symptoms by improving skin care. Indeed, the intervention showed a significantly higher cream use in IG as compared to CG.(28) However, looking at the electronically collected data in the IG (28), the average frequency of 0.4 cream applications per nurse per shift was
much lower than recommended in the present study and in current guidelines (2 applications/shift).

In this trial we provided, both in the IG and CG, basic educational lessons to achieve the same level of knowledge trying to avoid differences between the two arms. As previously studied, education itself could have an positive effect on severity of HD.(2, 31) This may at least partly explain why skin symptoms were not only improved in the IG but also (although less pronounced) in the control group during the trial.

Secondary outcome
Interestingly, NMF levels in both groups significantly decreased at follow-up compared to baseline. Exposure to water and soap has been shown to reduce NMF levels by different mechanisms (32-35), however this cannot explain decrease in NMF as wet work exposure did not change during the trial. One of the possible explanations might be introduction of a new disinfectant containing n-propanol instead of the formerly used ethanol, shortly before the start of our trial. In addition to the stronger skin barrier damaging effect of n-propanol compared to ethanol (36), our recent work also demonstrated a NMF level-decreasing effect of n-propanol. (33) The introduction of this new disinfectant might also explain another discrepancy in the NMF results. Reduction of NMF has been suggested as a contributing factor for dry skin in HD and one may expect that a decrease in NMF would parallel increase in skin symptoms, which was however not the case in the present study. The new disinfectant formula contained glycerol, a frequently used humectant in skin care products.(37) Notably, glycerol was also present in the hand creams provided to the IG. It may be speculated that the addition of glycerol in the new disinfectants could have at least partly compensated the NMF-decreasing effect on skin hydration associated with propanol. These positive effects of glycerol might also have interfered with the primary outcome and may explain why HECSI also was improved in the control group at follow up.

Strengths and limitations
Strengths of our study included stratified randomization, controlling for cross-contamination and comparable groups with regard to baseline values of the primary and secondary outcomes and covariates. In contrast to studies focused on patients, we included one high risk occupational group (HCW) under real occupational conditions. Assessment of outcomes and statistical analysis were performed blinded for allocation. To our best knowledge, this is the first study to
use real-time monitoring to quantitatively assess the consumption of creams. In addition, we applied an objective and validated tool (HECSI-score) to clinically assess the severity of HD by a trained physician. A quantitative biomarker of early skin barrier damage (NMF) was included as our secondary outcome.

There are several factors that might explain the lack of effect in the present RCT. One of the main drawbacks of this trial is the substantial loss of power due to overestimation of the number of available clusters, higher lost-to-follow-up and ICC values than estimated. Driven by organizational constraints during recruitment, we had to regard several sub-departments together as one cluster. Although these wards were at different locations, they shared the same management and cross-contamination was likely as HCW rotated within such large clusters. However, this aspect was not foreseen when power was calculated, leading to a considerable loss of power (post hoc power of 56%) because of the lower number of clusters that could be included. The included number of HCW, however, was very close to the planned sample size.

At the individual level there was a considerable lost-to-follow up, which was similar in both groups (41% in the IG and 39% in CG). We did not systematically record reasons for drop out, but from informal discussions with the supervisors, the most reported reasons for loss to follow-up were change of ward or job during the trial period, sick leave, maternity leave, annual leave during final measurements period at follow up or simply no desire to participate anymore. The sensitivity analysis revealed that loss-to-follow-up was independent of severity of hand symptoms and therefore the risk of attrition is not likely.

This trial was not designed to exclude atopic dermatitis of the hands, allergic contact dermatitis, nor non-occupational hand dermatitis (e.g. due to wet-work activities in leisure time). It is known that several types of occupational hand dermatitis exist, which overlap and are difficult to distinguish. (38)

Moreover, another factor that might have diluted the effect of the intervention with regard to severity is the mild symptoms of HD (mostly only slight erythema) observed in most HCW at entry, implying that there was limited room for improvement. As our study population was a working population, the HECSI scores were markedly lower than in patients in secondary prevention studies.(21) According to the classification proposed by Hald et al.(21), our study population was considered to represent mainly mild dermatitis (HECSI-score <11). There is no clear lower threshold value for HECSI to define HD as there is no clear agreement on when irritant skin changes are defined as HD. As emphasized by Bauer et al. (30) in a recent Cochrane study, future research should be directed towards further developing and validating hand dermatitis scoring systems for
a better and standardized discrimination of irritant skin changes from irritant contact dermatitis.(30)

Finally, the change in hand alcohol (from an ethanol to a propanol-containing formula and the addition of glycerol) on the wards just before this trial started is also considered a limitation. This is relevant as it may have influenced both primary and secondary outcomes.

**Conclusion**

This is the first trial to report the effectiveness of a prevention program in the healthcare environment focused on facilitation of creams combined with continuous monitoring and feedback on skin care performance. This intervention was reported to improve hand cream use, as is published separately.(28) However, the intervention group did not show significantly higher improvement in the primary (ΔHECSI) and secondary outcome (ΔNMF) as compared to the control group. Still, the intervention proved overall positive effects on severity of HD symptoms, supporting the benefits of creams in the workplace, in particular in HCW with mild HD.

As occupational health interventions tend to be complex and dependent on context, evaluation strictly based on the primary and secondary outcome in the total group might not reflect the overall benefit of the intervention.(39) In the present study we did not focus on the barriers and facilitators for hand cream use, however understanding why cream use, despite its improvement during the trial, still remains quite low is intriguing. To design successful HD prevention strategies in the future further investigation of these factors is needed.

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**Supplementary table 1. Symptoms of hand dermatitis in the intervention group (IG) and control group (CG) at baseline and follow-up**

| Symptoms    | T0          | T12         | T0          | T12          |
|-------------|-------------|-------------|-------------|--------------|
|             | IG (285)    | IG (167)    | CG (216)    | CG (132)     |
| Erythema    | 261 (92%)   | 98 (58%)    | 200 (93%)   | 111 (84%)    |
| Infiltration| 31 (11%)    | 9 (5%)      | 19 (9%)     | 6 (5%)       |
| Vesicles    | 9 (3%)      | 1 (1%)      | 18 (8%)     | 4 (3%)       |
| Fissures    | 94 (33%)    | 23 (14%)    | 81 (38%)    | 55 (42%)     |
| Scaling     | 141 (50%)   | 68 (41%)    | 123 (57%)   | 100 (76%)    |
| Oedema      | 1 (1%)      | 1 (1%)      | 4 (2%)      | 0% (3%)      |
Evaluating the effect of electronic monitoring and feedback on the hand cream use in health care workers: Healthy Hands Project

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Abstract

Background
Healthcare workers (HCW) are at high risk for developing hand dermatitis (HD). Current guidelines on HD prevention recommend use of emollients; however, in practice adherence is poor.

Objective
To assess whether provision of creams, electronic monitoring and feedback on cream consumption can improve skin care in HCW.

Methods
A cluster randomized controlled trial was conducted on 19 academic hospital wards including 501 HCW during 12 months. The intervention wards (n=9; 285 HCW) were provided with hand cream dispensers equipped with an electronic system to monitor use, which was regularly communicated to the HCW using posters. The process outcomes were self-reported cream consumption in both groups and electronically measured consumption per ward in the intervention group (IG) vs the control group (CG).

Results
The self-reported cream use at follow up was significantly higher in the IG compared to CG, before (OR=2.27; 95%CI 1.29-3.97; p=0.004) and during (OR=3.30; 95%CI 1.80-6.06, p<0.001), the shift, while at baseline there was no difference between the groups. In the IG, the electronically measured cream use was on average 0.4 events/shift/HCW.

Conclusion
The intervention improved hand cream usage and therefore may be considered as a practical strategy to promote skin care in HCW. Notwithstanding, application frequency remained lower than recommended in the present study and current guidelines.
Introduction

Prevention of hand dermatitis (HD) in the health care sector is critical for health care workers (HCW) as well as for the safety of patients. National guidelines for the prevention of occupational skin diseases (OSD) have been established in various countries following common hierarchal structure in prevention: elimination, reduction of exposure to the hazard or replacement of a hazardous substance by organisational or technical measures, and when this is not sufficient, also applying personal protection and behavioural measures.1 In many health care settings, avoidance of irritants is difficult due to specific patient care workflows and hygienic requirements. In these settings personal protection such as gloves and use of emollients is considered as the primary measure to maintain a competent skin barrier. Thus, the guidelines of the Dutch Society of Occupational Medicine (NVAB) recommend regular use of emollients, ideally after a wet-work activity, to enhance the skin barrier and prevent hand dermatitis (HD). 2 In a recent guideline developed by a working group of the European Society of Dermatology (ESCD) application of moisturizers on hands is recommended during the working day but especially after work and before bedtime.3 A recent Cochrane review, reported that moisturizers may result in a clinically important protective effect.4 However the main obstacle remains poor adherence to hand care recommendations.5 To improve skin care in HCW, we developed an intervention based on provision of hand cream, continuous electronic monitoring and repeated feedback of cream use to the wards. A similar approach has been applied in the intervention studies on hand hygiene, showing improvement in compliance.6 In general, monitoring and feedback are widely used as a strategy to induce behaviour change and have been shown to be in particular effective when: 1) baseline performance is low; 2) the source is a supervisor; 3) it is provided more than once; and 4) it is provided both verbally and in written form.7,8 Group monitoring is widely recognized as being more effective than other monitoring systems based on tracking individuals’ actions, which can be seen by staff as punitive or a breach of their privacy, and which do not exploit the powerful effect of peer group coherence.9

The primary outcome, change in HD severity, will be reported in a separate paper.10 Here we focus on the process outcome defined as the effect of the intervention on the use of hand creams. Further, practical aspects regarding favoured time and location of cream use will be discussed in the context of current recommendations.
Methods

The study population consisted of 501 HCW recruited from 19 wards and allocated to the intervention or control arm. Randomization was performed at ward level and the study was run between May 2016 – December 2017. Inclusion criteria were written informed consent and employment as a nurse or nutrition assistant at one of the included wards with exposure to ‘wet work’. Participants were excluded if they were employed at more than one ward during the trial period.11 Ethics approval to conduct the trial has been granted by the Medical Ethics Review Board of the Academic Medical Center (AMC) (reference number NL54372.018.15). Trial registration: NTR 5564, date of registration, November 2, 2015. An informed consent to participate in the trial was obtained from all participants.

Intervention

The study design has been described in detail in a previously published study protocol and flow chart.11 The intervention group was provided with hand cream-dispensers placed at accessible locations at wards, continuous electronic monitoring of cream use and feedback on the frequency of cream use on ward-level. The feedback was regularly provided by means of posters presenting the compliance on ward level to skin care recommendations, that is, application minimum 2 times/shift.

Education lessons

Education on skin care and protection was provided in both intervention group and control group by the research team in the form of lessons every 3 months from baseline to the end of study. More than 1 session was planned per ward each time to reach as many HCW as possible. The research team consisted of a physician and trained medical students. The HCW on each ward were invited via the nursing manager to join the lesson (approximately 5-10 minutes) held at the wards. In small groups (5-20), HCW were trained in basic knowledge about the skin, the development of hand dermatitis and recommendations for skin protection and skin care, as proposed by the NVAB guidelines.2 Based on the ‘3 moments of skin cream application’- approach, as recently proposed, 12 the HCW were recommended to apply creams at three moments; 1) before starting a shift 2) after washing hands and 3) after work, with setting a goal of at least 2 times/shift.
Electronic monitoring of cream consumption

Hand cream (Stokoderm Aqua Sensitive, Deb-STOKO Europe GmbH, Krefeld, Germany) was provided in electronically enabled dispensers at the wards in the IG on several most accessible locations. Per ward on average 5-10 dispensers were located at sinks next to hand alcohol dispensers, in the toilet, staff room (where staff has meeting or takes breaks), at entrance of ward, corridors, patient rooms and medication rooms. The electronic monitoring system (DebMed GMS System) registered real-time of use of creams for each dispenser, allowing insight in the total consumption of creams per ward, frequency of application and detailed pattern of use regarding time of the day and location. The system allows electronically enabled dispensers to communicate via a wireless network to local hubs and then via a 3G signal to a remote server where data is collated and can be retrieved for analysis (Fig. 1). The system also includes analysis software and web based reporting tools to provide user centred feedback with the data.

Feedback

Electronically acquired data on cream consumption and trends (e.g. total number of hand cream application events, popular moments or periods of use and popular locations) were used for feedback reports to the management. Per protocol this feedback was intended to reach the HCW via the managing nurse during regular meetings of the staff. As this didn’t seem to be feasible for the managing nurses, we switched after the first feedback session to a visual prompt directly available to all HCW. For this purpose, every month, starting from April 2017, workplace posters presenting the compliance data were placed at noticeable locations at the wards to remind staff of their performance and motivate them to reach their skin care goals (Fig. 2). On the poster, the compliance rates of the past month were graphically presented in two colors: showing instantly whether compliance was improved (green) or worsened (red) compared to last month. Furthermore, on the posters a reminder to use creams at least two times per shift was stated.
Fig. 1. DebMed® GMS™ installation System

Fig. 2 Feedback Posters
Data collection

Indicators aimed at detecting change in behavior towards enhanced skin care were assessed using questionnaires in both groups and electronic monitoring of cream consumption in the IG. Questionnaires were completed at baseline and follow up to record individual consumption of creams and individual exposure to skin irritants (the estimated frequency of handwashing, use of hand sanitizers and glove use per shift). The electronic monitoring system provided real time registration of application events per dispenser.

Questionnaires were taken at baseline and after 6, 12 and 18 months. The questionnaires at baseline and 12-months were completed by HCW and collected by the research team during the organized visits on the wards. The 6- and 18-month questionnaires were delivered to the team manager at the wards who were asked to distribute them among the HCW after explaining the purpose of the survey. The questionnaire at baseline included general data including gender, period of employment as a HCW, ward, history of atopic dermatitis and hand dermatitis. Furthermore, it included questions regarding exposure to wet work (estimated frequency of handwashing and use of hand rubs and gloves per shift) and hand cream use before, during and after the shifts. At 12 months, demographic questions were omitted, but additional questions were asked addressing attendance during the education sessions and visibility/memorability of the feedback posters. At 6 months only questions regarding wet work and hand care were asked. The questionnaire at 18 month included some additional questions regarding individual perceptions regarding the use of creams during work and reach and acceptance of the intervention (attendance and opinions on the effects of the educational sessions and the feedback posters in this trial).

Statistical analysis

The HCW were randomized to the intervention or control groups at the ward level. Wards (as the unit of randomization) were randomized in fixed size blocks of two and stratified into “high” or “low” levels of exposure to “wet work”. Wet work exposure was estimated at the ward level from the quantity of soap purchased in the period January to May 2016. The first half of the wards with highest soap purchase were categorized as high-exposure, the lower half as low-exposure.

Characteristics of wards, working years, working hours, sex, self-reported hand dermatitis in the last half year, self-reported use of creams, alcoholic hand rubs and handwashing are presented by using descriptive statistics and no formal statistical testing was carried out, except for cream use data. We used counts and percentages to present categorical variables. P-values of less
than 0.05 were considered statistically significant and statistical uncertainty of descriptive measures was expressed using two-sided 95% confidence intervals. The analyses were performed by an investigator (MS) supervised by the principal investigator (SK). All statistical analysis was performed using IBM SPSS statistics version 24 (IBM, Armonk, New York).

The analyses on the cream consumption at baseline was performed in all participants, while the difference in cream consumption between IG and CG at follow up was performed in HCW who completed the follow up questionnaires (per protocol population). We obtained odds ratios (OR) and P-values for the difference between the IG and CG using mixed-effect ordinal regression analysis with exchangeable working correlations matrix to account for clustering within wards. For association analysis, we calculated non-parametric correlation (Spearman’s rho, \( \rho \)) between frequency of moisturizing hands before/during and after shift.

Results

At baseline, 501 HCW were recruited from 19 wards randomized into an intervention group (IG) (9 wards, 285 HCW) and control group (CG) (10 wards, 216 HCW). The demographic characteristics of the study population are described in details elsewhere. At baseline, there were no marked differences concerning a history of self-reported hand dermatitis, exposure to wet work and hand cream use between the two arms (Table 1).

At baseline, all 501 participants were informed on the design and goals of the study and completed a baseline questionnaire. At 12 months follow up, the response rate was 59% (167 HCW) in the IG and 61% (132 HCW) in the CG completing the 12 month- questionnaire (“per protocol” population). At 18 months 61% (102 HCW) of the “per protocol” population in the IG returned the questionnaires and 56% (74 HCW) in the CG. For the “intention to treat” population this was respectively 36% and 34% at 18 months. At 6 months less than 25% of the questionnaires were returned.

In total, as planned, 6 small group education sessions were given by the research team during the trial and repeated 3-5 times separately per ward to reach as many HCW as possible. As assessed by questionnaire at 12 months, 81% of all participants who completed the trial took part in at least one of the education sessions; 95% of the IG and 64% of the CG. Ninety per cent of participants who attended found the education to be useful.
Self-reported cream use

The cream consumption was reported by the HCW separately for three time points ‘before’, ‘during’ and ‘after’ the shift and categorized as ‘never’, ‘less than 50% of my shifts’, ‘more than 50% of my shifts’ and ‘almost always’. As presented in Fig.3 and Table 1, the baseline use of hand creams was low, 70-80% of HCW never applied cream before and during the shift. After the shift this proportion is somewhat lower (≤50%). At baseline, however, there was no difference between IG and CG. Analysis by a mixed-effect ordinal regression revealed that the cream consumption at follow-up was significantly higher in the IG as compared to the CG, while at baseline there was no difference between the groups. At follow up HCW in the IG were 2.27 (95% CI 1.29 to 3.97, P=0.004) times more likely to report a higher frequency of hand cream use before the shift compared to the CG. During the shift this was 3.30 (95% CI 1.80 to 6.06, P<0.001) times more likely. There was no significant difference in cream use after the shift between both groups. (OR=1.55,95%CI 0.91-2.64, P=.11). When looking at overall cream use (i.e. per entire shift), at baseline 38% of HCW in the IG and 43% in the CG reported to never use creams. After the intervention, the proportion of HCW reporting ‘NEVER’ to apply creams was 18% in IG and 32% in the CG. HCW with confirmed severe HD reported more frequently to always use creams (69%; 20/29) than HCW with no, mild, or moderate HD (4% 18/470).

