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ORIGINAL ARTICLE

Nickel deposition and penetration into the stratum corneum after short metallic nickel contact: An experimental study

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Background: Knowledge about the skin deposition and penetration of nickel into the stratum corneum (SC) after short contact with metallic items is limited.

Objective: To quantify nickel skin deposition and penetration into the SC after short contact with metallic nickel.

Methods: Sixteen nickel-allergic participants and 10 controls were exposed to 3 pure nickel discs and 1 aluminium disc on each volar forearm for 3 × 10 minutes. Before exposure, 1 forearm was irritated with 0.5% sodium lauryl sulfate under 24-hour occlusion. Immediately, as well as 24 and 72 hours after metallic disc exposure, outer SC layers were removed with adhesive tapes and the nickel content was measured.

Results: Nickel deposition and SC penetration capable of eliciting allergic nickel dermatitis were found immediately and after 24 hours. Significantly higher nickel amounts were found on normal skin and in the SC of nickel-allergic participants than in controls both immediately and after 24 hours, and on irritated skin immediately after exposure.

Conclusions: Nickel deposition and SC penetration is considerable after nickel skin exposure of 3 × 10 minutes. Combined with the allergic responses resulting from the same exposures reported previously, this study highlights that short skin exposure to nickel-releasing items may cause allergic nickel dermatitis.

KEYWORDS

contact allergy, nickel allergy, nickel penetration, nickel release, nickel skin dose, stratum corneum, tape stripping

1 | INTRODUCTION

Nickel remains the most prevalent cause of contact allergy worldwide. The sustained high prevalence of nickel allergy in Europe[1] is often explained by prolonged skin contact with nickel-releasing items, whereas the potential role of nickel exposure as a result of short and daily skin contact with nickel-releasing items has largely been overlooked.

The nickel skin dose is related to the amount of nickel penetration into the stratum corneum (SC) and viable epidermis, which, in turn, can lead to induction of nickel allergy and elicitation of allergic nickel dermatitis. Recent studies have shown that the rate of nickel release from metallic items is particularly high immediately after contact with artificial sweat, and that even short skin contact can result in deposition of nickel onto the skin.[2–5] These studies were conducted in both controlled laboratory settings with metallic items and occupational settings where exposure to metallic items had occurred. Penetration of nickel ions from different nickel salts into the SC has also been studied in humans.[6,7] However, knowledge on the penetration of nickel ions into the SC after short contact with metallic items is lacking.

The aim of this study was to quantify nickel skin deposition and subsequent penetration into the SC after relatively short contact with metallic nickel (3 × 10 minutes) in normal and irritated skin of nickel-allergic participants and controls.

2 | MATERIALS AND METHODS

2.1 | Study population and design

The study has been described in detail elsewhere.[8] A flow chart of the study design is shown in Figure 1. Briefly, a clinical experimental study