We analyzed data on hand cream use collected at baseline and 12 months, which were defined as process outcome measures. Although data on hand cream use and exposure was also collected at 6 and 18 months, these data were not analyzed due to low response rates.
Table 2. Characteristics of healthcare workers (HCWs) and wet-work activities at baseline (T0) and 12-month follow up (T12).

| HCW characteristics | Self-reported Outcomes | Intervention group T0 | Intervention group T12 | Control group T0 | Control group T12 |
|---------------------|------------------------|-----------------------|------------------------|------------------|------------------|
| Wards               |                        | 8 clinical ward, 1 outpatient clinic, 285 HCW | 8 clinical ward, 1 outpatient clinic, 167 HCW | 10 clinical wards, 216 HCW | 10 clinical wards, 133 HCW |
| History of hand dermatitis past 6 or 12 months; n (%) | 95 (33%) | 54 (32%) | 72 (33%) | 43 (32%) |
| Frequency of use of hand alcohol n(%) | Less than 5 times a shift | 9 (3%) | 3 (2%) | 6 (3%) | 4 (3%) |
|                      | 5 to 10 times a shift   | 9 (3%) | 7 (4%) | 9 (4%) | 11 (8%) |
|                      | 11 to 15 times a shift  | 26 (9%) | 17 (10%) | 26 (12%) | 11 (8%) |
|                      | More than 15 times a shift | 242 (85%) | 139 (83%) | 175 (81%) | 109 (82%) |
| Frequency of hand washing; n (%) | Less than 5 times a shift | 31 (11%) | 20 (12%) | 17 (8%) | 11 (8%) |
|                      | 5 to 10 times a shift   | 86 (30%) | 42 (25%) | 73 (34%) | 27 (20%) |
|                      | 11 to 15 times a shift  | 74 (26%) | 43 (26%) | 72 (33%) | 52 (39%) |
|                      | More than 15 times a shift | 97 (34%) | 62 (37%) | 52 (24%) | 44 (33%) |
| Frequency of glove use n(%) | Less than 5 times a shift | 20 (7%) | 12 (7%) | 30 (14%) | 17 (13%) |
|                      | 5 to 10 times a shift   | 34 (12%) | 17 (10%) | 48 (22%) | 23 (17%) |
|                      | 11 to 15 times a shift  | 60 (21%) | 32 (19%) | 56 (26%) | 41 (31%) |
|                      | More than 15 times a shift | 171 (60%) | 107 (64%) | 82 (38%) | 53 (40%) |
| Frequency use of moisturising creams before shift; n (%) | Never | 210 (74%) | 82 (49%) | 160 (74%) | 89 (67%) |
|                      | About half of shifts    | 23 (8%) | 33 (20%) | 26 (12%) | 23 (17%) |
|                      | More than half of shifts | 16 (6%) | 18 (11%) | 9 (4%) | 12 (9%) |
|                      | Almost always           | 34 (12%) | 32 (19%) | 22 (10%) | 12 (7%) |
| Frequency of use of moisturising creams during shift; n (%) | Never | 197 (69%) | 62 (37%) | 151 (70%) | 85 (64%) |
|                      | About half of shifts    | 48 (17%) | 37 (22%) | 43 (20%) | 25 (19%) |
|                      | More than half of shifts | 6 (2%) | 27 (16%) | 9 (4%) | 17 (13%) |
|                      | Almost always           | 34 (12%) | 42 (25%) | 15 (7%) | 7 (5%) |
| Frequency of use of moisturising creams after shift; n (%) | Never | 131 (46%) | 43 (26%) | 108 (50%) | 48 (36%) |
|                      | About half of shifts    | 22 (13%) | 37 (22%) | 26 (12%) | 37 (20%) |
|                      | More than half of shifts | 29 (10%) | 27 (16%) | 24 (11%) | 37 (20%) |
|                      | Almost always           | 88 (31%) | 60 (36%) | 58 (27%) | 33 (25%) |
Electronically measured cream use
The electronic system was activated in August 2016 and continuously monitored cream application events for the duration of the trial. Fig. 4 shows the total number of cream application events per month averaged for all 9 intervention wards. The average number of cream applications per HCW per shift was 0.4. There seemed to be an increasing trend in use during the trial with a peak in May and in December/January. Fig. 5 shows the most popular moments of cream use and the location of cream dispensers. The dispensers with highest consumption of creams were located at the toilet, in staff rooms and the corridor and the least used location were patient rooms. The times of day with the highest frequency of cream use for all the locations together were at 10 am, 12pm and 3 pm. There were no significant differences between electronic measured cream use between wards with high -exposure to wet work and to low-exposure wards (resp. median=181.8 and median=174.5 total cream applications per month per HCW. The estimated median values were based on 20 working days/month and a 8 working hours/shift ).
Figure 3. Self-reported hand cream use before, during and after the shifts in the intervention group vs the control group. HCW, healthcare

Feedback Posters in the IG

From April to December 2017 a total of 8 feedback posters (see Fig 2 for an example) were placed at prominent locations (e.g. next to a sink, in the toilet, entrance/exit of the ward) on the intervention wards on a monthly basis.
The opinion of HCWs about the posters in the IG was assessed by means of questionnaires at 12 months and 18 months of follow up. At 12 months 87% (145) of the HCW reported to have noticed the posters. At 18 months, this proportion increased to 98% (100 of 102 HCW). A large proportion of HCW (86%; 88 HCW) understood the message displayed on the posters, and the majority (78%; 80 HCW) found that the posters were useful reminders to stimulate skin care. 43% of HCW reported that the posters moved them to use creams.

![Graph showing cream application events and posters feedback](image)

**Fig. 4.** Average number of total cream application events per month illustrated for all intervention wards together. Education was provided every 3 months. Feedback posters were delivered every month starting from April.

**Attitudes towards creams and cream use in the IG**

As assessed from the questionnaires at 18 months, 65% (66 HCW) reported to use creams provided in the electronic dispensers and 38% (39 HCW) reported to use personal creams. The most reported barrier for not using hand creams was belief that creams interfere with their workflow (81% of HCW). 94% reported they are aware of the benefits of cream use. The available cream in the dispenser was rated for quality/likeability as ‘good’ (median=4 on a Likert scale ranging from 1=very poor to 5= excellent). 86% reported that the creams were located where they needed them.
Fig. 5. Toilets, staff rooms and corridors are illustrated as the most popular locations, and patient rooms as the least popular. Between 10 AM and 3 PM, creams are used most often. Data were recorded during the trial from August 2016 to December 2017.

Exposure to wet work
Self-reported frequency of wet work activities (hand washing, gloves and hand disinfectants) at baseline and follow up are shown in Suppl. Figure. 1. At baseline, more than 80% of HCW in both groups belonged to the highest category of hand disinfectant users (more than 15 times per shift). A similar pattern was reported at follow-up. At baseline and at follow-up at least 60% in the IG and CG group reported to wash hands more than 10 times per shift. At follow-up, the respective percentages were 63% and 72%. The frequency of glove usage differs between the IG and CG at baseline, and a similar pattern has been observed at follow up.

Self-reported outcomes on hand dermatitis
At baseline, 33% (95) HCW in the IG and 33% (72) in the CG reported having hand dermatitis in the past year. At 12-months, a similar prevalence (32%) of hand dermatitis was reported in both IG (54 HCW) and CG (43 HCW).
Discussion

Improving skin care behavior is an important goal to prevent HD in HCW. In an intervention study focused on prevention of HD in the healthcare setting we investigated whether provision of hand creams accompanied with electronic monitoring and feedback on hand cream use may prevent hand dermatitis. Here, we show that the intervention was successfully implemented and resulted in an improved adherence to recommended skin care practices. This study provides, for the first time, real-time data on hand cream use in HCW regarding frequency of use as well as indicating favored locations and times of application in HCW.

Self-reported cream use

At follow-up, the self-reported hand cream use before and during the shifts was significantly higher in the IG compared to the CG, while at baseline there was no difference between groups. Consistently, the proportion of HCW that report to never use hand creams was lower in the IG than in the CG at follow-up (18% and 32%, respectively). At baseline, the respective percentages had been 38% and 43%. Such a large proportion of HCW reporting to never use hand creams is surprising and alarming. In the Netherlands, the guidelines on the prevention of contact dermatitis recommend use of emollients on regular base and creams are often provided by the employer. Similar recommendations have been proposed by the working group for diagnosis, prevention and treatment of hand eczema of the European Society of Contact Dermatitis (ESCD), stating that moisturizers should be applied on the hands during the working day and especially after work and before bed time. Interestingly, almost half of HCW (46% and 50% in respectively IG and CG) reported at baseline never to use hand creams ‘after the shift’, a ‘key application time’ recommended by the ESCD working group. After the intervention, this proportion decreased to 26% in the IG and 36% in the CG. The literature data on cream use in occupational settings is scarce. In a study of Große-Schütte et al 10% of HCW reported to never use hand care products. This study based on questionnaires reported that approximately 15% of HCW apply moisturizers after hand washing. The present study revealed that having severe symptoms of HD is associated with a higher frequency of cream use in HCW, which is in agreement with findings of van der Meer et al. 15
Electronic monitoring of cream use

Despite the improvement of self-reported hand cream use in this trial, the electronically monitored cream use of 0.4 events per HCW per shift in the IG remained below the recommended frequency of at least 2 times/shift. As more than one third of HCW reported that next to electronic dispensers they (also) used their own creams, this electronically measured application frequency might be somewhat underestimated, but still likely below the current recommendations of 2-3 times per day.2,3,12

The finding that almost 20% of the HCW at follow-up still reported to never use hand cream is worrisome, especially realizing that at least a third of HCW reported skin problems. Hand dermatitis is not only a problem for the affected individual, but may also pose a health hazard for patients as damaged skin increases bacterial flora.16 Furthermore, HCW with damaged skin seem to avoid hand disinfectants due to stinging sensation. 17

Although the majority of HCW did report to be aware of the benefits of hand creams, most of them reported that creams interfered with their workflow, especially when wearing gloves. Consistently, the highest frequency of cream use was recorded at moments when HCW didn't perform direct patient care activities: around 10 am during their coffee break, around 12 am during lunch break and around 3 pm during clinical handover when the shifts change (ends or starts). Other reported barrier to use hand care was the “greasiness” of the available creams, but nonetheless the likeability of the dispenser-cream was rated good (median=4 on a Likert scale ranging from 1=very poor to 5=excellent).

The dispensers in the staff-only rooms such as coffee-, break- and meeting rooms and toilets were used more often than the dispensers in rooms where patient care is delivered (patient rooms or medication rooms). There seemed to be an increasing trend in use during the trial with a peak in May and in December. It could be speculated that the increase in May is related to the introduction of feedback posters in April. The first feedback poster seems to have had the most impact, which could be explained by issues of user-fatigue, desensitization by the prompt (posters) and loss of novelty, which is illustrated well by citations of HCW like: ‘at first, the posters evoked competitiveness, but after a while I didn’t really notice them anymore’. The increase in cream use in December/January is most likely caused by the cold and dry weather leading to skin dryness in winter.

Feedback

The feedback posters, showing whether compliance in comparison with last month improved (green) or worsened (red), was well noticed by HCW and most
of them perceived them as useful. To increase visibility, the posters were placed in the staff toilets and staff break rooms as suggested previously as optimal display locations. Almost half of HCW (43%) felt additionally motivated by the posters. As this was reported at the end of the trial, it could be argued whether loss of novelty played a role in the motivating effect of the posters. Initially the intention was that the feedback would be provided verbally by the managing nurses during regular meetings. This did not prove practical and it would be interesting to learn whether addition of this recommended step would further improve the effectiveness of the intervention.

4.4 Education
Several studies have suggested that the low use of hand creams by HCW could be due to lack of knowledge. To avoid differences in the level of knowledge between IG and CG in the present trial, we provided small group education lessons meant to increase awareness on risk behavior and importance of skin care. The educational program was implemented well and was visited by the majority (81%) of the HCW who completed the trial. The attendance rates in the IG (95%) were higher than in the CG (64%). Likely, awareness of HCW being allocated to the control group, might have affected their motivation. Issues of preference and disappointment are not uncommon in trials where participants are aware of allocation. Higher attendance of the education lessons in the IG may have influenced the cream use, however this was not possible to evaluate as we didn’t assess the level of knowledge of HCW.

Exposure to wet work
The majority of HCW reported to wash their hands with soap more than 10 times per shift. Previously, Visser et al found that washing of hands >10 times per shift doubles the risk for HD. Consistently, in this intervention study we found that exposure to wet work estimated from soap use at ward level was a significant risk factor for HD. Also, we found high use of disinfectants; more than 80% of HCW used these more than 15 times per shift. Notably, the disinfectant (Sterilium) used by HCW in this trial contained glycerol, a known moisturizer which prevents skin dryness. As addition of a moisturizer to disinfectants has previously showed to prevent skin irritation, it might be speculated that the addition of glycerol in the disinfectants used in the present study have diminished the need for hand creams.
Strengths and limitations

Strengths of our study included the stratified randomized control design and the generalizability of our findings due to the large number of participants in a hospital setting and the relatively long follow-up period. For the first time cream consumption in HCW has been assessed by an electronic monitoring system. Real-time monitoring of cream use provided detailed data on the preferred locations for dispensers and moments of use, which can be valuable in designing future strategies to set up best practices for skin care in HCW.

One of the study limitations was not blinding participants regarding allocation, which might have caused performance bias in the control group. Another limiting factor was using self-reported data of the cream use to enable comparison of arms. This is known to be less accurate than electronic data, which could only be measured in the IG. The electronic system we used in this trial could not provide individual usage but only cream consumption at ward level. Electronic data on cream use of HCW might have caused underestimation of the total cream use as HCW not only used the dispenser creams, but also their own hand creams. Also, HCW in both IG and CG very frequently use disinfectants containing emollients. This may also have influenced hand care behavior.

Conclusion

Our findings show that electronic monitoring of hand cream use combined with feedback improves skin care behavior among HCW and therefore should be considered as a practical strategy to promote skin care. Our approach was easy and feasible to incorporate in daily practice in a health care setting without interfering with the workflow of HCW.

Acknowledgments

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Supplementary files

Suppl. Fig. 1. *Self-reported wet work activities*
PART II
Experimental studies on the *in vivo* effects of skin irritants on the skin barrier and inflammatory response
Specific barrier response profiles after experimentally-induced skin irritation in vivo

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Abstract

Background
Recently, natural moisturizing factors (NMF) and corneocyte surface topography were suggested as biomarkers for irritant dermatitis.

Objectives
To investigate how exposure to different irritants influences corneocyte surface topography, NMF levels and barrier function of human skin in vivo.

Methods
Eight healthy adult volunteers were exposed to aqueous solutions of 60% n-propanol, 0.5% SLS, 0.15% sodium hydroxide, 2.0% acetic acid and distilled water in a repeated irritation test over 96 h. Erythema, transepidermal water loss (TEWL), skin hydration, the dermal texture index (DTI) and NMF were measured at baseline, and after 24 and 96 h.

Results
SLS and sodium hydroxide caused the most pronounced effect on erythema and TEWL. Although, n-propanol caused only slight changes in TEWL and erythema, it showed a pronounced effect on skin hydration, NMF and DTI. NMF was the only parameter which was significantly altered by all investigated irritants. The changes in DTI were inversely associated with the NMF levels and skin hydration.

Conclusion
Skin barrier impairment and inflammatory response are irritant-specific, emphasizing the need for a multi-parametric approach for studying skin irritation. The NMF levels seem to be the most sensitive parameter in detecting irritant-induced skin barrier alterations.
Introduction

Irritant contact dermatitis (ICD) is a common inflammatory skin disease that can be elicited by single or repeated exposure to chemicals with irritating properties such as detergents, organic solvents, alkaline agents or acids. The patho-mechanism of ICD remains incompletely understood, however impairment of the skin barrier function plays a central role, triggering homeostatic and innate immune responses. (1,2) Recently Koppes et al. (3) reported two novel biomarkers of irritant skin reaction to SLS: the dermal texture index (DTI) as a measure of the corneocyte topography and the levels of the natural moisturizing factors (NMF). As demonstrated by Atomic Force Microscopy (AFM), SLS dramatically increased the number of circular nano-objects protruding above the corneocyte surface, expressed by the DTI (3) and decreased the NMF levels at the same time. In contrast to the SLS-induced increase in DTI, no changes in DTI were observed in skin affected by allergic contact dermatitis (3). These observations suggest that the alterations in the corneocyte surface topography might be attributed to the skin barrier damage caused by the irritant, rather than inflammation itself (4). Therefore, we were interested in whether the changes in DTI and NMF were SLS-specific or also occur with other skin irritants. Furthermore, we wanted to investigate the relationship between DTI and NMF with skin bioengineering parameters including erythema, TEWL, and skin hydration. In the present study, we investigated the early and late changes in the morphological, biochemical and bioengineering parameters following repeated exposure to different classes of water soluble skin irritants in the human skin in vivo.

Methods

Study population

Healthy adult volunteers aged 20-65 years (n=8, 3 female and 5 male; median age 24.5 years) without history of skin or systemic diseases were recruited for the study. The exclusion criteria comprised intensive ultraviolet exposure in the test area within the last 6 weeks before inclusion and/or during the study, pregnancy or lactation. The participants were not allowed to use skin care products in the test area (back) within 24 hours prior to the baseline measurements and for the entire duration of the study (5 days). The protocol was approved by the Ethics Committee of the University of Lübeck (Nr. 14-111) and all participants gave written informed consent beforehand.
Irritants and mode of exposure

Exposure to the irritants and distilled water as control was performed under occlusion with large Finn Chambers® (12 mm diameter; Smart Practice, Barsbüttel, Germany). Two rows, comprising each of 4 test (irritant-exposed) and 2 control (water and non-exposed, normal skin) fields were marked left and right on the skin of the upper-mid back. Fifty microliters aqueous solutions of the following irritants were applied: 0.5% SLS (99.0% purity; Sigma-Aldrich, Steinheim, Germany), 0.15% sodium hydroxide (NaOH; Mallinckrodt Baker, Deventer, The Netherlands), 60.0% N-propanol (1-propanol, Merck, Darmstadt, Germany), and 2.0% acetic acid (AcA; Roth, Karlsruhe, Germany). The test chambers were applied twice daily for 30 min, within an interval of 3-4 h on four consecutive days using a previously validated and published protocol. (5) The fields of the first row were examined at baseline and 24 h later and the ones of the second row, respectively, at baseline and 96 h later.

2.3 Bioengineering parameters: erythema, TEWL and capacitance

All measurements were performed at baseline, 24 h and 96 h later under controlled ambient conditions (temperature 21±1 °C, 40-50% relative humidity). The skin irritant response was measured by non-invasive assessment of erythema, TEWL and skin hydration. Erythema was measured with the skin Colorimeter CL400 and expressed in the L*a*b*system; TEWL was measured with the open chamber system (Tewameter TM300) and skin hydration was assessed by measuring capacitance (Corneometer CM825), all devices from Courage and Khazaka Electronics (Cologne, Germany). For each parameter, two consecutive measurements per field were performed by the same trained observer and according to published guidelines(6-9).

Biochemical parameters: NMF

NMF was determined in SC tape strips collected by 14 mm D-Squame discs (CuDerm, Dallas, TX, USA). Tape stripping was performed on day (D)2 and on D5, respectively, 24 h and 96 h after the first application of the irritants as previously described (10). Six consecutive tapes from each field were obtained and stored in sterile 1.5 ml Eppendorf tubes (Eppendorf, Hamburg, Germany) at ~80°C until analysis; the fifth tape was used for analysis. The NMF components (histidine, 2-pyrrolidone-5-carboxylic acid, trans- and cis-urocanic acid) on the tape were extracted with 400 μl of 25% (w/w) ammonia solution, evaporated to dryness and reconstituted in 200 μl pure water. The extracts were diluted 1:1 with mobile phase prior to high-performance liquid chromatography (HPLC-UV) analysis.
To compensate for a variable amount of the SC on the tape, the amount of SC was estimated from the protein levels determined in the aqueous extract after ammonia extraction, as previously described (11). Briefly, the amount of proteins has been determined from an aliquot of 75 µl of the extract by using a Pierce Micro BCA protein assay kit according to the procedure of the manufacturer (Thermo Fischer Scientific, Rockford, IL, USA; referred to as the Pierce assay). Bovine serum albumin (BSA) was used as a standard to prepare calibration curve. The NMF concentration was normalized for the protein amount on the tape and expressed as mmol NMF/g protein.