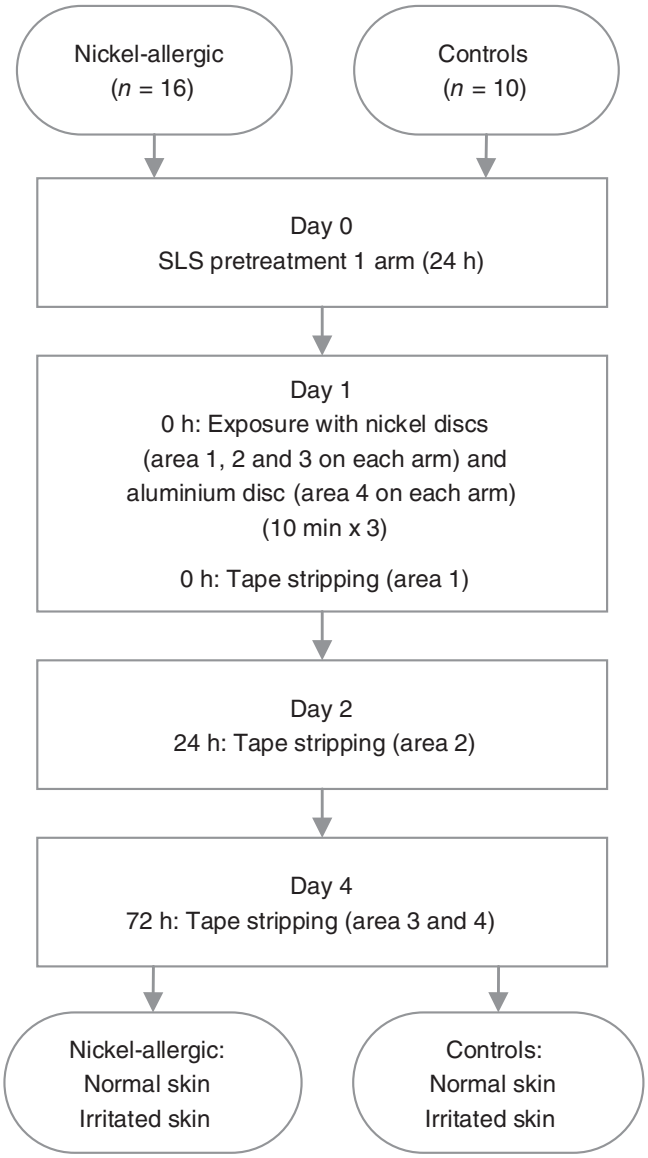

FIGURE 1 Flow chart of study, showing nickel exposure and sampling (separate tape stripping for 2-pyrrolidone-5-carboxylic acid is not included in the overview). SLS, sodium lauryl sulfate

was performed on 16 nickel-allergic participants from the Department of Dermatology and Allergy at Herlev and Gentofte Hospital, and 10 control subjects recruited by advertisement. Before the study, one of the volar forearms was randomized for skin irritation, and, on day 0, 4 exposure areas of each arm were marked. To induce skin irritation, the 4 areas were pretreated for 24 hours with 0.5% sodium lauryl sulfate (SLS) aq. (99% purity SLS; Sigma-Aldrich, St Louis, Missouri) under occlusion. All participants were exposed to 3 pure nickel discs and 1 aluminium disc (as a negative control) on each forearm for 3 × 10 minutes. Exposures were made in an exposure chamber with a temperature of 29.2°C ± 0.7°C (range 28.8°C-29.4°C). The discs were applied manually by the same investigator (M.G.A.) in three 10-minute contacts, separated by 10-minute intervals, with 3 different metallic discs for each exposure area. Each disc was applied with an initial 90° of rotation forth and back 2 times, to create friction between the skin and the metal surface. Participants were not allowed to take showers or use emollients during the study period. Before recruitment, the study was approved by the local ethics committee (H-16050296) and the Danish Data Protection Agency, and all participants gave written informed consent. The study was registered at ClinicalTrials.gov (NCT03309215).

2.2 | Tape stripping of the SC

Immediately (0-1 hour), 24 hours and 72 hours after exposure to the metallic discs, sequential tape stripping was used for measurement of nickel penetration into the SC in one of the nickel-exposed areas on each arm (normal and irritated skin). The aluminium-exposed skin on each forearm was tape-stripped 72 hours after exposure and used as a negative control (Figure 1). Each area was tape-stripped once with 15 consecutive tapes (3.8 cm^2, D-Squame; Monaderm, Monaco, France). Tapes were placed on the most central part of all test areas, and, after use of a pressure applicator (225 g/cm^2) (D-squame; Monaderm) for ~10 seconds, the tape was gently removed with a quick uniform movement with a plastic tweezer. In addition, 8 consecutive tape strips were taken from normal skin of both upper inner arms for analysis of 2-pyrrolidone-5-carboxylic acid (PCA). The method for tape stripping of the inner arms for PCA analysis was identical, except that the skin around the first strip was marked. A new tweezer was used for each test site. Optical density with D-Squame Scan 850A (Heiland Electronic, Wetzlar, Germany) was used on each tape to normalize for the variable amount of protein content. After protein measurement, all tapes were stored separately in 2-mL microtubes (Thermo Fisher Scientific, San Diego, California) at room temperature, and transferred to the laboratory at Karolinska Institutet (Karolinska Institutet) for nickel analysis.