Morphological parameters: corneocyte surface topography

The corneocyte surface morphology was analysed by AFM as described previously (12). Briefly, the third consecutive tape strip was subjected to AFM measurements carried out with a Multimode atomic force microscope equipped with a Nanoscope III controller and software version 5.30sr3 (Digital Instruments, Santa Barbara, CA, USA). Silicon nitride tips on V-shaped gold-coated cantilevers were used (0.01 N/m, MLCT; Veeco, Mannheim, Germany). Imaging was performed at ambient temperature with forces less than 1 nN at one to three scan lines per second (1–3 Hz) with a resolution of 512 × 512 pixels. For texture analysis, subcellular scan areas of 20 × 20 µm² were recorded. For a larger overview, images of 70 × 70 µm² were recorded. Topographical data of the corneocyte surfaces were analysed with the nAnostic™ method, by the use of custom-built, proprietary algorithms (Serend-ip, Münster, Germany) evaluating each nanostructure protruding from the mean surface level, referred to as circular nanosize object (CNO). The CNOs were automatically filtered according to their size and shape; only structures of positive local deviational volume smaller than 500 nm in height and with an area of <1 µm² were considered. The DTI counted these features for an area of 20 × 20 µm² of cell surface per image (13).

Statistical analyses

Statistical analyses were performed by GraphPad Prism 7.0 (Graphpad software, La Jolla, CA, USA). A p-value<0.05 was considered statistically significant. Data distribution was assessed using the Shapiro-Wilk test. The differences in the bioengineering parameters (erythema a*-value, capacitance and TEWL) between baseline and the respective readings at 24 and 96 h were compared by using two-sided paired Student t-test. To test the effect of water, differences between the water control (occlusion) and the non-exposed skin site at respectively 24 and 96
h were analysed by two-sided paired Student t-test. In order to assess the neat
effect of the irritant, the differences between the values of the bioengineering
parameters measured at 24 and 96 h and the corresponding baseline values
have been calculated for each irritant (Δ values) and compared with the Δ values
of the water control by using repeated measures ANOVA analysis followed by
Dunnett’s multiple comparisons test. The correlation between the investigated
parameters was evaluated by the Pearson’s correlation analysis (normal
distribution) or by the Spearman’s rank correlation test (skewed distribution).
In the tables and figures, the data are presented as mean values ± standard error
(SEM), unless otherwise indicated.

Results

Bioengineering parameters
The values of erythema (a*-value), transepidermal water loss (TEWL) and
capacitance at baseline, 24 and 96 h later are shown in Table 1. At baseline there
were no significant differences in any of the measured parameters between the
test and control fields (data not shown). Of the studied irritants, SLS exerted
the most pronounced effect and after 96 h there were significant differences
compared to the baseline values for all bioengineering parameters (Table 1). The
water exposed skin site showed a significant increase in TEWL and decrease
in capacitance after 96 h as compared to the corresponding baseline values.
Furthermore, the water-occluded skin site showed a significantly higher TEWL
and a lower capacitance at 96 h as compared to non-exposed skin (Fig. 1).
|                | a*-value | TEWL (g/m²/h) | Capacitance (AU) |
|----------------|----------|---------------|------------------|
|                | MEAN±SEM | MEAN±SEM      | MEAN±SEM         | MEAN±SEM               |
|                | Baseline | Baseline 96 hr| Baseline 24 hr   | Baseline 96 hr         | Baseline 24 hr         | Baseline 96 hr |
| AcA            | 10.0±0.7 | 9.8±0.6      | 10.3±0.7        | 10.6±0.6              | 5.5±0.2                | 5.7±0.2        | 5.5±0.2        | 6.3±0.4*     | 36.5±2.5 | 34.0±3.5 | 32.2±2.5       | 32.1±1.5*     |
| N-propanol     | 9.8±0.7  | 9.5±0.7      | 9.8±0.7         | 10.3±0.7             | 5.3±0.2                | 6.2±0.7        | 5.4±0.2        | 9.4±1.1*    | 37.8±3.3 | 25.3±3.5 | 37.7±3.3   | 23.1±4.3***  |
| SLS            | 9.8±0.8  | 10.0±0.8     | 9.9±0.6         | 16.0±0.9***         | 5.6±0.2                | 6.7±0.5        | 5.6±0.2        | 37.1±3.5*** | 36.8±2.6 | 27.1±3.7** | 37.1±2.5*** | 18.1±4.4**   |
| NaOH           | 9.9±0.7  | 10.2±0.7     | 10.0±0.7        | 12.1±1.2             | 5.6±0.2                | 7.3±0.5*       | 5.5±0.2        | 13.2±1.6** | 35.3±2.1 | 22.2±1.7** | 35.3±2.1*** | 11.3±1.3***  |
| Aq             | 9.6±0.6  | 9.7±0.6      | 9.9±0.6         | 9.4±0.6              | 5.6±0.2                | 5.7±0.3        | 5.6±0.2        | 6.8±0.3*    | 36.1±2.0 | 34.0±2.9 | 36.0±2.0*       | 31.1±2.4***  |
| Non-exposed    | 9.4±0.6  | 9.2±0.6      | 9.7±0.6         | 9.0±0.7              | 5.4±0.2                | 5.4±0.2        | 5.5±0.1        | 5.5±0.2     | 36.6±2.1 | 37.0±2.1 | 36.4±2.1 | 37.0±3.1*    |
As all investigated irritants were applied in water which apparently affected the skin barrier, the effect of the irritants was assessed by comparing the changes from the baseline values (Δ values) at 24 h and 96 h with the respective Δ values of the water control. The Δ values for erythema (a*-value), capacitance and TEWL are shown in Fig 1. At 24 h, only n-propanol showed a significant difference in the Δ values of erythema, while concerning TEWL a significant difference was found for NaOH. In contrast, for capacitance a significant change from baseline was observed for exposure to n-propanol and SLS and a tendency of decrease for NaOH (p=0.05). At 96 h, repeated exposure to SLS and NaOH resulted in significant differences in the Δ values for all measured parameters (Fig.1). At the same time-point, AcA exposure did not induce significant difference in any of the outcome parameters and n-propanol showed a significant difference (i.e. decrease) only with regard to capacitance.

**Biochemical and morphological parameters: NMF and DTI**

The values of NMF and DTI, measured in the SC tapes collected at 24 and 96 h after the first irritant exposure are shown in Figure 2. Repeated application of SLS, NaOH and n-propanol led to significantly lower NMF levels as compared with the corresponding water control as early as 24 h after initial exposure (Fig. 2 a, b). After 96 h, there was a reduction from the baseline value of 22% for AcA, 55% for n-propanol, 75% for SLS and 65% for NaOH. Furthermore, 96 h after the first application of the test chambers, the NMF levels of the water exposed skin site were significantly lower compared to the non-exposed site.

The representative images of the corneocyte surface topography of the irritant-exposed and control fields captured by AFM at, respectively, 24 and 96 h after initial exposure are shown in Figure 3. Already after 24 h (Fig. 3,C upper panel), thinning of fibres, wrinkles and elongated spots have been observed for the n-propanol and SLS exposed sites. After 96 h, characteristic circular nano-size objects appear after exposure to n-propanol, SLS and NaOH (Fig. 3,C lower panel). The AFM images of the AcA-exposed site were similar to the control skin sites and also in agreement with their DTI values. The changes in topography observed after n-propanol, SLS and NaOH exposure were consistent with the elevated DTI (number of circular nano-objects per surface area), shown in Figure 2 b,d. It has to be noted that the AFM measurements after 24 h have been done only in two subjects. Although statistical evaluation of these data was not meaningful, the increasing trend in DTI suggests that the changes in surface topography occur early.
Fig. 1 Comparison of the changes in the bioengineering parameters at 24 and 96 hrs from baseline (Δ values). Results are shown as mean value ± SEM. Test fields: acetic acid (AcA), n-propanol, sodium lauryl sulfate (SLS), sodium hydroxide (NaOH), occlusion with water (Aq) and non-exposed skin site. The Δ values were compared to the water exposed skin site by using Repeated measures ANOVA analysis followed by Dunnett’s multiple comparisons test. The difference between the water exposed site (Aq) and non-exposed skin site was compared by a Student t-test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. AU Arbitrary Units
Fig. 2 Natural moisturizing factors (NMF) and Dermal Texture Index (DTI) measured on the stratum corneum tapes collected at 24 and 96 hours after initial exposure. Test fields: acetic acid (AcA), n-propanol, sodium lauryl sulphate (SLS), sodium hydroxide (NaOH), distilled water (Aq) and non-exposed skin. The values for the irritants were compared to the water exposed skin site by using Repeated measures ANOVA analysis followed by Dunnett’s multiple comparisons test (mean ± SEM). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

To examine the association between the measured parameters, a correlation analysis was performed. The results of the correlation analysis are presented in the supplementary Table 1. Fig. 4 shows the linear regression lines with corresponding coefficients of determination (r2) for the relationship between DTI (log values) and the parameters relevant for skin hydration: NMF and capacitance. For the regression analysis data for all irritants measured at 96 h after initiation of exposure were included. As shown in Figure 4, there was an inverse correlation between DTI and, respectively, NMF and capacitance. Furthermore, NMF was positively associated with capacitance.
**Fig. 3** Nanostructure of human corneocytes after irritant exposure in vivo. 

a) Corneocytes were obtained by the tape stripping method. The basal (bottom) face of corneocytes adhering to the tape was imaged by scanning force (AFM). 

b) 20 μm scans were analysed by computer vision to count circular structures (= Dermal texture index, DTI); the presented DTI values are the mean of 10 images. 

c) Representative images of the corneocyte surface, assessed on the third consecutive tape strip after irritant exposure. Irritant exposures: acetic acid (AcA), n-propanol, sodium lauryl sulphate (SLS), sodium hydroxide (NaOH), water occlusion and non-exposed (normal) skin. Thick white arrows show elongated fibres (upper panel) and thin white arrows circular nano-objects (CNOs).
Fig. 4 The relationship between DTI, NMF and skin hydration (capacitance) measured after 96 hrs. $r^2$: determination coefficient of the linear regression analysis

Supplementary Table 1. Spearman correlation coefficients for the relationship between investigated parameters measured at 24 and 96 hrs.

| 24 hrs          | Erythema (a*values) | Capacitance | TEWL  | DTI  | NMF  |
|-----------------|---------------------|-------------|-------|------|------|
| Erythema (a*values) | -0.207              | -0.256      | -0.071 | -0.009 |
| Capacitance     | -0.207              | -0.098      | -0.252 | 0.507*** |
| TEWL            | 0.256               | -0.098      | 0.024  | -0.228 |
| DTI             | -0.071              | 0.252       | 0.024  | -0.738* |
| NMF             | -0.009              | 0.507****   | -0.228 | -0.738* |

| 96 hrs          | Erythema (a*values) | Capacitance | TEWL  | DTI  | NMF  |
|-----------------|---------------------|-------------|-------|------|------|
| Erythema (a*values) | -0.470***           | 0.546****   | 0.345** | 0.543**** |
| Capacitance     | -0.470***           | 0.617****   | 0.594*** | 0.596**** |
| TEWL            | 0.546****           | -0.617****  | 0.542**** | 0.792**** |
| DTI             | 0.345**             | -0.594****  | 0.542**** | 0.672**** |
| NMF             | 0.543****           | 0.596****   | 0.792**** | 0.672**** |

Level of significance: $p < 0.05^*; p < 0.01^{**}; p < 0.001^{***}; p < 0.0001; ^{****}p < 0.00001$
Discussion

In the present study we investigated and compared the effects of repeated exposure to different classes of water soluble irritants on functional, biochemical and morphologic parameters of the skin barrier in vivo. Exposure to the skin irritants led to increase in DTI reflecting changes in the corneocyte topography. DTI was inversely correlated with the NMF levels, which showed decrease after exposure to all irritants. Regarding the bioengineering parameters the results showed a clear irritant-specific effect, which has been shown previously (5,10,17,24). These irritant-specific responses may be explained by different mechanisms underlying the skin barrier damage, dependent on the intrinsic nature of the irritant (7,14) and suggest that the parameter of choice to assess irritation should largely depend on the type of irritant applied. Whereas erythema traditionally has been the most commonly used parameter to visually assess the irritating properties of a chemical, in the present investigation we did not find significant changes from baseline after exposure to acetic acid (AcA) and n-propanol. This might, at least partly, be explained by their weak irritant capacity, which has been demonstrated previously (15-17). However, after exposure to n-propanol, skin capacitance, which is commonly used as a measure of skin hydration, was strongly reduced as early as 24 h after the first exposure. Skin hydration is of ultimate importance for the skin barrier function as it influences not only the skin plasticity, but also the activity of various proteases involved in desquamation, lipid synthesis and inflammatory responses (18). The water content of the skin is largely dependent on the levels of hygroscopic NMF within the corneocytes (19), and furthermore on the SC lipid organization and size of the individual corneocytes which regulate TEWL. The strong and rapid decrease in NMF and skin hydration, with TEWL remaining unchanged, suggests that the effect of n-propanol on skin hydration is likely caused by a decrease in NMF rather than through its interference with the lipid bilayers. In addition to its marked effect on NMF, n-propanol also caused significant changes in the corneocyte surface topography, showing an increase in the number of the circular nano-size objects, expressed as DTI. In contrast to the effects exerted by n-propanol predominantly on skin hydration and DTI, exposure to SLS and NaOH caused significant changes in all measured biophysical parameters (erythema, TEWL and capacitance) as well as NMF and DTI. This might be explained by multiple mechanisms by which these alkaline irritants affect the skin barrier (20). Literature data shows that SLS can affect both, protein and lipid structures of the SC. For example, SLS interacts with the SC proteins resulting in transient
swelling of corneocytes (21). After initial increase in hydration, subsequently, the water-holding capacity of the SC decreases, which in turn will result in skin dryness (22). In addition to its effect on NMF, SLS induces changes in the lipid lamellae organisation (23) which is consistent with the significant increase in TEWL, also found in the present study.

The pronounced decrease in skin hydration found after exposure to the irritants can at least partly be explained by the marked decrease in NMF ranging from 22% to 75% from the baseline value with most marked effect induced by SLS and least by AcA which is in agreement with previous studies using the same irritant concentrations and mode of exposure (24-26). Among all investigated parameters, NMF showed to be the most sensitive with regard to irritant damage, as it showed a significant decrease by all studied irritants. For example, for AcA, NMF was the only parameter that was different from the corresponding water control. Moreover, NMF was the only parameter that was able to detect changes between the non-exposed skin and water-occluded skin sites. The precise mechanisms for NMF reduction remain so far unknown, but possible pathways include irritant-induced effects on the proteolysis of filaggrin to NMF components, reduced expression of filaggrin or leakage of NMF from the corneocytes due to damage of the cornified and/or lipid envelope. For example, both SLS and NaOH are alkaline substances which cause elevated skin pH and might decrease the activity of the enzymes involved in filaggrin proteolysis (27,28).

However, Koppes et al. observed increased activity of two key enzymes involved in filaggrin proteolysis, bleomycin hydrolase and Calpain-1 after exposure to SLS in the human skin in vivo (3). On the other hand, SLS is known to denature proteins of the cornified envelope and increase its permeability (29) which may lead to the leakage of NMF components from the corneocytes (28). Similar mechanism is also likely to explain the effects of a strong corrosive agent with alkaline pH such as NaOH (14,30). Further evidence that the reduction in the NMF levels may be caused by skin barrier damage is provided by findings for increased plasmin activity after exposure to SLS (3). Elevated plasmin activity has previously been associated with a damaged skin barrier (3). Also n-propanol is known to induce changes in the SC e.g. it has pronounced denaturing effect on the SC proteins, profilaggrin processing and desquamatory SC enzymes (31), which is consistent with marked decrease in skin capacitance and NMF and changes in surface topography. Recent study shows the presence of another aliphatic alcohol, 2-propanol, between the solid keratin rods inside the corneocytes which might explain the morphological changes observed in the present study for n-propanol (32). Our hypothesis that reduction in NMF might
be associated with skin barrier damage is consistent with the significant inverse correlation of NMF with DTI as a measure of the corneocyte surface topography. All irritants, except AcA, induced a significant increase in DTI and changes such as thinning of fibres, wrinkles and elongated spots were observed as early as 24 h after initial exposure to n-propanol and SLS. After 96 h, characteristic circular nano-size objects appeared, most abundantly for n-propanol and SLS.

DTI has recently been suggested as a parameter which might be used to distinguish allergic from irritant contact dermatitis (3), based on findings that SLS induced a strong increase in DTI, while in the same study no changes in DTI after exposure to contact allergens were observed. Here, we extend these findings by showing that the effect on DTI can also be induced by unrelated irritants with different physico-chemical properties. The nature of DTI has not been clarified yet, but data from recent studies in atopic- and contact dermatitis patients (13) consistently show that DTI is inversely associated with the NMF values, as also confirmed by the results of our present study. The finding that DTI was increased in SLS-exposed skin, but not in experimentally induced allergic contact dermatitis (3), suggests that inflammation per se is unlikely to be the underlying cause of the observed changes in the corneocyte surface topography. These observations are in agreement with the findings of the present study, which showed a stronger correlation of DTI with NMF and capacitance than with erythema as one of the main signs of inflammation. It may be speculated that the changes in the corneocyte surface texture are caused by decreased skin hydration due to reduced NMF and/or increased TEWL. This is consistent with the published literature reporting similar changes in the morphology of the corneocyte surface in diseases associated with skin dryness such as diabetes (33), psoriasis or ichthyosis vulgaris (34). Reduction of NMF due to exposure to skin irritants or genetic factors such as filaggrin gene loss-of-function mutations in ichthyosis would lead to decreased hydration and shrinkage of the corneocytes which might in turn cause the observed changes in the cell surface texture.

The present study has several limitations. The study had an explorative character and the sample size was small, partly due to the fact that the preceding power calculation was based on the previous data on SLS, a potent irritant with a strong effect on NMF and DTI. Larger scale studies are ongoing. Furthermore, the application of the irritants in only one concentration does not allow conclusions to which extent the observed effects on the measured parameters were dose-dependent. Nevertheless, our results reveal significant differences in the barrier response to common water-soluble irritants and substantiate the need for the use of a multi-parametric approach based on functional, biochemical and morphologic parameters to assess skin irritancy in vivo.
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Barrier damaging effects of n-propanol in occlusion-modified tandem repeated irritation test: modulation by exposure factors and atopic skin disease

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Abstract

Though recent studies have shown significant and previously unknown negative effects of n-propanol on the barrier function and corneocyte surface topography in healthy skin, the effects of the applied irritant concentration and the outcomes of cumulative exposure to the irritant in at risk populations have not been studied. We therefore investigated the relationship between the applied irritant concentration and the barrier damaging effects of repeated n-propanol exposure in healthy and atopic individuals in vivo. Furthermore, we studied the modulation of the outcomes by previous permeability barrier function impairment by co-exposure and host-related factors. For the purpose, healthy adult volunteers and individuals with a history of atopic dermatitis (AD) were exposed to n-propanol concentrations from 30% to 75% in occlusion-modified tandem repeated irritation test (om-TRIT). The outcomes included non-invasive measurement of erythema, transepidermal water loss, capacitance and the natural moisturising factors (NMF) levels at baseline and 96 hours later.