2.3 | Determination of nickel in tape strips

On the basis of experience of analysing samples from pilot exposures,[8] we analysed 7 tape trips from each test site for nickel content. For the extraction of nickel in tapes, the microtubes with tape strips were filled with 2 mL of 67% HNO_3 (Normatom; VWR, Leuven, Belgium), completely covering the tape. After 72 hours, 1 mL of the acid extract was transferred to a new microtube (1.5 mL; Sarstedt, Nümbrecht, Germany). Prior to analysis, 150 μL of acid extract was transferred to a 12-mL PP-tube (Sarstedt) prefilled with 4.85 mL of Milli Q water (Millipore, Solna, Sweden; 18.2 MΩ/cm), 5.0 mL of 2% HNO_3 (67% Normatom [VWR, Leuven, Belgium], diluted in Milli Q water), and indium added as an internal standard (5 ppb, prepared from stock solution 1000 μg/mL Spectrascan; Teknolab, Kungsbacka, Sweden). Standard solutions of an 8-point (0, 0.1, 1, 5, 10, 50, 100 and 500 μg/L) calibration curve were diluted from a nickel stock solution (1000 μg Ni/mL, Spectrascan; Teknolab), by the use of 2% HNO_3 with the addition of indium as an internal standard (5 ppb). Samples were analysed in kinetic energy discrimination mode with helium as the collision gas and argon as the carrier gas, by use of an iCAP Q inductively coupled plasma mass spectrometry (ICP-MS) system from Thermo Scientific (Waltham, Massachusetts). Nickel was monitored at masses 58 and 60, and indium was monitored at mass 115. The method detection limit (MDL) was evaluated on the basis of the results from 72 blank tapes, and set to 0.08 μg/L (3 × SD of blank

tape samples). The instrument limit of detection was calculated for each run as 3 × SD by the use of 3 acid blanks, and ranged from 0.000771 to 0.122639 μg/L.

2.4 | Estimation of the nickel exposure concentration

To study nickel release from the nickel discs used on the skin, discs were immersed in artificial sweat for 3 × 10 minutes and for 1 week (168 hours). Samples were kept in a heating cabinet (Memmert, Schwabach, Germany) at 30°C during the respective immersion period. Additionally, a wipe test was performed to mimic the nickel release from nickel discs during skin contact (a full description is given in Appendix S1). The released concentrations of nickel in artificial sweat and wipe extract were determined with ICP-MS. The MDL was 0.0073 μg/L for nickel.

2.5 | Filaggrin gene (FLG) genotyping and analyses of PCA

For all participants, buccal mouth swaps were taken for *FLG* genotype analysis. The *FLG* mutation status for R501X, 2282del4 and R2447X were determined by multiplex analysis of buccal swabs at Herlev and Gentofte Hospital, as previously described in detail.[9]

To quantify FLG degradation products, the tape-stripping technique was performed on both inner upper arms. PCA from tape strip number 3 was analysed by ultra-performance liquid chromatography at the Department of Autoimmunology and Biomarkers at Statens Serum Institut (a full description is given in Appendix S1). The sample preparation was performed as described by Kezic et al.[10]

2.6 | Statistical analysis

For presentation of data and statistical analysis, nickel in the first 2 tape strips on each sampling occasion was interpreted as being taken from the skin surface, and was thus analysed separately. Nickel in tapes 3 to 7 was considered to indicate penetration into the SC.[11] The participants were divided into 4 groups according to nickel allergy and skin status (nickel-allergic vs control; irritated vs normal skin). *FLG* mutation status was categorized as 'wild type' or 'null mutation', the latter including any of the 3 mutations tested for. A mean value of the PCA from tape strip 3 of both upper arms was used in the analysis. Non-parametric analyses were used. A *P*-value of ≤.05 was considered to be significant. The Mann-Whitney *U* test was used for comparison of nickel doses between nickel-allergic and control participants, and for testing of differences in nickel doses in participants with or without *FLG* mutation. To test for differences between patch test reactivity and nickel penetration into the SC or nickel deposition onto the skin surface, the Kruskal-Wallis test was used. Spearman's correlation coefficient (r_s) was used to correlate PCA levels and nickel penetration into the SC or deposition onto the skin surface. For comparison of nickel in normal and irritated skin or of the nickel area with the aluminium control area on the same participants, the Wilcoxon signed rank test was used.

TABLE 1 Characteristics of the study population

	Nickel-allergic (*N* = 16)	Controls (*N* = 10)	*P*[a]
Sex (*n*)			
Women	13	9	NS
Men	3	1	
Age at test (years), median (25/75)	55.5 (44.0-63.5)	34 (25.0-58.0)	NS[a]
Atopic dermatitis ever[b]	2	0	NS
Hand dermatitis ever[b]	4	0	NS
Filaggrin gene mutation carriers[c]	2	2	NS

NS, not significant.
[a] The Mann-Whitney *U* test was used.
[b] Participants were asked whether a doctor had ever told them that they had atopic dermatitis or hand dermatitis.
[c] Genotyping was performed for the 3 most common loss-of-function mutations in the filaggrin gene (501X, 2282del4, and R2447X). Two nickel-allergic participants and 1 control were heterozygous in 1 filaggrin gene, and 1 control was compound heterozygous.
*Fisher's exact test was used.