N-propanol exerted significant barrier damaging effects even at the lowest concentration in both groups. The relative changes in the outcome parameters were more pronounced with increasing n-propanol concentrations. Preceding low-grade trauma by occlusion/water exposure reduced the skin irritation threshold in both groups. Exposure to all irritant concentrations reduced significantly the NMF levels. The differences in the severity of the barrier function impairment after exposure to the same irritant concentrations under the same conditions between the AD and the control group were significant.
Introduction

Alcohol-based hand disinfection, repeated washing and occlusion are important exposure factors for development of cumulative irritant hand eczema (HE). N-propanol (1-propanol) is a short-chain alcohol and a common constituent of workplace or household hand disinfectants. Similarly to isopropanol (2-propanol) or ethanol, n-propanol exerts antimicrobial effects at a concentration range from 15% up to 85%. It is frequently combined with isopropanol (2-propanol) and 30% or 45% concentrations of the irritant are found in some of the most common marketed formulations recommended for hand disinfection in hospital settings.[1,2] Compared to alkaline soaps and detergents, short chain alcohols are considered to have a relatively low skin irritation potential.[3] In two recent studies however, we found considerable and previously unknown negative effects of 60% n-propanol on the permeability barrier function and corneocyte surface topography in healthy skin.[4,5] Therefore, in the present study we aimed to extend our previous findings by investigating the effects of the applied irritant concentration and the relative contribution of workplace relevant co-exposures in healthy volunteers and individuals with atopic dermatitis (AD), known to have an increased risk for irritant HE. To the best of knowledge, the cumulative effects of n-propanol on the barrier function and properties in at risk populations have not been investigated under experimental conditions so far.

Methods

Study population
Twenty healthy adult volunteers without history for skin or systemic diseases (16 female and 4 male; mean age 26.2 years) and twenty individuals with AD in stage of remission for more than 6 weeks (17 female and 3 male; mean age 25.3 years) were included in the study. UV-exposure within the 6 weeks preceding the study, pregnancy or lactation were defined as exclusion criteria. The protocol (No. 14-111) was approved by the Ethics Committee of the University of Lübeck and all volunteers gave written informed consent beforehand.

Irritants and mode of exposure
Fifty microliters aqueous solutions of n-propanol (1-propanol, Merck KGaA, Darmstadt, Germany) in concentrations of respectively 30.0%, 45.0%, 60.0% or 75.0% were applied on 8 previously marked test fields on the upper-mid back on 4
consecutive days (D1-D4) twice daily for 30 min according to a recently validated occlusion-modified tandem-irritation test (om-TRIT) protocol.[5] The irritants were applied to the respective test fields at the same time of the day (±1 hour) using large Finn chambers (12 mm diameter, SmartPractice, Reinbek, Germany). On 4 of the test fields, exposure to the respective irritant concentrations was preceded by occlusion with distilled water for 30 min and on 4 fields the same irritant concentrations were applied without preceding occlusion; 2 adjacent fields, respectively, exposed to distilled water only (occlusion) or left untreated (normal skin) served as controls. The volunteers were allowed to take shower as usual; the use of skin care products or UV-exposure in the test area was not allowed for the entire duration of the study (5 days).

Bioengineering assessment of the skin irritant response
Visual scoring, non-invasive assessment of erythema, transepidermal water loss (TEWL) and skin hydration (capacitance) were used to monitor the skin irritant response. The assessments were performed before the first application of the irritants (baseline) and 96 hours later (end of the study), before tape stripping. Visual scoring was based on Kligman and Frosch, with assessment of erythema, scaling and fissuring based on a 0–4, respectively 0–3 point scale.[6] Erythema was measured with the Colorimeter CL400 (CK Electronics, Cologne, Germany) and the values were expressed in the L*a*b* system; the a*-value on the green-red axis was used for assessment of erythema. TEWL was measured with the open chamber system (Tewameter TM300) and skin hydration was assessed by measuring capacitance (Corneometer CM825; both devices from CK Electronics, Cologne, Germany). For all parameters 2 consecutive measurements per field were performed by the same investigator under controlled environmental conditions (temperature 21 ± 1°C; average relative humidity 40-45%) and according to the published guidelines.[7-10]

Natural moisturizing factor analysis
The samples for stratum corneum NMF analysis were taken 24 hours after the last irritant exposure on day 5 using commercially available 14 mm D-Squame® discs (CuDerm Corp., Dallas, TX, USA) and stored in sterile 1.5 ml Eppendorf tubes (Eppendorf, Hamburg, Germany) at −80°C until analysis. Six samples per test field per volunteer were collected as previously described.[11] The stratum corneum NMF components (histidine, 2-pyrrolidone 5-carboxylic acid, trans- and cis-urocanic acid) on the tapes were extracted with 400 µl of 25% (w/w) ammonia solution, evaporated to dryness and reconstituted in 200 µl pure
water before high-performance liquid chromatography (HPLC-UV) analysis.[12] Extracts from two D-Squame® discs were pooled for analysis; the NMF levels were corrected for the amount of protein and expressed as mmol NMF/g protein.

**Statistical analysis**

GraphPrism Version 7 (GraphPad Software Inc., San Diego, CA, USA) was used to perform statistical analysis. The level of significance was p<0.05. Repeated-measures ANOVA or non-parametric Friedmann tests with additional correction for multiple comparisons (Dunn's test) were used to evaluate the changes between the respective fields in respect to time and occlusion. The difference between AD and healthy controls was tested by 1-way ANOVA with Bonferroni’s multiple comparison test or Kruskal-Wallis test in combination with Dunn’s multiple comparison test. The data in the respective tables and figures are presented as mean and standard error of the mean (SEM) or as median with interquartile range, as indicated.

**Results**

The erythema (a*), TEWL and capacitance values at baseline and after 96 hours repeated exposure to the different concentrations of n-propanol with and without previous damage to the skin barrier by occlusion in the AD and healthy controls group are shown in Table 1. Within the study groups there were no significant differences in the visual irritation score and the barrier function parameters between the test and control fields at baseline.

Repeated occlusion and water exposure modulate the concentration-dependent barrier damaging effects of n-propanol in healthy and atopic skin

Cumulative exposure to 45% n-propanol without previous barrier damage by occlusion led to significantly increased a*-values in the AD group after 96 hours compared to baseline (p<0.01). In contrast, at the same time point there were no significant changes in the a*-values of test field exposed to the same irritant concentration in the healthy controls (Table 1). Previous barrier impairment by repeated exposure to water/occlusion enhanced the irritant-induced effects and led to a significant a*-value increase after exposure to the lowest applied irritant concentration (30%) in both groups (p<0.05 and p<0.01 after 96 h compared with baseline in respectively, the healthy controls and AD group). For both groups, no significant difference in the a*-value following occlusion alone were found.
The relative changes of the $a^*$-values after 96-hour cumulative exposure to the different n-propanol concentrations, with and without previous occlusion, compared to baseline ($\Delta a^*$) are shown in Fig. 1a. In both groups the $a$-value* increased parallel to the increasing applied irritant concentration. In the healthy controls, the differences in the $\Delta a^*$-values between the previously occluded and non-occluded fields were significant for all studied irritant concentrations ($p<0.01$, $p<0.0001$, $p<0.01$ and $p<0.001$ for respectively 30%, 45%, 60% and 75% n-propanol; Table 2).
Table 1. Erythema (a*- value), transepidermal water loss (TEWL) and capacitance at baseline (D1) and after 96 hours (D5) repeated exposure to different concentrations of n-propanol (n-PrOH), with and without previous damage by repeated occlusion/water exposure in healthy controls (Healthy) and individuals with atopic dermatitis (AD), (N = 20, per group). The data are presented as mean and standard error of the mean (SEM). Level of significance <0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared with baseline (D1). AU: arbitrary units. Occl. Ctrl: occlusion with distilled water; Ctrl: non-exposed skin site.

| Without previous damage by repeated occlusion/water exposure | With previous damage by repeated occlusion/water exposure |
|---------------------------------------------------------------|----------------------------------------------------------|
|                  | Ctrl 30% n-PrOH 45% n-PrOH 60% n-PrOH 75% n-PrOH | Occl. Ctrl 30% n-PrOH 45% n-PrOH 60% n-PrOH 75% n-PrOH |
|                  | mean ± SEM  mean ± SEM  mean ± SEM  mean ± SEM  mean ± SEM  mean ± SEM  mean ± SEM  mean ± SEM  mean ± SEM  mean ± SEM |
| **a*-value (AU)** | |
| Healthy D1       | 10.98 ± 0.36  | 11.38 ± 0.43  | 11.27 ± 0.45  | 10.94 ± 0.39  | 11.24 ± 0.37  | 11.30 ± 0.44  | 11.44 ± 0.45  | 11.13 ± 0.44  | 11.55 ± 0.40  | 11.28 ± 0.44  |
| Healthy D5       | 10.82 ± 0.39  | 11.57 ± 0.43  | 11.30 ± 0.44  | 11.60 ± 0.39  | 12.08 ± 0.43  | 11.88 ± 0.50  | 12.23 ± 0.45  | 12.45 ± 0.36  | 13.29 ± 0.42  | 13.40 ± 0.58  |
| AD D1            | 9.98 ± 0.31   | 10.37 ± 0.25  | 10.14 ± 0.27  | 10.02 ± 0.27  | 10.14 ± 0.27  | 10.01 ± 0.25  | 10.28 ± 0.29  | 10.17 ± 0.25  | 10.65 ± 0.32  | 10.49 ± 0.27  |
| AD D5            | 11.04 ± 1.15  | 11.70 ± 0.76  | 11.74 ± 0.62**| 11.92 ± 0.62**| 12.75 ± 0.70**| 11.47 ± 1.04  | 11.96 ± 0.71**| 11.97 ± 0.40**| 13.33 ± 0.54**| 12.93 ± 0.54**|
| **TEWL (g/m²/h)**| |
| Healthy D1       | 4.75 ± 0.30   | 4.83 ± 0.24   | 4.64 ± 0.27   | 4.58 ± 0.26   | 4.59 ± 0.26   | 4.45 ± 0.24   | 4.56 ± 0.25   | 4.32 ± 0.22   | 4.14 ± 0.20   | 4.26 ± 0.24   |
| Healthy D5       | 4.56 ± 0.24   | 5.29 ± 0.31   | 5.31 ± 0.33** | 5.67 ± 0.29****| 6.08 ± 0.31***| 5.79 ± 0.31** | 6.24 ± 0.30** | 6.97 ± 0.35** | 7.40 ± 0.35** | 8.07 ± 0.47** |
| AD D1            | 4.89 ± 0.21   | 4.81 ± 0.19   | 4.55 ± 0.19   | 4.72 ± 0.20   | 5.12 ± 0.25   | 4.36 ± 0.18   | 4.73 ± 0.18   | 4.34 ± 0.23   | 4.35 ± 0.19   | 4.41 ± 0.15   |
| AD D5            | 5.08 ± 0.21   | 6.66 ± 0.36***| 8.24 ± 1.05***| 10.56 ± 1.45***| 12.12 ± 1.33***| 6.35 ± 0.27** | 9.10 ± 0.41** | 11.86 ± 0.77**| 13.37 ± 1.40***| 13.67 ± 1.30***|
| **Capacitance (AU)**| |
| Healthy D1       | 49.60 ± 1.53  | 48.14 ± 1.47  | 49.04 ± 1.53  | 49.38 ± 1.63  | 49.93 ± 1.50  | 49.76 ± 1.76  | 50.17 ± 1.59  | 50.55 ± 1.88  | 49.20 ± 1.71  | 49.56 ± 1.69  |
| Healthy D5       | 42.90 ± 1.81**| 37.96 ± 1.70**| 32.71 ± 1.74***| 29.03 ± 1.63***| 26.42 ± 1.63***| 42.90 ± 1.81***| 37.96 ± 1.74***| 32.71 ± 1.74***| 29.03 ± 1.63***| 26.42 ± 1.81***|
| AD D1            | 30.14 ± 1.65  | 29.41 ± 1.70  | 30.24 ± 1.60  | 30.19 ± 1.77  | 29.77 ± 1.77  | 30.35 ± 1.67  | 30.25 ± 1.74  | 29.56 ± 1.72  | 29.85 ± 1.74  | 30.11 ± 1.76  |
| AD D5            | 31.97 ± 1.67  | 23.23 ± 1.30***| 21.74 ± 1.33***| 20.40 ± 1.31****| 19.19 ± 1.39****| 27.94 ± 1.81** | 21.34 ± 1.30****| 17.70 ± 1.43***| 16.11 ± 1.44** | 15.90 ± 1.47****|
Table 2. Differences in the Δvalues of the test fields exposed to the same irritant concentrations with and without previous damage by occlusion/water exposure in the healthy and atopic dermatitis (AD) group. Level of significance <0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns-not significant.

| n-PrOH concentration | Δa*-value (AU) | ΔTEWL (g/m²/h) | ΔCapacitance (AU) |
|----------------------|----------------|----------------|--------------------|
|                      | Healthy AD     | Healthy AD     | Healthy AD         |
| 30 %                 | p<0.01 ns      | p<0.01 ns      | ns ns              |
| 45 %                 | p<0.0001 ns    | p<0.0001 ns    | ns p<0.01          |
| 60 %                 | p<0.01 ns      | p<0.0001 ns    | ns p<0.0001        |
| 75 %                 | p<0.001 ns     | p<0.001 ns     | p<0.01 p<0.05      |

Repeated occlusion with water alone resulted in impaired epidermal barrier function and significant TEWL increase on D5 compared to baseline in both groups (in AD and the healthy controls group respectively p<0.01 and p<0.05; Table 1). In AD cumulative exposure to 30% n-propanol alone was sufficient to induce a manifest damage to the epidermal barrier and the TEWL values of the respective test field on D5 were significantly higher compared to baseline (TEWL on D1 and D5 4.81±0.19 and 6.66±0.36 g/m²/h; p<0.001). In contrast, in the healthy controls cumulative exposure to 30% n-propanol led to significant TEWL increase only if the barrier function was previously compromised by occlusion/repeated water exposure. In both groups, TEWL after exposure to 45%, 60% and 75% n-propanol was significantly increased on D5 compared to D1 and the observed effect was independent of barrier damage by previous occlusion with water (Tabl.1). Barrier damage prior to n-propanol exposure enhanced the irritant-induced effects and in the healthy controls the relative TEWL increase on D5, assessed as ΔTEWL (ΔTEWL= TEWL D5 - TEWL D1), was significantly higher for all irritant concentrations (Table 2). In the AD group, the differences in ΔTEWL between the previously occluded and non-occluded fields on D5 were significant only after exposure to 30% and 45% n-propanol.
Fig. 1. Comparison of the changes in the a*-values (a), transepidermal water loss (b) and capacitance (c) after repeated exposure to different concentrations of n-propanol (30%-75%) with and without preceding damage to the epidermal barrier by occlusion/water in atopic (AD) and healthy individuals. The data are presented as Δ values (median and interquartile ranges) compared to baseline. Level of significance <0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. TEWL-transepidermal water loss, AU-arbitrary units, Occl.Aq-occlusion with distilled water, n-PrOH-n-propanol
In the presence of atopic skin disease repeated exposure to n-propanol resulted in more severe impairment of the barrier function and on D5, ΔTEWL of the test fields exposed to the same irritant concentration under the same conditions in the AD group were significantly higher than in the healthy controls (Fig. 1b).

In both groups cumulative exposure to all studied n-propanol concentrations with as well as without previous occlusion led to significant decrease of capacitance of the respective fields on D5 compared to D1 (Table 1). The decrease in capacitance was concentration-dependent in both the healthy and atopic group as shown by the increasing Δvalues parallel to the increase of the applied n-propanol concentration (Fig. 1c). In AD, the Δvalues on D5 compared to baseline were significantly greater if exposure to 45%, 60% and 75% n-propanol was preceded by occlusion (p<0.01, p<0.001 and p<0.05 for respectively 45%, 60% and 75% n-propanol). In the healthy controls the differences between the previously occluded and non-occluded fields exposed to the same irritant concentrations were significant only after exposure to 75% n-propanol (Table 2).

The relative decrease of stratum corneum hydration on D5 was more pronounced in the healthy controls group; the differences between the healthy and AD group were significant for the test fields exposed to 45%, 60% and 75% n-propanol with as well without previous damage by occlusion/water exposure (Fig. 1c)

Cumulative exposure to n-propanol reduces significantly the NMF levels in healthy and atopic skin

There were no significant differences in the baseline NMF levels between the groups. On D5, in both groups the NMF levels of all irritant-exposed fields, with as well as without previous damage by occlusion/water exposure, were significantly lower compared to the control fields (non-exposed/normal skin, respectively occlusion with water); at the same time point there were there were no significant differences between the control fields (Fig. 2). In the AD and the healthy controls group, there were no significant differences in the relative reduction of the NMF levels between the previously occluded and the corresponding, non-occluded irritant-exposed fields. In contrast to the barrier function parameters, no significant differences in the relative NMF reduction of the test fields exposed to the same concentrations of the irritant under the same conditions between the healthy and the AD individuals were found.
Figure 2. Stratum corneum natural moisturising factor (NMF) levels after repeated exposure to different concentrations of n-propanol (30%-75%) with and without previous barrier damage by occlusion/water in atopic (AD) and healthy skin. Absolute values (median and interquartile ranges), level of significance <0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to non-exposed (normal skin) site. Occl.Aq-occlusion with distilled water, n-PrOH-n-propanol, Ctrl-non-exposed (normal skin) site.

Discussion

The aim of the present om-TRIT was to investigate the relationship between the applied n-propanol concentration and the relative contribution of workplace relevant co-exposures on the outcomes of cumulative exposure to the irritant in healthy and AD individuals. The results of the study show that the irritant and barrier damaging effects of n-propanol in vivo are dependent on the applied irritant concentration and modulated by both exposure and host-related factors. In this line, whereas we found objectively measurable negative effects after exposure to even the lowest studied concentration, the relative changes in the inflammatory, barrier function and biochemical parameters were more pronounced with increasing n-propanol concentrations. Furthermore, using an
om-TRIT which was recently shown to differentiate the neat irritant-induced effects from the relative contribution of relevant co-exposures, we showed that repeated low-grade trauma by occlusion/water exposure alone impaired the barrier function and reduced the skin irritation threshold in both groups. As the cumulative duration of occlusion/water exposure under om-TRIT conditions corresponds to the definition of wet work, the findings of the study have important implications for development of irritant HE under real-life exposure conditions in both healthy and at risk individuals. In the same context, the different outcomes of the same exposures in atopic and healthy skin, shown in the study, may contribute to both the risk and recalcitrance of irritant HE in AD. In agreement with our earlier publications based on exposure to 60% n-propanol, in the present study we found reduced NMF levels after exposure to the entire range of investigated n-propanol concentrations and these findings were independent of the presence of atopic skin disease or previous damage by occlusion.

The number of published in vivo studies investigating the cumulative barrier damaging effects of repeated exposure to short-chain alcohols applied as single irritants has been limited. Lübbe et al. found no significant increase in TEWL after cumulative exposure to 60% n-propanol following previous damage to the epidermal barrier by overnight water exposure under occlusion in healthy skin.[13] Based on these findings the authors suggested that the irritant potential of 60% n-propanol was comparable to water. Similarly, Löffler et al. observed no significant changes in TEWL after repeated patch testing with ethanol, n-propanol and isopropanol applied on healthy skin in concentrations ranging from 60% to 100%.[14] In contrast, the findings of an independent study based on experimental repeated open application test showed that neat n-propanol exerted an irritant effect comparable to that of 1.0% sodium lauryl sulfate (SLS) and underlined the importance of the test model used in experimental studies for prediction of the skin irritation potential.[15]

The changes in the barrier function after cumulative exposure to 60% n-propanol following a tandem repeated irritation test (TRIT) model in healthy skin were initially studied by Kappes et al.[16] Using the same application mode, in a recent publication we found significant impairment of the permeability barrier function along with alterations in the corneocyte surface topography after exposure to the same concentration of the irritant and pointed to the previously underestimated skin irritant potential of short-chain alcohols.[4] In addition, the findings of our previous and present study are in agreement with the observations of an earlier om-TRIT, studying the effects of 60% n-propanol and 0.5% SLS in
the human skin in vivo and the results of a recent forearm controlled application
test by Cartner et al., showing significant impairment of the epidermal barrier
function after exposure to 70% n-propanol in healthy female volunteers.[5,17]
The pronounced in vivo irritant effect of n-propanol, observed by Cartner et al.,
was to shown to translate in vitro to a marked cellular toxicity and significant
TNF-α release by the skin residential cell. These findings are in agreement with
earlier in vitro observations for significantly increased expression and release
of key primary keratinocyte-derived cytokines such as IL-1α, TNF-α and IL-6 in
response to n-propanol exposure.[18,19]Taken together, the above mentioned in
vivo and in vitro observations provide a strong line of evidence for the negative
effects of n-propanol on the permeability barrier function and show its important
contribution to development of cumulative irritant HE in occupations with
workplace relevant exposure to short-chain alcohols.

Skin dryness and scaling are main and early signs of irritant HE. The
maintenance of skin hydration depends on both the intercellular lipids which
regulate the transport of water across the stratum corneum and a mixture of
low molecular weight, water-soluble compounds such as amino acids, organic
acids, urea and inorganic ions, collectively known as natural moisturising
factors (NMF). Short-chain alcohols are known penetration enhancers and
their pronounced effects on the intercellular lipids, including disruption of the
lipid lamellae, lipid phase transition and alterations in the lipid organisation
have been described in the literature.[20-26] Apart from these effects, a recent
study showed that short-chain alcohols and in particular, n-propanol, reduced
the activity of phospholipase A2 (PLA2), one of the key enzymes involved in
maintenance of the barrier homeostasis and lipid processing in the skin.[17] In
an earlier publication, we provided first evidence for significant reduction in the
stratum corneum NMF levels after om-TRIT with n-propanol and/or SLS and
suggested that short-chain alcohols may cause skin dryness through interaction
with both the skin lipids and reduction of NMF.[5] The results of the present study
confirm and extend these initial observations by showing a significant decrease
in the NMF levels after exposure to all of the investigated irritant concentration
in both healthy and atopic skin. The relative reduction of NMF in the healthy
controls ranged from 54.8% to 62.6% after exposure to the different irritant
concentrations without previous barrier damage by occlusion and, from 46.3% to
61.6% if the corresponding fields had been previously exposed to water under
occlusion. In the AD group, the relative changes in NMF after irritant exposure
without and with previous occlusion ranged between 46.2% and 61.4% and 42.3%
and 54.3%, respectively. Importantly, as in our previous study, in both groups
occlusion with water alone did not have a significant impact on NMF and these findings confirm that the observed effects are induced solely by the irritant. The significantly decreased NMF levels after cumulative exposure to even the lowest (30%) n-propanol concentration and the lack of significant differences in the relative NMF changes between the lowest and the highest (75%) irritant concentration in both groups correspond to the desiccation effects, reported under real-life exposure conditions and point to the contribution of a broad range of n-propanol concentrations to the sum of low-grade events, leading to manifest irritant HE. The pathomechanisms leading to NMF decrease after repeated exposure to n-propanol remain incompletely understood. Short-chain alcohols, including n-propanol, are known to exert protein denaturing and cytotoxic effects as well as compromise the epithelial cell membrane integrity which may facilitate the NMF escape from the cells.[17,27-29] As more than 50% of the total NMF content is known to be derived by the proteolytic degradation of filaggrin, the results of a recently published in vitro investigation showing that n-propanol and to a lesser extent, isopropanol and ethanol, decrease significantly the activity of the profilaggrin-processing enzyme kallikrein 5 (KLK5) provide another possible explanation for our findings.[17] Whether the previously reported, reduced activity of PLA2 by n-propanol could negatively impact the maintenance of the acidic skin pH and thus affect filaggrin processing under om-TRIT exposure conditions in vivo, remains at present speculative.

In contrast to the numerous epidemiologic studies showing increased susceptibility to occupational irritant HE in the presence of atopic skin disease, there have been few publications on the endpoints of cumulative exposure to single or multiple irritants under controlled exposure conditions in AD. The first evidence for significant impairment of the permeability barrier function after repeated exposure to 0.3 ml of 0.1% SLS, 2.3% di-sodium lauryl 3-ethoxysulphosuccinate or 2.0% Shellsol K in atopic skin was provided by Tupker et al.[30] Using a TRIT design, in an earlier study we showed that atopic skin is more susceptible to damage by even low concentrations of weak workplace irritants, such as 2% acetic acid, that would not exert a significant negative impact on healthy skin.[31] Additionally, in another study based on the same model, we observed significant differences in the severity of the barrier function impairment and inflammatory response induced by repeated single and concurrent exposure to detergents and alkaline agents in AD.[11] In the present study we found that cumulative exposure to 30% n-propanol, applied as a single irritant, was sufficient to induce a manifest damage to the epidermal barrier in AD whereas the same exposure had no significant effect on healthy skin unless
the barrier function had been previously impaired. The pattern of skin reactivity to cumulative exposure to n-propanol was similar to the one observed in our previous studies and there were significant differences in the severity of barrier impairment of the test fields exposed to the same irritant concentration under the same conditions in the AD compared with the healthy controls group. To the best of knowledge, this is the first study on cumulative exposure to short-chain alcohols and at the same time, first om-TRIT study in atopic skin and its findings provide further experimental evidence for the increased susceptibility to irritant damage in AD.

The mechanisms underlying the more severe barrier impairment after cumulative irritant exposure in AD are incompletely understood however could be attributed to alterations in the barrier properties and inflammatory responses in the presence of atopic skin disease. Compromised barrier function with increased baseline TEWL even in uninvolved skin is a major characteristic of AD and several groups in the past have shown a positive correlation between the pre- and post-exposure TEWL values after single as well as repeated SLS-induced irritation in both healthy and atopic individuals.[30,32-35] Whereas these observations could partly explain our results, it remains controversial if the barrier responses to experimentally-induced SLS irritation may predict the irritant responses to unrelated primary irritants, such as short-chain alcohols.[36]

Additionally to the increased baseline TEWL, the more severe barrier impairment after cumulative exposure to n-propanol shown in the present study, may be explained by earlier findings for increased percutaneous penetration of both hydrophilic and lipophilic compounds in AD. In this context, Jakasa et al. observed significantly higher SLS and polyethylene glycols of different molecular mass diffusivity through the clinically normal, uninvolved skin of AD volunteers as compared to healthy, non-atopic controls.[37,38] Independently of Jakasa et al., an earlier study showed increased penetration of dimethyl sulfoxide and theophylline through the stratum corneum of AD patients.[39] Similarly, using photoacoustic spectrometry Hata et al. found accelerated penetration of dyes of different water solubility such as Yellow 4 (hydrophilic) and Red 215 (lipophilic) in the clinically uninvolved skin of atopic volunteers.[40]

Beyond the impaired barrier function and increased skin diffusivity, the findings of earlier studies showing that increased skin surface pH, as found in AD, results in the activation of proteases involved in the processing of the pro-forms of the IL-1 cytokines in the epidermis, provide another possible explanation for the enhanced barrier responses in atopic compared to healthy
skin, observed in the present om-TRIT.[33,41-43] As the volunteers in the study were not genotyped, our results do now allow conclusions on the influence of filaggrin mutations carrier state on the outcomes of repeated exposure to n-propanol in atopic or healthy skin.

Taken together, the results of the study provide novel evidence for significant barrier damaging effects of even low concentrations of n-propanol in the human skin in vivo. The consistent effects found in the present and in our earlier studies confirm the validity of the om-TRIT model and point to the need for critical re-evaluation of the role of n-propanol in the pathogenesis of cumulative irritant HE in healthy and atopic skin.
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GENERAL DISCUSSION
This chapter first discusses the main findings and their interpretation, then methodological considerations. Finally, it presents conclusions and recommendations for future research and practice.

This project focused on the prevention of hand dermatitis (HD) in healthcare workers (HCWs). The main objective was to assess the effectiveness of an intervention program aimed at reducing HD symptoms and natural moisturizing factor (NMF) levels by increasing skincare use among HCWs. In addition, we aimed to investigate in vivo the effects of commonly used skin irritants on the skin barrier and the inflammatory response.

The corresponding research questions were defined accordingly.

Objective I. To assess the effectiveness of an intervention program aimed at reducing HD symptoms by increasing skincare use among HCWs

Research question I. Is a prevention program focused on skincare monitoring and feedback effective in reducing the severity of hand dermatitis in HCWs?

Research question II. Does a prevention program focused on skincare monitoring and feedback improve NMF levels in the skin?

Research question III. Does a new technology in the form of electronic monitoring combined with feedback increase skincare use among HCWs?

Objective II. To investigate in vivo the effects of commonly used irritants on the skin barrier and inflammatory response

Research question IV. What are the effects of various skin irritants on the skin barrier and inflammation?

Research question V. What is the effect of the concentration, occlusion and of atopic dermatitis on the barrier function and inflammatory response after experimental exposure to n-propanol?

PART I

MAIN FINDINGS

Research question I. Is a prevention program focused on skincare monitoring and feedback effective in reducing the severity of hand dermatitis in HCWs?

A cluster RCT (chapter 3) conducted on HCWs with or without signs of HD could not provide evidence for the effectiveness of an intervention of skincare monitoring and feedback with respect to the main outcomes: change in HECSI score and NMF stratum corneum levels from baseline to one-year follow-up.
However, the results did show significant effects in specific subgroups, as well as overall positive effects.

In both the intervention and control group (resp. IG and CG), HECSI score improved (resp. 6.2 and 4.2 points). However, the relative improvement proved to be significantly greater in the IG than in the CG (56% vs 44%; \( P<0.001 \)).

Although the reduction of 6.2 points in HECSI score at follow-up in the IG, versus a reduction of 4.2 in the CG, was not significantly different, only the IG achieved a reduction larger than the minimal detectable change (MDC) of 5.1 HECSI points, which can be considered a meaningful improvement.

To explore whether HD severity plays a role in the effectiveness of the intervention, a post-hoc analysis was performed in two subgroups based on their clinical symptoms at baseline. A HECSI score of 11 points was used as a cut-off value to divide the groups into mild HD (HECSI≤11) and moderate to severe HD (HECSI≥11), a classification proposed by Hald et al. (2009). The results revealed a significant effect of the intervention on HD severity in the subgroup of HCWs with mild HECSI (\( P<0.0001 \)), while no significant difference between IG and CG was found in the subgroup of HCWs with moderate to severe HD. This suggests that this intervention might be effective in averting progression of early skin-barrier damage into clinical dermatitis, and may therefore be specifically relevant in primary prevention.

**Research question II. Does a prevention program focused on skincare monitoring and feedback improve NMF levels in the skin?**

Stratum corneum levels of NMF, collected from the hands, were measured at baseline and after 12 months follow-up in a cluster RCT performed among HCWs (chapter 3). Contrary to expectations, NMF levels in both groups were significantly decreased at follow-up compared to baseline, despite their exposures to wet work not changing during the trial. Changes in NMF levels from baseline to follow-up were not significantly different between the control and intervention groups, indicating no effect of the intervention on the NMF levels.

In the literature, NMF levels are shown to be useful to detect effects of various irritants including SLS, NaOH, n-propanol and acetic acid, suggesting that NMF might be a general biomarker of skin irritation. Reduction of NMF has been suggested as a contributing factor for dry skin in HD, and one might expect that a decrease in NMF would parallel or lead to an increase in skin symptoms. However, the latter was not observed in this trial. This might have been caused by the introduction of a new disinfectant shortly before the trial started, with a
formula based on n-propanol rather than the ethanol formerly used. Compared with ethanol, n-propanol has previously shown a stronger damaging effect on the skin barrier. (1) In chapter 5 we demonstrated that n-propanol strongly decreased NMF levels. (2) Surprisingly, despite this decrease in NMF both the IG and the CG showed improvement in their clinical symptoms. One of the reasons for this might be the addition of glycerol in the new alcohol formulation. Glycerol is a frequently used humectant in skincare products. (3, 4) It might therefore be speculated that the NMF-decreasing effect on skin hydration associated with propanol was offset – at least partly – by the addition of glycerol in the new alcohol formulation. These changes in the composition of the frequently used disinfectants might not have only interfered with our primary outcome HECSI, but also with NMF as the quantitative biomarker of early skin-barrier damage. This suggests that NMF is not a suitable biomarker to monitor hand eczema in HCWs.

Research question III. Does a new technology in the form of electronic monitoring combined with feedback increase skincare use among HCWs?

Self-reported cream use
Chapter 4 shows that the studied intervention improved hand-cream use. Skincare behaviour in the IG improved and was significantly higher compared with the CG. At follow up, HCWs in the IG were 2.3 times more likely to report a higher frequency of cream use before their shift than the CG (P = 0.004; 95%CI: 0.26-1.38). During the shift this trend was even higher: HCWs in the IG were 3.3 times more likely to report a higher frequency of cream use (P < .001, 95%CI: 0.59-1.8). At baseline there was no difference between the two groups.

When looking at overall cream use (i.e. per entire shift), at baseline 38% of HCWs in the IG and 43% in the CG reported never using creams. After the intervention, the proportion of HCWs reporting ‘NEVER’ to applying creams decreased to 18% in the IG and 32% in the CG. HCWs with confirmed severe HD reported ‘ALWAYS’ using creams more frequently (69%; 20/29) than HCWs with no, mild or moderate HD (4% 18/470).

Electronically measured cream use and attitudes
Despite the increase in self-reported hand-cream use in this trial, the electronically monitored cream use of 0.4 events per HCW per shift in the IG remained below the recommended frequency of two times per shift. As more than one third of HCWs reported that, as well as electronic dispensers, they also
used their own creams, this electronically measured application frequency might be somewhat underestimated but is still likely below the currently recommended 2-3 times a day. (2, 3, 12 evaluation art.) This is surprising, as the majority of HCWs reported being aware of the benefits of hand creams and rated the likeability of the creams as ‘good’ (median 4 on a Likert scale ranging from 1 (0 = very poor to 5 = excellent). The most frequently reported (81%) reason for not using creams was the perception that it interfered with workflow. From the data collected electronically, it appeared that the dispensers in the staff-only rooms were used more frequently than those at locations where patient care is delivered. Most of the HCWs (86%) reported that the cream dispensers were well-located. Popular hours with the highest frequency of cream use were 10 AM, 12 PM and 3 PM.

**Feedback**

The feedback posters showing the compliance rates (with the recommended practice of two applications per shift) were noticed by most HCWs and perceived as useful. Almost half of the HCWs (43%) felt additionally motivated by the posters. As this was reported at the end of the trial, it could be argued that ‘loss of novelty’ played a role in the reported motivating effect of the posters.

**METHODOLOGICAL CONSIDERATIONS**

It is well recognized that designing a cluster randomized trial (CRT) is a more complex process than preparing individually randomized trials. (5) Among the methodological challenges associated with design and analysis, CRTs require more participants in order to obtain equivalent statistical power and more complex analyses. (5) On the other side of the coin, cluster design prevents the risk of experimental contamination; this was considered critical for the present trial.

Unsurprisingly, the cluster design of the trial presented here offered a number of methodological challenges. During recruitment, several sub-departments had to be considered collectively as one large cluster. These sub-departments shared management and a risk of cross-contamination was present as staff rotated between them despite their different locations. Moreover, the inclusion of a smaller number of clusters than originally anticipated reduced the trial’s statistical power (post hoc power 56%) as the total number of clusters has a greater impact than the number of participants sampled per cluster.

Another drawback in the study was the considerable level of loss to follow-up at the individual level. However, this seems not to have compromised the
validity of the trial as the main findings still hold, even assuming the ‘worst-case scenario’ regarding missing data. Moreover, the loss to follow-up was similar in both groups (41% in the IG and 39% in the CG) and sensitivity analysis revealed this to be independent of the severity of hand symptoms, therefore making the risk of attrition unlikely.

This trial included elements of both primary and secondary intervention, since it included “cases with increased risk of HD symptoms but no onset yet” as well as cases with actual symptoms of HD. A subgroup analysis based on HCWs with and without HD at baseline was not possible as there is no cut-off value available for HECSI to diagnose HD. Primary prevention usually consists of the identification of subjects who are at risk but have not yet progressed to the point of showing symptoms. However, as almost all the HCWs showed signs of erythema, a symptom included in the HECSI, the number representing a HECSI value equal to zero was so small that the potential of this intervention being assessed for primary prevention (i.e. incidence) was questionable.

Another aspect that should be considered is the lack of an authentic control group, as educational sessions were provided to both groups to achieve the same level of knowledge about risk factors and the prevention of HD. Recent studies have reported that education itself could have a positive effect on the severity of HD in a secondary prevention setting. This might at least partly explain why HD also improved in the CG. As a consequence, the provision of education to both groups could have minimized the contrast between the IG and CG.

Because our trial was conducted in the workplace, addressing HCWs in their natural working environment, it was not possible to eliminate all potential confounding variables. The hospital-wide change in hand-alcohol formulation (from an ethanol to a propanol-based formula, and the addition of a moisturizer) just before the start of our trial is considered an additional limitation, as this event had the potential to affect both the primary and the secondary outcomes.

The primary outcome in this trial was the change in skin symptoms, which was expressed as a HECSI score – shown previously to be a reliable, standardized and validated scoring system for a trained physician to clinically assess the severity of HD. Among other advantages, the HECSI is particularly suited to monitoring the severity of HD symptoms, as it did in this trial. More importantly, compared with studies that rely on self-reporting, an objective severity assessment is methodologically much stronger. (9, 10)
Objective II. To investigate in vivo the effects of commonly used irritants on the skin barrier and inflammatory response

MAIN FINDINGS

Research question IV. What are the effects of various skin irritants on the skin barrier and inflammation?

To investigate the effects of commonly used irritants on the skin barrier and inflammatory response, two clinical trials were performed. In the first (chapter 5), the effects of repeated exposure to different classes of commonly used irritants (n-propanol, sodium lauryl sulfate (SLS), sodium hydroxide and acetic acid) were assessed in healthy volunteers. For this purpose, functional (erythema, transepidermal water loss and hydration), biochemical (natural moisturizing factors, NMF) and morphological parameters (topography of the corneocyte surface) of the skin barrier were investigated and compared.