REDCAP electronic data capture tools were used for data collection.[12] Statistical analysis and graphs were created in SAS, Version 9.4 for Windows (SAS Institute, Cary, North Carolina), GRAPHPAD PRISM version 6.07 for Windows (GraphPad Software, La Jolla, California), and Microsoft Excel (Excel 2010; Microsoft, Redmond, Washington).

3 | RESULTS

All participants completed the study. The study population and their clinical reactions and blood flow measurements have previously been described in detail.[8] In brief, we showed that 63% of nickel-allergic participants reacted with allergic nickel dermatitis on irritated skin, and 19% on normal skin with previous dermatitis, whereas none of the controls had any clinical reactions or blood flow increases. The study population characteristics are shown in Table 1.

A substantial amount of nickel was deposited onto the skin after the exposures (3 × 10 minutes). Nickel was found in all of the analysed tapes from the nickel-exposed areas; immediately, 24 hours and 72 hours after exposure (Figure 2). At all 3 time points, the highest amount was present in the first tape, ranging from 8.7 μg/cm^2 (range: 5.9-14.4 μg/cm^2) in normal skin of controls to 13.3 μg/cm^2 (range: 7.4-44.2 μg/cm^2) in normal skin of nickel-allergic participants immediately after exposure. The nickel content in tapes decreased in the same pattern in tapes from deeper SC layers for the 4 groups. The variation in nickel penetration was largest immediately after exposure and higher in nickel-allergic participants.

The proportions of nickel that had penetrated into the SC (tapes 3-7) and nickel on the skin surface (tapes 1 and 2) are shown in Figure 3.[11] In normal skin, the nickel in SC/nickel on surface ratio was similar in nickel-allergic participants and controls at all time points, except for a lower ratio in controls immediately after exposure. However, the actual amount of nickel on the skin surface in normal skin was significantly higher in nickel-allergic participants than in controls both immediately (mean difference: 13.4 μg/cm^2) ($P \leq .03$) and 24 hours after exposure (mean difference: 2.0 μg/cm^2) ($P \leq .01$). In

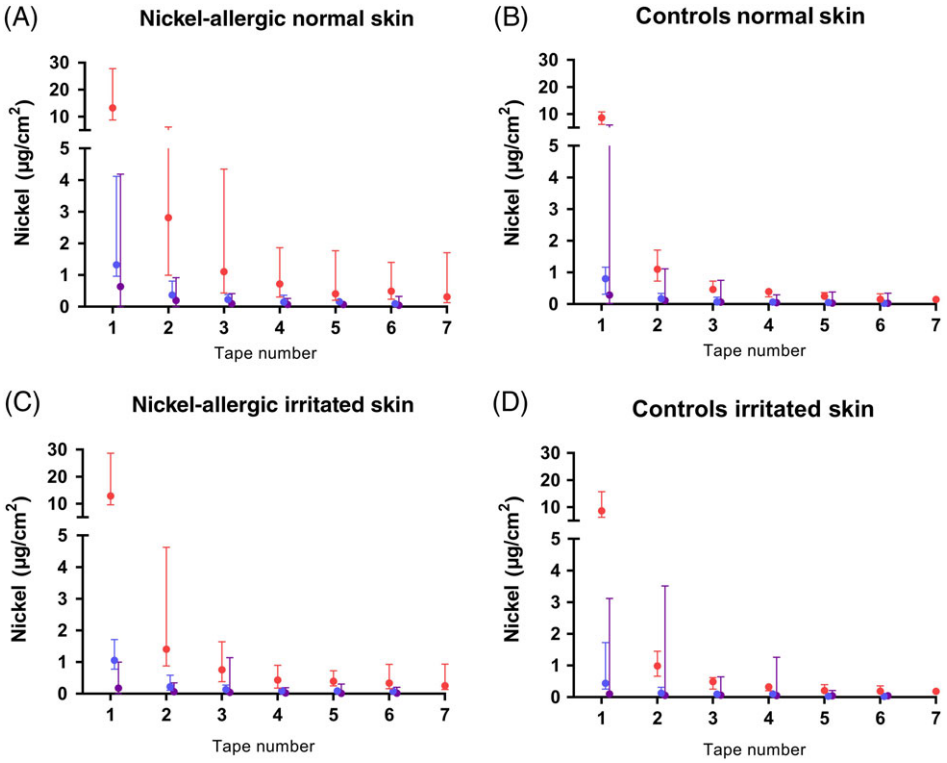

FIGURE 2 Measured nickel amount per tape (µg/cm^2) at different time points after exposure (0, 24 and 72 hours) in normal or irritated skin of nickel-allergic participants (**A, C**) and controls (**B, D**). The results are indicated by bars for interquartile range, markers for median value, and colours for time points: red, 0 hours; blue, 24 hours; and violet, 72 hours

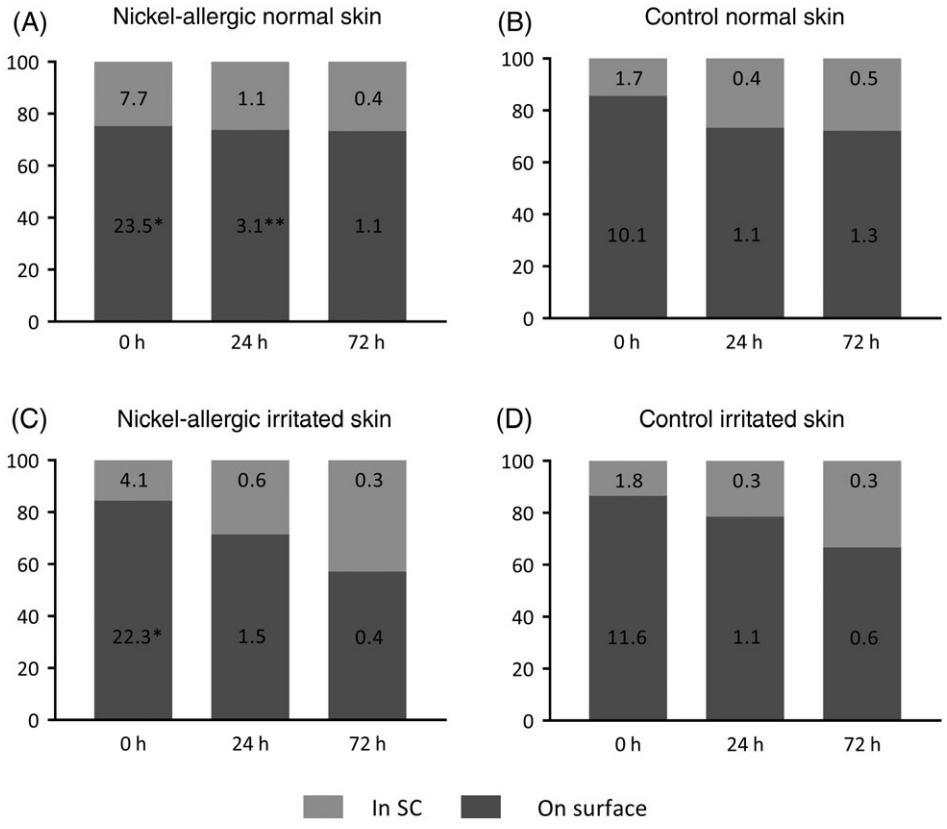

FIGURE 3 Comparison of nickel on the skin surface (tapes 1 and 2) and amount penetrating into the stratum corneum (SC) (tapes 3-7) at different time points (0, 24 and 72 hours) after exposure for nickel-allergic (n = 16) (**A, C**) and control (n = 10) (**B, D**) participants in normal skin (**A, B**) and skin irritated with sodium lauryl sulfate (**C, D**). The actual mean amount of nickel (µg/cm^2) is noted on the bars; differences between nickel-allergic and control participants were tested with the Mann-Whitney U test. *$P \leq .05$, **$P \leq .01$