The results showed an irritant-specific pattern in the irritant response, implying that these irritants damage the skin barrier by different mechanisms. Alkaline substances like SLS and sodium hydroxide were the only irritants that caused significant changes in all the parameters investigated.

N-propanol, the main ingredient of alcohol disinfectants used in the health sector, caused only slight changes in the skin-barrier function as assessed by transepidermal water loss (TEWL) and erythema. However, it showed pronounced effects on skin hydration and NMF levels. The finding that n-propanol markedly reduces NMF levels and skin hydration, with TEWL remaining unchanged, suggests that the effect of n-propanol on skin hydration is probably caused by a decrease in NMF levels rather than through alterations in the lipid bilayers.

In addition to its effect on NMF levels and hydration, n-propanol also caused significant changes in corneocyte surface topography that were reflected by an increase in the number of the circular nano-size objects expressed as the DTI (dermal texture index). Recently, an increase in DTI was found in SLS-exposed skin but not in experimentally induced allergic contact dermatitis, suggesting that inflammation as such is unlikely to be the underlying cause of the observed changes. This is further supported by the results of the present study, which show a strong increase in DTI after exposure to n-propanol without the presence of inflammation. Furthermore, DTI correlated strongly with NMF and capacitance but not with erythema (as one of the main signs of inflammation).

Of all the parameters investigated, only the NMF level was significantly altered by all the irritants studied. Moreover, NMF level was the only parameter
able to detect changes between the non-exposed skin and water-occluded skin sites. Consequently, at least in experimentally induced irritation, NMF might be regarded as the most sensitive effect parameter.

Altogether, these findings show that skin-barrier impairment and the inflammatory response are irritant-specific and emphasize the need for a multi-parametric approach when studying skin irritation. NMF proved to be the most sensitive biomarker of irritant-associated changes.

**Research question V. What is the effect of the concentration, occlusion and of atopic dermatitis on the barrier function and inflammatory response after experimental exposure to n-propanol?**

In the second study, which was performed in healthy and atopic individuals (chapter 6), erythema, TEWL, skin hydration and natural moisturizing factor (NMF) levels were measured at baseline and then 96 hours after exposure to n-propanol, a common ingredient in commercially available hand disinfectants used in healthcare. The results confirm and extend our findings from chapter 5 and demonstrate that skin-barrier impairment, especially regarding skin hydration, and inflammatory response after exposure to n-propanol are concentration-dependent and potentiated by previous damage to the permeability barrier by occlusion and the presence of atopic dermatitis. The threshold concentration of n-propanol exerting significant changes in erythema was lower in atopic individuals than in healthy subjects (resp. 45% and 60%). A similar trend has been found for TEWL (threshold concentrations of 30% and 45% in atopic and healthy subjects, respectively). Unlike with TEWL and erythema, however, reduction in skin hydration was greater in healthy subjects than in those with atopic dermatitis. This is likely caused by the pre-existent low hydration in AD subjects at baseline, meaning that the water content in the stratum corneum that is measured by capacitance could not drop any further.

Previous damage to the permeability barrier by repeated occlusion showed an enhancing damaging effect on erythema and TEWL in both groups. After occlusion, the threshold concentration of n-propanol for TEWL and erythema was 30% in both atopic and healthy subjects.

Interestingly, by contrast with the skin bioengineering parameters TEWL and erythema, no significant differences were found between the healthy and atopic individuals in the relative NMF reduction of the test fields exposed to the same concentration of the irritant under the same conditions. The lowest n-propanol concentration applied already appeared to exert such a strong effect on the finite
pool of hygroscopic molecules within the corneocytes that further increases in the n-propanol concentration had no substantial additive effect. Obviously, the effect of n-propanol is so strong that even the lowest n-propanol concentration achieves the maximum possible effect.

Taken together, these results support our previous findings about the negative effects of n-propanol on the permeability barrier function and highlight its importance in the pathogenesis of ICD in HCWs. Furthermore, these findings clearly show the aggravating effect of external (occlusion) and intrinsic (atopic dermatitis) factors on the skin-damaging effect of n-propanol, which should be considered in the risk analysis and for the prevention of ICD in health sector.

METHODOLOGICAL CONSIDERATIONS
The investigated parameters of skin irritation were measured at the same time points, i.e. at baseline, 24 and 96 hours after starting the first of four successive exposures. Although these are standard moments in experimental studies, in practice it is likely that the kinetics of irritant response are both irritant-specific and individual-specific. Other limitations, as is often the case in experimental irritation studies, are the relatively small group sizes and short follow-up times.

INTERPRETATION OF RESULTS AND RELEVANCE FOR OCCUPATIONAL HEALTH
The Healthy Hands Project (HHP) presents a successful strategy to promote improved skincare in healthcare workers, but a significant effect regarding the skin condition was detected only in a subgroup of HCWs with mild HD. We now discuss what this means for the overall value of the intervention.

*Effect of the intervention on skincare behavior*
The present intervention was designed to change skincare behaviour, so as ultimately to reduce HD symptoms among HCWs. Regular hand-cream use has been widely recognized as an effective approach to reduce HD. A recent Cochrane review on interventions for the primary prevention of HD concluded that moisturizers might be effective, but also noted that this conclusion was based on moderate quality of evidence. According to previous studies, however, the consumption of creams in the workplace setting remains low. In the present study, we therefore aimed to improve hand-cream use through electronic monitoring of the consumption of creams supplied and by providing feedback — an approach previously applied successfully in hand hygiene campaigns (ref).

The findings from the HHP provide evidence that this approach was effective in increasing skincare use. Self-reported skincare behaviour in both groups
improved, but was significantly higher in the IG at follow-up, while at baseline there was no difference between the intervention and control groups (resp. IG and CG). Furthermore, the considerable proportion of HCWs who reported ‘never’ using hand creams decreased significantly in the IG, from 63% to 37%; this was far less in the CG (from 65% to 56%). A similar but opposite pattern was observed in the proportion of HCWs who reported ‘always’ (defined as at least once per shift) using hand creams. These findings might seem encouraging at first sight, but the electronically monitored cream consumption of 0.4 events per HCW per shift in the IG (averaged for all intervention wards together) remained far below the recommended frequency of two times per shift. It should be noted that the electronic data did show a trend of increase during the trial, and moreover might have underestimated actual cream use as one third of HCWs reported using their own creams, either instead or as well as those supplied.

To understand the effect on behaviour, it is important to study such as aspects as HCW beliefs, attitudes and satisfaction with the intervention. In the trial, these were evaluated by means of a short questionnaire at the end of the follow-up period. The results show that the HCWs were largely positive regarding such intervention characteristics as the likeability of the creams and the location of dispensers, and most reported that they were aware of the beneficial effects of creams. From the questionnaires, the most commonly reported reason (81%) for not applying creams during a shift was perceived interference with workflow. HCWs regarded the application of creams as time consuming, as they could not continue their work activities immediately and did not have the time to wait until the cream was absorbed.

Another possible explanation provided in chapter 4 for the low cream use in the present study is the introduction of a new disinfectant containing glycerol, a moisturizer that prevents dryness of the skin. Glycerol is a common ingredient of skin moisturizers, and was also an ingredient in our creams. In line with this explanation, it is likely that frequent use of disinfectants (on average more than 15 times per shift) containing glycerol lowered the necessity of hand-cream use. The effects of glycerol being included in the new disinfectant formula available in both the intervention and the control wards might therefore have affected skin-cream use (a process outcome in the present study), but could also have diluted the effect of the intervention on skin symptoms (primary outcome). In experimental studies, glycerol has been shown to increase hydration and prevent erythema.(11-13) It could be argued that the additional effect of cream use at work, when exposure to glycerol-containing disinfectants is high, is limited. It might therefore be beneficial to encourage the use of glycerol-containing
disinfectants in practice, although clearly only after this beneficial effect has also been confirmed in epidemiological studies.

**Effect of the intervention on hand dermatitis**

Small changes in behaviour observed across an entire population are likely to have a larger effect on health outcomes, assuming both that the targeted behaviour is appropriate and that a causal link with health exists. (14) It may therefore be surprising to learn that the improvement in the clinical outcome (HECSI) in the intervention group was not significantly greater than in the control group, despite the improved skin care in the IG. However, considering the limited magnitude of the increase in cream use, this might not have been sufficient to cause a significant effect in terms of clinical signs. In this trial, we included all HCWs regardless of whether or not they exhibited symptoms of HD. A large proportion of the HCWs had mild dermatitis (70%), but there were also others with more severe symptoms. Emollients are in general recommended to combat dry skin, which is regarded as the primary event in the development of ICD. (15) Hence, it might be expected that creams could prevent the first signs of HD (e.g. dryness, erythema) and improve the skin barrier. But in the case of more severe HD symptoms, creams will not be sufficient and the use of topical therapy with corticosteroids is recommended. (16, 17). In line with this reasoning, it is not surprising that the present intervention did indeed show a significant effect in HCWs with mild disease (defined as HECSI <11) by comparison with those with moderate to severe HD. This suggests that our intervention is effective in averting progression of early skin-barrier damage into clinical dermatitis, and may therefore be particularly relevant in primary prevention.

**Wet work in hcw**

To establish equal groups with regard to wet work exposure, pre-stratification based on the ward-level exposure (high or low) was used. The exposure at ward level was derived from the supply of soaps in the year preceding the trial, since hand washing – as opposed to use of disinfectants – has previously been reported as the main risk factor for HD. (18) This has been confirmed in the present study, as exposure to wet work was shown to be a risk factor for HD. This conclusion also holds when data on exposure (i.e. supply of soaps) during the trial was used.

In the HHP, the use of disinfectants was not regarded as a risk factor, as suggested by existing literature. (18) The use of disinfectants was high (almost all HCWs used disinfectants at least 15 times per shift), which is in accordance
with previous trials in healthcare. In chapter 3 we hypothesized that the introduction of a new formula containing n-propanol, replacing the previous ethanol-based disinfectant used in the hospital, shortly before the start of the trial caused negative effects on the skin condition of HCWs. N-propanol has previously been shown to have more damaging effects on the skin barrier than ethanol. In chapter 5 we carried out an experimentally-induced irritation study to investigate the irritating properties of n-propanol. Here, n-propanol showed a strong effect on NMF levels and skin hydration. However, it hardly affected transepidermal water loss (TEWL) or erythema, the parameters most commonly used to assess irritating properties. We argued that this emphasizes the need for careful selection of a relevant parameter to assess skin irritation, and demonstrated this in chapter 5 by using several commonly used irritants. In chapter 6 it was shown that the effect of n-propanol is concentration-dependent and potentiated by the presence of atopic dermatitis (AD). This is consistent with epidemiological data showing increased susceptibility of individuals with (a history of) atopic dermatitis to the development of irritant. As mentioned before, in the HHP the distribution of HCWs with AD was similar in the IG and the CG.

Another interesting effect of n-propanol on the skin barrier is alterations in the corneocyte surface texture, similar to the changes previously reported in carriers of filaggrin loss-of-function mutations in atopic dermatitis patients. The number of nano-size protrusions correlated inversely with NMF levels. In chapter 5 this was confirmed by showing a negative association between these protrusions and NMF levels. The development and physiological consequences of the protrusions have not yet been elucidated, but recently it has been shown that the adhesion of staphylococcus aureus (SA) bacteria was stronger in the presence of these protrusions and low NMF levels. Patients with AD are known to have increased colonization of SA. Following this line of reasoning, it would seem likely that HCWs with low NMF are more prone to skin infections.

In chapter 5 we showed that n-propanol strongly reduces NMF and skin hydration, which could be expected to negatively influence the symptoms of HD in our HCWs. In our intervention study (chapter 3), however, HD symptoms declined in both the IG and the CG despite a reduction in NMF levels and frequent use of disinfectants containing n-propanol. Two aspects seem to be important here. First, the addition of moisturizers to the propanol-based disinfectants as discussed above. Second, it has to be noted that most experimental irritation studies, including ours, are performed under occlusion. In chapter 5 we showed that occlusion enhances the adverse effect of n-propanol. In the real-world
setting, alcohols including n-propanol evaporate easily from the skin, so the actual duration of exposure is shorter than in experimental studies. In practice, however, exposure time might be prolonged when gloves are used immediately after applying disinfectants. For this reason, various studies and guidelines recommend minimizing exposure to detergents and other irritants before putting on gloves, wearing gloves for as short a time as possible and wearing cotton gloves under the occlusive gloves as ways to reduce skin-barrier damage. (22, 23)

FUTURE PERSPECTIVES AND RECOMMENDATIONS

Our study emphasizes the importance of promoting skincare in HCWs, especially in a primary prevention setting. To generate more robust evidence of the effectiveness of interventions like the Healthy Hands Project, more large randomized controlled trials (RCTs) are needed, preferably over extended time periods and in a prospective cohort design, e.g. in student nurses or new employees. Below, we reflect upon what can be learned from our research and focus on future directions.

First of all, low compliance, regardless of the measures taken, is a common hurdle in preventive programs. In spite of the observed improvements in skincare, this remained an issue in our trial, too. To understand how to improve implementation of such a program in the occupational setting, more barriers and facilitators of hand-cream use in HCWs need to be identified and translated into action. One of the strategies worthy of future attention is the use of electronic monitoring and feedback programs, in particular to drive hand care compliance. Technology is useful for providing fast and targeted feedback.(24) In the present research, we aimed to optimize the design and delivery of such a feedback strategy. The existing hand-hygiene literature proposes several strategies for improved effectiveness of feedback(24): (1) having the feedback provided by a supervisor; (2) providing it at least monthly; (3) providing it in both verbal and written form; and (4) setting clear goals with specific instructions on how to improve.(25) Most of these characteristics were part of our program, but there remain opportunities to further enhance future programs by incorporating the approaches mentioned, such as involving supervisors or managers in providing feedback, more emphatically. Another aspect to consider for the design of future programs is the development of an electronic system able to monitor individual rather than only ward-level cream consumption. If possible, parallel electronic measurement of soap and disinfectant consumption would also be valuable.
A second recommendation for future trials is to perform the study in the winter, as skin symptoms are seasonal and therefore likely to be greater higher and more relevant in the colder months of the year. Due to time constraints and practical limitations, the HHP began in April and the skin measurements were performed in May and June.

A third point to bear in mind for future programs is the culture of HCWs with regard to hand dermatitis symptoms, which – as reported over the years – are underestimated for their implications and often perceived as part of the job. (26, 27) This culture was clearly encountered on the nursing wards where we conducted interviews. We believe that having front-line staff ‘on board’ to proactively advocate improved hand care on wards, rather than outsiders, would be hugely helpful in bringing about positive change.(26, 28)

In chapter 3 we also argue that it is of vital importance for the prevention of HD to increase awareness among HCWs and hospital management that having HD can pose a risk to patient safety. Not only are HCWs with HD reported to have more colonization of *staphylococcus* on their skin, but this has also been identified as one of the primary reasons for avoiding skin disinfection. (29) Hospital management and infection-control professionals have essential roles in informing and educating workers about the importance of hand care and should advocate improved skincare to reduce both hospital-acquired infections (HAIs) and occupational dermatitis among HCWs.

A fourth point to emphasize is the importance of detection of the early symptoms of HD and early diagnosis. This is one of the key components in HD prevention and therefore should be given more attention in prevention programs. Which is not only up to the HCW, but also to management, occupational physicians and health-and-safety professionals. As reported by Van der Meer, HD seems to have little impact on work in terms of absenteeism and, not being seen as a reason not to work, its prevalence may therefore be underestimated by occupational physicians and by workers themselves.(31) However, relatively high ‘presenteeism’ rates (defined as “going to work despite feeling you should have taken sick leave because of hand eczema”) have also been reported in a study by Oosterhaven et al.(30) Occupational physicians should therefore be encouraged to use different tools for estimating HD prevalence; for example, performing risk assessments for health.(31) Also, based on our informal conversations with HCWs, employees do not seem to be sufficiently instructed on what action to take if they start to develop HD and how to access an occupational physician specialized in OSD. More guidance here would support a culture of better prevention.
Based on the electronic data on hand-cream use from our trial, advice regarding the best locations for cream use and its best timing can now be added to the available occupational health recommendations for skincare.

RECOMMENDATIONS FOR FUTURE STUDIES

1. Investigation of the effectiveness of an HD prevention program focused on skincare monitoring and feedback, in the form of large randomized controlled trials (RCTs) with a prospective cohort design in a healthcare primary prevention setting (e.g. student nurses or new employees).

2. Side-to-side comparison of different hand-cream formulations, investigating not only their protective effect but also HCW preferences regarding their greasiness, absorption time and odour. The finding that n-propanol significantly affects NMF and skin hydration suggests that humectants in particular (e.g. glycerol or urea) might be effective in optimizing skin hydration.

3. Comparison of hand disinfectants with and without the addition of glycerol in a prospective cohort study in an occupational setting. In this trial it was assumed that the unanticipated addition of glycerol to the disinfectants played a role in improving HD, given the barrier-enhancing effects of glycerol as shown in experimental studies. It is recommended that these findings be further verified in an occupational setting.

4. Studies to develop standardized measures for the detection of HD in trials. One significant concern with epidemiological studies is the lack of well-defined criteria for diagnosing HD and for distinguishing irritant skin changes from irritant contact dermatitis. In this context, the currently available scoring systems, like HECSI, should be further studied with regard to their interpretation and determination of clinical relevance and severity classifications.

5. Investigation of the skin microbiome in relation to the severity of hand dermatitis, corneocyte surface texture and NMF.

CONCLUSION

This thesis shows that a prevention program based on the electronic monitoring of skincare and feedback was an effective strategy in improving skincare behaviour in HCWs. Despite this, a significant effect in terms of reducing skin symptoms of HD was detected only in the subgroup of HCWs with mild symptoms of HD. Further implementation of this intervention may only be recommended in the context of primary prevention of HD among HCWs (e.g.
in student nurses). Altogether, the findings of the Healthy Hands Project are promising and support the benefits of skincare in the workplace. This work could form a good basis for further optimization of the design and delivery of such a strategy, but more research is needed to identify the barriers and facilitators affecting skin care compliance to optimize implementation of similar strategies.
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8

SUMMARY / SAMENVATTING
Healthcare workers’ skin is daily exposed to ‘wet work’. Frequent and cumulative exposure to various irritants and contact allergens can result in development of contact dermatitis (CD), especially hand dermatitis (HE). When disease becomes chronic, the burden is particularly high for individuals due to effects on quality of life. In addition, the high rates of presenteeism, absenteeism and medical costs associated with hand dermatitis also create a serious economic burden to society. It has been argued that having HD can also pose a risk to patient safety. Not only are HCWs with HD reported to have more colonization of *Staphylococcus aureus* on their skin, but impaired skin has also been identified as one of the primary reasons for avoiding skin disinfection leading to higher infection rates. The importance of prevention of hand eczema by using moisturizers is one of the crucial recommendations, emphasized in guidelines. The use of moisturizers by HCW is low and changing the culture towards better skin care remains a hurdle in preventive programs. For this reason an effective strategy is needed to improve hand cream use in HCW to prevent hand dermatitis symptoms. In addition, we investigated the effects of commonly used skin irritants on the skin barrier and the inflammatory response important in the pathogenesis of ICD in HCW. These results are considered to be helpful in the risk analysis for prevention of ICD in healthcare.

This lead to the objectives of this research:

**Objective I.** To assess the effectiveness of an intervention program aimed at reducing HD symptoms by increasing skincare use among healthcare workers

**Objective II.** To investigate in vivo the effects of commonly used irritants on the skin barrier and inflammatory response in healthy and atopic individuals

The corresponding research questions are addressed in separate chapters, which are summarized below.

**PART I: Intervention study in healthcare workers**

**Research question I:** *Is a prevention program focused on skin care monitoring and feedback effective in improving severity of hand dermatitis in healthcare workers?*

In chapter 3 the results are described of a RCT focused on increasing skin care to improve symptoms of hand dermatitis in HCW. This intervention of skin care monitoring and feedback presents a successful strategy to promote improved skin care in health care workers, but a significant effect regarding the skin condition was only detected in a subgroup of HCW with mild HD.
The clinical severity of hand dermatitis measured by the Hand Eczema Severity Index-score (HECSI-score) reduced in the IG for -6.2 (95%CI -7.7,-4.7) and in the CG -4.2 points (95%CI -6.0,-2.4). There was no difference in absolute decrease in HECSI (ΔHECSI) between groups, however relative improvement in HECSI was significantly higher in the IG compared to the CG (56% vs.44%; P<0.001).

To explore whether HD severity plays a role in the effectiveness of the intervention a post hoc analysis was performed in two subgroups based on the clinical symptoms at baseline. An HECSI score of 11 points was used as a cut off to divide the groups into no or mild HD (HECSI≤11) and moderate to severe HD (HECSI≥11). The results revealed a significant effect of the intervention on HD severity in the subgroup of HCW with at baseline no or mild HECSI (P<0.0001) while in the subgroup of HCW with more severe HD there was no significant difference between IG and CG. This suggests that this intervention might be effective in averting progression of early skin barrier damage into clinical dermatitis, and may therefore be specifically relevant in primary prevention.

Overall, the intervention showed positive effects and emphasizes the importance of promoting skin care in HCW, despite the lack of a significant effect on the primary outcome. To generate more robust evidence of effectiveness of interventions, like the Healthy Hands Project, more large randomised controlled trials (RCTs) are needed, preferably over extended time periods and in a prospective cohort design, e.g. in apprentices nurses or new employees.

**Research question II: Does a prevention program focused on skin care monitoring and feedback improve ‘natural moisturizing factors’ (NMF) levels in the skin?**

The stratum corneum levels of NMF, collected from the dorsal skin of the hands of HCW, were measured at baseline and after 12 months follow-up (chapter 3). Surprisingly, NMF levels in both groups were significantly decreased at follow-up compared to baseline, despite steady ‘wet work’ exposure levels during the trial. Changes in NMF levels from baseline to follow-up were not significantly different between the control and intervention groups, indicating no effect of the intervention on the NMF levels.

NMF is suggested as a general biomarker of skin irritation as it is shown to be useful to detect effects of various irritants, including SLS, NaOH, n-propanol and acetic acid (Chapter 5). Dry skin in HD is associated with a decrease in NMF, which is assumed to parallel or lead to an increase in skin symptoms. Yet, the latter was not observed in the clinical data of the trial. An explanation for
this may be the changed disinfectants formula provided at the wards, shortly before the start of the trial: from an ethanol based formula to a propanol one. Compared with ethanol, n-propanol has previously shown a stronger damaging effect on the skin barrier. In chapter 5 we demonstrated that n-propanol strongly decreased NMF levels. Surprisingly, despite this decrease in NMF both the IG and the CG showed improvement in their clinical symptoms. One of the reasons for this might be the addition of glycerol in the new alcohol formulation. Glycerol is a frequently used humectant in skincare products. It might therefore be speculated that the NMF-decreasing effect on skin hydration associated with propanol was offset – at least partly – by the addition of glycerol in the new alcohol formulation. These changes in the composition of the frequently used disinfectants might not have only interfered with our primary outcome HECSI, but also with NMF as the quantitative biomarker of early skin-barrier damage. This suggests that NMF is not a suitable biomarker to monitor hand dermatitis in HCWs.

**Research question III: Does a new technology of electronic monitoring combined with feedback increase skin care use among HCW?**

**Self-reported cream use**

In Chapter 4 the effect of the intervention on skin care behaviour is described. The findings from the Healthy Hands Project provided evidence that the intervention was effective in improving cream use. Self-reported skin care behaviour in both groups improved at follow up, but was significantly higher in the IG as compared to the CG. HCWs in the IG were 2.3 times more likely to report a higher frequency of cream use before their shift than the CG (P = 0.004; 95%CI: 0.26-1.38). During the shift this trend was even more pronounced: HCWs in the IG were 3.3 times more likely to report a higher frequency of cream use (P < .001, 95%CI: 0.59-1.8). At baseline there was no difference between the two groups. Importantly, HCWs with confirmed severe HD reported ‘ALWAYS’ using creams more frequently (69%; 20/29) than HCWs with no, mild or moderate HD (4%; 18/470).

**Electronically measured cream use and attitudes**

Although the increased self-reported hand cream use seems encouraging, the electronically monitored cream use of 0.4 events per HCW per shift in the IG remained below the recommendations (at least 2 times/shift). This electronically measured application frequency might be somewhat underestimated as one
third of HCWs reported to use their own creams. From questionnaires the most frequently reported (81%) reason for not using creams was the perception that it interfered with workflow and is time-consuming. From the data collected electronically, it appeared that the dispensers in the staff-only rooms were used more frequently, especially around 10 AM, 12 PM and 3 PM, than those at locations where patient care is delivered. Another explanation for the low cream use might have been the newly introduced hand disinfection that contained glycerol, a moisturizer that prevents dryness of skin. The frequent use of this disinfectant is likely diminished the need for cream use in HCWs.

**Feedback**
The feedback posters, showing the compliance rates with the recommended practice of two applications per shift, were noticed by almost all HCWs and perceived as useful. Almost half of the HCWs (43%) felt additionally motivated by the posters, although they reported that this effect ceased after some time, i.e. ‘loss of novelty’ decreased the motivating effect of the posters.

Altogether, electronic monitoring of hand cream use combined with feedback showed to be an effective strategy to improve skin care behaviour.

**Part II: Experimental studies on the in vivo effects of skin irritants on the skin barrier and inflammatory response**

**Research question IV:** What are the effects of various skin irritants on the skin barrier and inflammation?

The effects of commonly used irritants on the skin barrier and inflammatory response has been investigated in two experimental studies. In the first study (chapter 5), the effects of repeated exposure to different classes of commonly used irritants (n-propanol, sodium lauryl sulfate (SLS), sodium hydroxide and acetic acid) were assessed in healthy volunteers. For this purpose, functional (erythema, transepidermal water loss and hydration), biochemical (natural moisturizing factors, NMF) and morphological parameters (topography of the corneocyte surface) were investigated and compared. The results showed an irritant-specific pattern in the irritant response, implying that these irritants damage the skin barrier by different mechanisms. Alkaline substances, like SLS and sodium hydroxide, were the only irritants that caused significant changes in all the parameters investigated. N-propanol, the main ingredient of alcohol disinfectants used in the health sector, caused only slight changes in the skin-barrier function as assessed by transepidermal water loss (TEWL) and erythema.
However, it showed pronounced effects on skin hydration and NMF levels. The finding that n-propanol markedly reduces NMF levels and skin hydration, with TEWL remaining unchanged, suggests that the effect of n-propanol on skin hydration is probably caused by a decrease in NMF levels rather than through alterations in the lipid bilayers.

In addition to its effect on NMF levels and hydration, n-propanol also caused significant changes in corneocyte surface topography that were reflected by an increase in the number of the circular nano-size objects expressed as the DTI (dermal texture index). Recently, an increase in DTI was found in SLS-exposed skin but not in experimentally induced allergic contact dermatitis, suggesting that inflammation as such is unlikely to be the underlying cause of the observed changes. This is further supported by the results of the present study, which show a strong increase in DTI after exposure to n-propanol without the presence of inflammation. Furthermore, DTI correlated strongly with NMF and capacitance but not with erythema, as one of the main signs of inflammation.

Of all the parameters investigated, only the NMF level was significantly altered by all the irritants studied. Moreover, NMF level was the only parameter able to detect changes between the non-exposed skin and water-occluded skin sites. Consequently, at least in experimentally induced irritation, NMF might be regarded as the most sensitive effect parameter.

Altogether, these findings show that skin-barrier impairment and the inflammatory response are irritant-specific and emphasize the need for a multi-parametric approach when studying skin irritation.

**Research question V:** What is the concentration-dependent effects of n-propanol on the barrier function and inflammatory response with and without previous damage to the permeability barrier by occlusion in healthy and atopic individuals?

The second study focused on n-propanol, a common ingredient in commercially available hand disinfectants used in healthcare. The effect of the concentration of n-propanol, previous barrier damage by occlusion and presence of atopic dermatitis have been assessed by measuring erythema, TEWL, skin hydration and natural moisturizing factor (NMF) levels at baseline and 96 hours after exposure to n-propanol. The findings demonstrated that skin-barrier impairment and inflammatory response after exposure to n-propanol are concentration-dependent and potentiated by previous damage to the permeability barrier by occlusion and the presence of atopic dermatitis. Atopic individuals showed a lower threshold concentrations of n-propanol that exerted significant changes
in erythema than healthy subjects (resp. 45% and 60% n-propanol). A similar trend was found for TEWL (threshold concentrations of 30% and 45% in atopic and healthy subjects, respectively). In contrast, reduction in skin hydration was larger in healthy subjects than in those with atopic dermatitis. This could be explained by pre-existent low skin hydration in AD subjects at baseline, meaning that the water content in the stratum corneum that is measured by capacitance could not drop any further.

Previous damage to the permeability barrier by repeated occlusion showed an enhancing adverse effect on erythema and TEWL in both groups. After occlusion, the threshold concentration of n-propanol for TEWL and erythema was 30% in both atopic and healthy subjects.

Interestingly, in contrast with the skin bioengineering parameters TEWL and erythema, no effect of the concentration, occlusion or presence of atopic dermatitis has been found on the NMF levels. The lowest n-propanol concentration applied, already appeared to exert such a strong effect on the finite pool of hygroscopic molecules within the corneocytes that further increases in the n-propanol concentration had no substantial additive effect. Obviously, the effect of n-propanol is so strong that even the lowest n-propanol concentration achieves the maximum possible effect.

Taken together, these results support our previous findings about the negative effects of n-propanol on the skin barrier and highlight its importance in the pathogenesis of ICD in HCWs. Furthermore, these findings support the hypothesis from chapter 3, which is the NMF-decreasing effect of the new n-propanol containing hand disinfection. The aggravating effects of external (occlusion) and intrinsic (atopic dermatitis) factors on the skin-damaging effect of n-propanol, prove the importance of risk analysis for the prevention of ICD in the health sector.
Samenvatting

De huid van zorgverleners wordt dagelijks blootgesteld aan ‘nat werk’. Frequentie en cumulatieve blootstelling aan irritatieve stoffen zoals zepen, water en oplosmiddelen kunnen resulteren in irritatief contact eczeem (CE), en vooral in handeczem (HE). Het chronisch worden van de ziekte gaat gepaard met een hoge ziektebelasting voor patiënten met grote gevolgen voor de kwaliteit van leven. Mogelijke gevolgen zijn arbeidsongeschiktheid, arbeidsverzuim en productieverlies. Dit gaat gepaard met hoge kosten voor werkgevers en de maatschappij. Ook staat het ter discussie of HE een risico met zich mee brengt voor de patiëntveiligheid. Niet alleen is de huid van zorgverleners met HE meer vatbaar voor infecties, het weerhoudt hen er ook van handdesinfectie middelen te gebruiken, met als gevolg een groter risico op infectie en verspreiding.

Irritatief CE ontstaat als gevolg van cumulatieve schade aan de huidbarrière. Derhalve wordt het belang van de juiste handverzorging, ter bescherming en het verhogen van de belastbaarheid van de huid, benadrukt in verschillende richtlijnen voor de preventie van (arbeid gerelateerd) CE. Hierin wordt beschreven dat indifferentie behandeling (het gebruik van verzorgende, geen geneesmiddelen bevattende crèmes) de basis vormt voor adequate huidverzorging. Echter, in de praktijk is het gebruik van handcrèmes onder zorgverleners vrij laag en blijft het bevorderen van de benodigde gedragsverandering een uitdaging in preventieve programma’s.

Het hoofddoel van dit onderzoek was daarom gericht op het ontwikkelen van een strategie om handverzorging onder zorgverleners te bevorderen. Hiertoe werd er een interventie ontwikkeld gebaseerd op het elektronisch monitoren van handcrème gebruik en feedback, een strategie die succesvol bleek te zijn in betere naleving van de handhygiëne richtlijnen in de gezondheidszorg. Naast deze interventiestudie werden, in een experimentele setting, de effecten van veelgebruikte irritatieve middelen op de huidbarrière en de inflammatoire respons bestudeerd, welke in de context van de gezondheidszorg van belang zijn voor risico-inventarisatie en uiteindelijk de preventie van HE.

Dit heeft geleid tot de volgende doelstellingen:

**Doelstelling I.** Het onderzoeken van de effectiviteit van een interventie programma voor het verminderen van HE symptomen door het verbeteren van handverzorging onder zorgverleners

**Doelstelling II.** Het onderzoeken van de effecten van veelgebruikte irritatieve stoffen op de huidbarrière en de inflammatoire respons bij gezonde
proefpersonen en proefpersonen met (een geschiedenis van) atopische dermatitis

De overeenkomende onderzoeksvragen zijn beantwoord in afzonderlijke hoofdstukken, hieronder samengevat.

Deel I: Effectiviteit van een interventie programma gericht op het verbeteren van handverzorging onder zorgverleners

Onderzoeksvraag I: Is een preventieprogramma gericht op het monitoren van handverzorging, gecombineerd met feedback, effectief in het verbeteren van de ernst van handeczeem?

In hoofdstuk 3 zijn de resultaten beschreven van een Randomized Control Trial (RCT) gericht op het verbeteren van handeczeem (HE) symptomen bij zorgverleners. Deze interventie, gebaseerd op het monitoren van handcrème gebruik en feedback, presenteert een succesvolle strategie voor het verbeteren van handverzorging bij zorgverleners, hoewel een significant effect ten aanzien van de HE symptomen enkel werd gedetecteerd in een subgroep van zorgverleners met mild eczeem.

De klinische ernst van handeczeem gemeten met de ‘Hand Eczema Severity Index’ score (HECSI-score) was verlaagd in de interventie groep (IG) met -6.2 (95%CI -7.7,-4.7) en in de controle groep (CG) met -4.2 punten (95%CI -6.0,-2.4). Er was geen verschil in de absolute afname in HECSI (ΔHECSI) tussen de groepen, hoewel de relatieve verbetering in HECSI significant hoger was in de IG vergeleken met de CG (56% vs. 44%; P<0.001).

Om te exploreren of de ernst van handeczeem een rol speelt in de effectiviteit van de interventie werd er een post-hoc analyse uitgevoerd in twee subgroepen gebaseerd op de klinische symptomen op baseline. Een HECSI score van 11 punten werd gebruikt als de afkapwaarde om de groepen te verdelen in ‘geen’ tot ‘mild’ eczeem (HECSI≤11) en ‘matig’ tot ‘ernstig’ eczeem (HECSI≥11). De resultaten onthulden een significant effect van de interventie op de ernst van HE in de subgroep van zorgverleners die bij aanvang ‘geen’ tot ‘mild’ eczeem hadden (P<0.0001), terwijl in de subgroep van zorgverleners met bij aanvang ‘matig’ tot ‘ernstig’ eczeem er geen significant verschil kon worden aangetoond tussen de IG en CG. Dit suggereert dat deze interventie mogelijk effectief is in het verhinderen van progressie van vroege schade aan de huidbarrière naar gemanifesteerd eczeem, waardoor het met name relevant zou zijn voor de primaire preventie.
De interventie liet globaal positieve effecten zien en benadrukt het belang van huidverzorging, ondanks het gebrek aan een significant effect op de primaire uitkomst (absolute afname in HECSI, ΔHECSI).

Om meer robuust bewijs te genereren ten aanzien van de effectiviteit van deze interventie zijn er meer grote gerandomiseerde studies nodig, bij voorkeur met een langere follow-up en in een prospectieve studie.

**Onderzoeksvraag II: Verbetert een preventieprogramma gericht op het monitoren van handverzorging en het geven van feedback de ‘natural moisturizing factors’ (NMF) waarden in de huid?**

De stratum corneum waarden van de NMF, verzameld van de dorsale zijde van de handen van zorgverleners, werden bepaald op baseline en na follow-up. (hoofdstuk 3.1). Opmerkelijk genoeg waren de NMF waarden in beide groepen significant verlaagd tijdens follow-up vergeleken met de uitgangswaarde, ondanks de vergelijkbare blootstelling aan ‘nat werk’ tijdens de trial. Verandering in de NMF waarden van baseline tot follow-up waren niet significant anders tussen de controle en de interventie groep, wijzend op het ontbreken van effect van de interventie op de NMF waarden.

NMF wordt beschouwd als een belangrijke biomarker voor huidirritatie en werd in experimentele studies gebruikt voor het monitoren van de barrière effecten van irriterende stoffen, zoals sodium lauryl sulfaat (SLS), natrium hydroxide (NaOH), n-propanol en azijnzuur (AcA) (hoofdstuk 4.1). Verlaging van NMF in deze trial zou verklaard kunnen worden door de vervanging van de handalcohol op basis van ethanol door een nieuwe handalcohol op basis van n- en iso-propanol, net na het starten van de trial. Het is bekend dat, vergeleken met ethanol, n-propanol een groter schadelijk effect op de huidbarrière heeft. In onze experimentele studies beschreven in hoofdstuk 4 werd aangetoond dat n-propanol de NMF concentratie sterk verlaagt. Een droge huid is één van de eerste symptomen van HD en wordt geassocieerd met lage NMF concentraties in het stratum corneum. Echter, zowel de interventie groep (IG) als de controlegroep (CG) toonden verbetering in klinische symptomen ondanks deze afname in NMF. Een mogelijke verklaring hiervoor is de toevoeging van glycerol in de nieuwe handalcohol formule. Glycerol is een veelgebruikt humectant, een waterbindinge stof, in huidverzorgingsproducten. Derhalve wordt gespeculeerd dat het NMF verlagende effect op de huidhydratatie veroorzaakt door n-propanol, mogelijk gecompenseerd is door de toevoeging van glycerol in de nieuwe handalcohol formule. Deze verandering in de samenstelling van de veelvuldig
gebruikte alcohol heeft mogelijk niet enkel de potentie te interfereren met de primaire uitkomstmaat HECSI, maar ook met NMF als een biomarker voor vroege huidbarrièreschade. AI met al kunnen we concluderen dat NMF, in contrast met experimentele irritatie studies, geen geschikte biomarker zou zijn voor het monitoren van huidbarrièreschade in zorgverleners.

**Onderzoeksvraag III: Leidt een nieuwe technologie van elektronisch monitor gecombineerd met feedback tot verbeterde handverzorging onder zorgverleners?**

**Zelf-gerapporteerd handcrème gebruik**
In hoofdstuk 4 werd het effect van de interventie op het gedrag van zorgverleners ten aanzien van handverzorging beschreven. De bevindingen toonden aan dat de interventie effectief was in het verbeteren van het crème gebruik. Zelf-gerapporteerd handcrème gebruik verbeterde in beide groepen tijdens de follow-up, maar was significant hoger in de IG vergeleken met de CG. Zorgverleners in de IG rapporteerden 2.3 keer vaker een hogere frequentie van crème gebruik ‘vóór de dienst’ dan de CG (P = 0.004; 95%CI: 0.26-1.38). ‘Tijdens de dienst’ bleek deze tendens meer uitgesproken: het rapporteren van een hogere frequentie van handcrème gebruik was 3.3 keer waarschijnlijker in de IG (P < .001, 95%CI: 0.59-1.8). Op baseline waren er geen verschillen tussen beide groepen. Ook rapporteerden zorgverleners met ernstig eczeem het ‘tijdens elke dienst’ gebruiken van handcrèmes veel frequenter (69%; 20/29) dan zorgverleners met geen, mild dan wel matig eczeem (4% ; 18/470).

**Elektronisch gemeten handcrème gebruik**
Hoewel het zelf-gerapporteerde handcrème gebruik veelbelovend lijkt, blijft het elektronisch gemeten handcrème gebruik van gemiddeld 0.4 applicaties per zorgverlener in de interventiegroep achter bij de aanbevelingen (van minstens 2 keer per shift). Deze elektronisch gemeten frequentie is echter mogelijk onderschat, omdat een derde van de zorgverleners rapporteert eigen handcrèmes te gebruiken. Uit de vragenlijsten blijkt dat de meest gerapporteerde reden om de handen niet te verzorgen de perceptie is dat handcrème gebruik tijdrovend is en interferereert met de werkzaamheden. De elektronisch verzamelde data wijzen uit dat de dispensers in de overleg- en overdrachtsruimten veel vaker werden gebruikt, met name rond 10 uur (pauze), 12 uur (lunch) en 15 uur (dienstwissel), dan de dispensers op locaties waar directe patiëntenzorg werd geleverd. Een andere verklaring voor het lage handcrème gebruik is mogelijk de nieuwe handalcohol waarin glycerol is toegevoegd, een humectant die het
uitdrogen van de huid voorkomt. Het frequente gebruik van deze alcohol heeft mogelijk geleid tot minder behoefte aan het gebruiken van handcrèmes onder zorgverleners.

*Feedback*
De feedback posters, die de mate van compliantie met de aanbevolen twee applicaties per shift tentoonstelden, waren door bijna alle zorgverleners opgemerkt en als nuttig beschouwd. Ongeveer de helft van de zorgverleners (43%) voelde zich extra gemotiveerd door de posters, hoewel dit effect wel afnam na verloop van tijd.

Concluderend, lijkt het elektronisch monitoren van handcrème gebruik gecombineerd met feedback een effectieve strategie te zijn om handverzorging te verbeteren onder zorgverleners.

**Deel II: Effecten van irritatieve stoffen op de huidbarrière en de inflammatoire respons:** *in vivo experimentele studies*

**Onderzoeksvraag IV:** *Wat zijn de effecten van verscheidene irritatieve stoffen op de huidbarrière en inflammatie?*

De effecten van veelgebruikte irritatieve stoffen op de huidbarrière en de inflammatoire respons zijn onderzocht in twee experimentele studies. In de eerste studie (hoofdstuk 5), werden de effecten van herhaalde blootstelling aan verschillende klassen veelgebruikte irritatieve stoffen (n-propanol, sodium lauryl sulfaat (SLS), natriumhydroxide en azijnzuur) bestudeerd bij gezonde vrijwilligers. Voor dit doel werden functionele (erytheem, transepidermaal waterverlies en hydratatie), biochemische (natural moisturizing factors, NMF) en morfologische parameters (topografie van de oppervlakte van de corneocyten) bestudeerd en vergeleken. De resultaten lieten per irritatieve stof een specifiek patroon in de irritatieve respons zien, wat impliceert dat deze irritatieve stoffen de huidbarrière beschadigen via verschillende mechanismen. Alkalische stoffen, zoals SLS en natriumhydroxide, waren de enige irritatieve stoffen die een significant verschil in alle onderzochte parameters toonden. N-propanol, het hoofdingrediënt van de handalcohol gebruikt in de gezondheidszorg, veroorzaakte alleen geringe veranderingen in de huidbarrièrefunctie, uitgedrukt in transepidermaal waterverlies (TEWL) en erytheem, hoewel het wel een uitgesproken effect liet zien op de hydratatie en de NMF concentraties. De bevinding dat n-propanol de NMF concentraties en hydratatie aanmerkelijk verlaagt, terwijl TEWL onveranderd blijft, suggereert dat het effect van n-propanol op de hydratatie
mogelijk eerder veroorzaakt is door een afname in NMF waarden dan door alteraties in de lipide dubbellagen.

Aanvullend op bovengenoemde effecten, veroorzaakt n-propanol ook significante veranderingen in de textuur van de oppervlakte van corneocyten, gemanifesteerd als talrijke protrusies. Het aantal van deze protrusies per oppervlakte wordt uitgedrukt als de DTI (dermal texture index). Recent is er een stijging in de DTI gevonden in de aan SLS-blootgestelde huid, echter een soortgelijke stijging werd niet bewerkstelligd in experimenteel geïnduceerd allergisch contact eczeem. Dit suggereert dat het onwaarschijnlijk is dat ontstekings op zichzelf de onderliggende reden zou zijn voor de geobserveerde veranderingen. Dit wordt verder ondersteund door de resultaten van de huidige studie, waarin de blootstelling aan n-propanol een forse stijging in de DTI bewerkstelligt zonder de aanwezigheid van ontsteking. Voorts, bleek DTI sterk gecorreleerd met NMF en hydratatie (capacitance), echter niet met erytheem, welke een belangrijke aanwijzing zou zijn voor ontsteking.

Van alle onderzochte parameters, was enkel de NMF concentratie significant veranderd na blootstelling aan alle irritatieve stoffen. Daarnaast bleek de NMF de enige parameter te zijn die een significant verschil toonde na occlusie met water. Dit impliceert dat NMF, tenminste in de setting van experimenteel geïnduceerde irritatie, beschouwd kan worden als de meest sensitieve effect parameter.

Alles bij elkaar genomen laten deze bevindingen zien dat huidbarrièreschade en de inflammatoire respons stof-specifiek zijn en benadrukken daarmee de behoefte aan een multi-parametrische benadering voor het bestuderen van een huidirritatie.

**Onderzoeksvraag V:** *Wat is de invloed van de concentratie, occlusie en aanwezigheid van atopisch eczeem voor het effect van n-propanol op de barrière functie en de inflammatoire respons?*

De tweede experimentele irritatie studie richtte zich op n-propanol, een veelgebruikt ingrediënt in commercieel beschikbare handalcoholen in de zorg. Het effect van de concentratie n-propanol, pre-existent barrièreschade door occlusie en aanwezigheid van atopisch eczeem werden bestudeerd door het meten van erytheem, TEWL, hydratatie en NMF-concentraties vóór en 96 uur na de blootstelling aan n-propanol. De bevindingen demonstreerden dat de huidbarrièreschade en de inflammatoire respons na blootstelling aan n-propanol concentratie-afhankelijk is en versterkt wordt door pre-existent barrièreschade door occlusie en atopisch eczeem. Voor atopici lag de
concentratie drempelwaarde waarbij significante veranderingen in erytheem werden gezien lager dan in gezonde individuen (resp. 45% en 60% n-propanol). Een vergelijkbare trend werd gevonden voor TEWL (drempelwaarde van resp. 30% en 45% in atopici en gezonde individuen). In contrast hiermee, was de daling in hydratatie groter in gezonde individuen dan in atopici. Dit is te verklaren door de pre-existent lage hydratatie bij atopici op baseline, waardoor de hoeveelheid water in het stratum corneum niet verder kan zakken. Pre-existente schade van de huidbarrière als gevolg van repetitieve occlusie liet een versterkt ongewenst effect zien op erytheem en TEWL in beide groepen. Na occlusie was de drempelwaarde van n-propanol voor TEWL en erytheem 30%, in zowel atopici als gezonde individuen.

In tegenstelling tot parameters als TEWL en erytheem, werd er voor de NMF geen concentratie-afhankelijk effect, noch een effect van occlusie dan wel aanwezigheid van atopisch eczeem gevonden. De laagste n-propanol concentratie bleek al een dusdanig sterk effect op de poel van wateraanrekkende moleculen in de corneocyten te hebben, dat verhoging van de concentratie geen substantieel toegevoegd effect had. Daarmee bereikt de laagste concentratie n-propanol al een maximaal ongewenst effect op NMF en hydratatie.

Tot slot, ondersteunen deze resultaten de eerdere bevindingen ten aanzien van de ongewenste effecten van n-propanol op de huidbarrière en tonen daarmee haar potentiële belang in de pathogenese van irritatief contact eczeem. Tevens onderstrepen deze uitkomsten de hypothese in hoofdstuk 3, namelijk het effect van de nieuwe n-propanol bevattende handalcoholen op het verlagen van de NMF concentraties. De versterkende effecten van externe (occlusie) en intrinsieke (atopisch eczeem) factoren op de huidbarrière-verstorende effecten van n-propanol, benadrukken voorts het belang van adequate risicoinventarisatie in de preventie van HE in gezondheidszorg.
ADDENDUM
Curriculum Vitae

Maryam Soltanipoor is geboren op 1 september 1987 in Teheran, Iran. Op haar tiende verhuiste zij naar Nederland. In 2006 voltooide zij het Gymnasium aan het Stedelijk College in Eindhoven. Na het succesvol afronden van haar propedeusejaar Biomedische Wetenschappen met een deeltijdse studie Rechtsgeleerdheid aan de Universiteit van Amsterdam, werd Maryam toegelaten tot de studie Geneeskunde aan dezelfde universiteit. Gedurende haar eerste studiejaar ontwikkelde Maryam een bijzondere interesse in de Public Health. Dit resulteerde in het mede-oprichten en besturen van een vereniging dat meer bewustzijn onder medische studenten wilde creëren ten aanzien van Public Health vraagstukken, middels het organiseren van maatschappelijke projecten, debatten en studiereizen. Daarnaast volgde Maryam ter verdieping de minor Society and Health op de Harvard School of Public Health in Boston. Ondertussen was zij als secretaris, van de Evaluatiecommissie van het Onderwijsinstituut (OWIGEN), Faculteit der Geneeskunde (AMC), betrokken bij het evalueren van het curriculum en het verbeteren van het leerklimaat en participeerde zij in meerdere extra-curriculaire projecten op de afdeling Dermatologie van het VUmc (onder supervisie van Prof. dr. Thomas Rustemeyer en Sylvie Franken) en de Maag-, Darm-, en Leverziekten (MDL) van het AMC. Dankzij beurzen en een scholarship award zette Maryam voorts een studie op naar galzoutsynthese en -transport in het levercentrum van de Yale School of Medicine, onder supervisie van prof. dr. U.H.W. Beuers en prof. M. Nathanson.

In 2015 behaalde Maryam het artsexamen aan de Universiteit van Amsterdam. Vervolgens startte zij met een promotieonderzoek, een samenwerkingsverband tussen de afdeling Dermatologie van het VUmc (onder begeleiding van Prof. dr. Thomas Rustemeyer) en het Coronel Instituut voor Arbeid en Gezondheid, AMC (onder begeleiding van dr. Sanja Kezic en wijlen Prof. dr. Judith Sluiter), zoals beschreven in dit proefschrift. In deze periode was Maryam hoofdzakelijk verantwoordelijk voor het opzetten en uitvoeren van klinische trials en experimentele studies. Ook was zij betrokken bij het onderwijs in de bachelor geneeskunde als mentor van het klinisch lijn onderwijs. Daarnaast was zij actief lid van het Europese onderzoeksnetwerk (COST Action- StanDerm) dat zich richt op de preventie van arbeid gerelateerd contacteczem. Gedurende haar PhD periode presenteerde Maryam haar werk op 10 internationale en nationale congressen.

Thans, werkt Maryam als arts op de soa-poli bij de GGD Amsterdam. In de toekomst ambieert zij een carrière als dermatoloog. Maryam woont in Amsterdam samen met haar vriend Roy van Wanrooj.
# PhD Portfolio

Name PhD student: Maryam Soltanipoor  
PhD period: April 2015- February 2019  
Name PhD supervisors: Dr. S. Kezic, Prof. T. Rustemeyer, Prof. J.K. Sluiter

## 1. PhD training

| General courses                                                                 | Year | Workload (ECTS) |
|--------------------------------------------------------------------------------|------|-----------------|
| The AMC World of Science                                                      | 2016 | 0.7             |
| BROK (Basiscursus regelgeving Klinisch Onderzoek)                              | 2015 | 0.9             |
| Oral Presentation                                                              | 2017 | 0.8             |
| Practical Biostatics                                                          | 2017 | 1.1             |
| Systematic Review                                                             | 2017 | 0.3             |
| Searching for Evidence                                                        | 2017 | 0.3             |
| Clinical Data Management                                                      | 2016 | 0.3             |

| Specific courses                                                             | Year | Workload (ECTS) |
|--------------------------------------------------------------------------------|------|-----------------|
| Trainingschool ‘Skinbarrier’, Split, Croatia                                 | 2015 | 1.5             |
| Trainingsschool ‘Patchtesting’, Erlangen, Germany                            | 2016 | 1.5             |
| London Short Scientific Mission, London, England                             | 2015 | 1.1             |

| Presentations                                                                 | Year | Workload (ECTS) |
|--------------------------------------------------------------------------------|------|-----------------|
| European Society of Contact Dermatitis (ESCD) Congres, Milan, Italy (oral)     | 2018 | 0.6             |
| International Congres of Occupational Health (ICOH), Dublin, Ireland (oral)    | 2018 | 0.7             |
| Nederlandse Vereniging voor Experimenterende Dermatologie (NVED), Lunteren, Nederland (oral) | 2018 | 0.6             |
| European Society of Contact Dermatitis (ESCD), Manchester, England (oral)     | 2017 | 0.7             |
| Standerm COST, Copenhagen, Denmark (oral)                                     | 2015 | 0.5             |
| AMC/VUmc dermatology residents’ education program, department of Dermatology, VUmc, Amsterdam (oral) | 2017 | 0.2             |
| Coronel research meetings, AMC, Amsterdam (4) (oral)                          | 2015-2018 | 1.8     |
| Heijermanslezing Arbeidsdermatologie                                          | 2019 | 0.5             |

| Posters                                                                        | Year | Workload (ECTS) |
|--------------------------------------------------------------------------------|------|-----------------|
| Standerm COST, Berlin, Germany                                                 | 2018 | 0.4             |
| Nederlandse Vereniging voor Experimenterende Dermatologie (NVED), Lunteren, Nederland | 2017 | 0.4             |
| American Academy of Dermatology (AAD), San Diego, USA                          | 2018 | 0.4             |
1. PhD training

| (Inter)national conferences | Year | Workload (ECTS) |
|-----------------------------|------|-----------------|
| European Society of Contact Dermatitis (ESCD) Congres, Milan, Italy | 2018 | 0.6 |
| International Congres of Occupational Health (ICOH), Dublin | 2018 | 0.8 |
| Nederlandse Vereniging voor Experimentele Dermatologie (NVED), Lunteren, Nederland (2) | 2018-2016 | 0.6 |
| European Society of Contact Dermatitis (ESCD), Manchester, England | 2017, 2018 | 0.8 |
| Standerm COST, Copenhagen and Berlin | 2015, 2018 | 0.4 |
| European Academy of Dermatology of Dermatology and Venereology (EADV), Copenhagen, Denmark | 2015 | 0.5 |
| SNNDV Nascholing, Utrecht en 's-Hertogenbosch, NL | 2017 | 0.2 |
| Jaarsymposium Dermatologie, Utrecht (3) | 2017-2019 | 0.3 |
| Dermatologendagen, Amsterdam (2) | 2017, 2018 | 0.2 |
| Wetenschappelijke Vergadering Nederlandse Vereniging voor Dermatologie en Venereologie, Amsterdam, Leiden, Groningen (NVDV) (3) | 2016, 2017 | 0.3 |
| Refereeravonden, AMC-VUMC, OLVG, Amsterdam | 2015, 2018 | 0.1 |
| Klinische demonstraties, Leiden (3) | 2018 | 0.1 |

2. Teaching

| Tutoring, Mentoring | Year | Workload (ECTS) |
|---------------------|------|-----------------|
| Mentoring Klein Klinisch Lijnonderwijs (KKLO), 3rd year | 2016-2017 | 3.0 |
| Teaching and supervision of medical students: | | |
| Course Academic Skills | 2017 | 1.5 |

| Supervising | Year | Workload (ECTS) |
|-------------|------|-----------------|
| Scientific Internship, Fleur de Wit (master student, VUmc) | 2016 | 2.0 |
| Scientific Internship, Federico Frison (master student University of Trieste, Italy) | 2016 | 1.5 |
| Extra-scientific Internship, Angela Bosma (master student, AMC) | 2016 | 1.0 |
| Scientific Internship, Ruth van Asperen (master student, VUmc) | 2017 | 1.5 |
List of Publications

Soltanipoor M, Kezic S, Sluiter JK, de Wit F, Bosma AL, van Asperen R, Rustemeyer T. Effectiveness of a skin care program for the prevention of contact dermatitis in healthcare workers (the Healthy Hands Project): a single centre, cluster randomized controlled trial. Contact Dermatitis. 2019. doi: 10.1111/cod.13214

Soltanipoor M, Rustemeyer T, Sluiter JK, Hines J, Frison F, Kezic S. Evaluating the effect of electronic monitoring and feedback on hand cream use in healthcare workers: Healthy Hands Project. Contact Dermatitis. 2019. doi: 10.1111/cod.13148

Soltanipoor M, Stilla T, Riethmuller C, Thyssen JP, Sluiter JK, Rustemeyer T, Fischer TW, Kezic S, Zillikens D, Angelova-Fischer I, Specific barrier response profiles after experimentally-induced skin irritation in vivo, Contact Dermatitis. 2018. doi: 10.1111/cod.12981

Soltanipoor M, Kezic S, Sluiter JK, Holman R, Statistical analysis plan for the Healthy Hands Project; single centre cluster-randomized clinical trial of a skin care program for the prevention of contact dermatitis in health care workers. Trials. 2018. doi: 10.1186/s13063-018-2703-7.

Soltanipoor M, Kezic S, Sluiter JK, Rustemeyer T. The effectiveness of a skin care program for the prevention of contact dermatitis in health care workers (the Healthy Hands Project): study protocol for a cluster randomized controlled trial. Trials. 2017. doi: 10.1186/s13063-017-1803-0.

Soltanipoor M, Angelova-Fischer I, Stilla T, Sluiter JK, Rustemeyer T, Fischer TW, Kezic S, Jakasa I. Barrier damaging effects of n-propanol in occlusion-modified tandem repeated irritation test: modulation by exposure factors and atopic skin disease (Accepted in Contact Dermatitis)

De Vries HCJ, Soltanipoor M, Kezic S, Vergunst CE, Sinecatechins ointment 10% (Veregen®) for genital warts: percutaneous penetration of Epigallocatechin Gallate concentrations in the stratum corneum collected by adhesive tape stripping method. Journal of the European Academy of Dermatology and Venereology. 2018 doi: 10.1111/jdv.14933
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