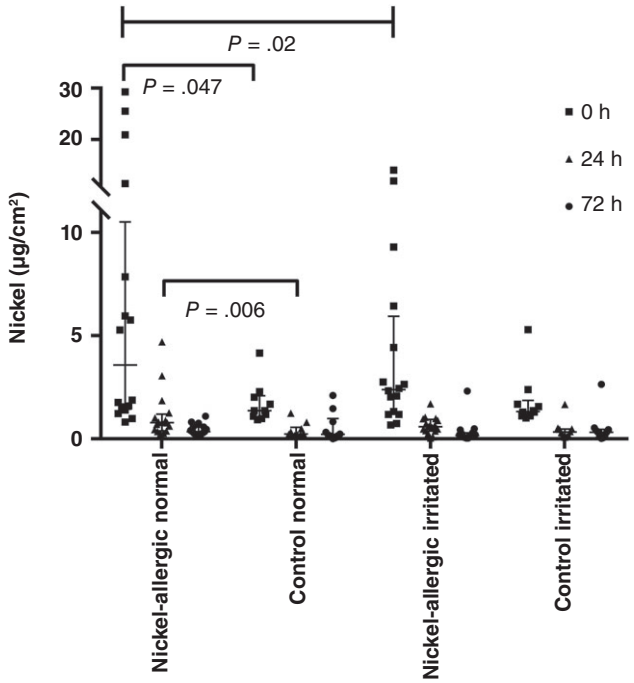

FIGURE 4 Amount of nickel (µg/cm^2) that had penetrated into the stratum corneum measured in tape 3 to tape 7 at different time points after exposure (0, 24 and 72 hours) for the 4 groups (nickel-allergic/controls, irritated/normal skin). Each dot represents 1 participant. Bars: interquartile range. Lines: median. At different time points after exposure to nickel discs, the Mann-Whitney *U* test was used to compare the amounts of nickel between nickel-allergic and control participants, and the Wilcoxon signed rank test was used to compare the amounts of nickel in normal and irritated skin of the same individuals

irritated skin, the amount of surface nickel was higher in nickel-allergic participants, but only immediately after exposure (mean difference: 10.7 µg/cm^2) ($P \leq .05$).

The amount of nickel on the skin surface (tapes 1 and 2) correlated with the amount penetrating into the SC (tapes 3-7) in both irritated and normal skin of all participants (normal $r_s = 0.91$) (irritated $r_s = 0.94$) ($P < .0001$) (Figure S1A,B). Also, a correlation was found between the amounts of nickel on the skin surface in normal and irritated skin of all participants (Figure S2). The amount of nickel penetrating into the SC in normal skin was higher in nickel-allergic than in controls both immediately (median difference: 2.2 µg/cm^2) ($P = .047$) and after 24 hours (median difference: 0.54 µg/cm^2) ($P = .006$) (Figure 4). No difference in the amount of nickel penetrating into SC of irritated skin was found between the groups. When nickel penetration into the SC was compared between normal and irritated skin of the same nickel-allergic participants, a higher amount was found in normal skin immediately after exposure (median difference: 1.85 µg/cm^2) ($P = .02$), but there was no difference in the deposition of nickel on the skin surface.

Nickel penetration into the SC (tapes 3-7) in normal skin decreased over time to 18%-22% and 10%-15% of the amount found in the SC immediately after exposure after 24 hours and after 72 hours, respectively. Seventy-two hours after exposure; nickel was still present in the SC at the nickel-exposed areas. At this time point, significantly more nickel was found on the surface of the nickel-exposed skin than on the surface of the aluminium-exposed skin (median difference: 0.68 µg/cm^2) ($P < .0001$) and in the SC (median difference: 0.28 µg/cm^2) ($P < .0001$).

TABLE 2 Mean nickel release per unit surface area from nickel discs in artificial sweat for 1 week (168 hours); 3 × 10 minutes (with 3 different nickel discs) and 10 minutes. Also shown is the mean amount of nickel deposited onto a wipe moistened with artificial sweat, from 1 nickel disc that was rotated 90° forth and back 2 times, simulating participant exposure

Procedure	µg Ni/cm^2 (mean)	SD
Release in artificial sweat		
168 h	2.60	0.31
3 × 10 min	1.82	0.70
1 × 10 min	0.30	0.04
Wipe		
1 × simulated participant exposure	166	37

All results are based on triplicate experiments.

The mean amounts of nickel released from nickel discs after immersion in artificial sweat for different time periods are shown in Table 2. The total amount of nickel released during 3 immersion periods of 10 minutes each (1.82 µg Ni/cm^2) corresponded to 70% of the release after 1 week (2.60 µg Ni/cm^2). One simulated participant exposure by use of a wipe moistened with artificial sweat resulted in released amounts of nickel orders of magnitude higher (165.7 µg Ni/cm^2) than measured after immersion in artificial sweat for 1 week. This is reflected by the nickel amount in the 7 tapes taken immediately after exposure; 7–83 µg Ni/cm^2.

Three participants were heterozygous *FLG* mutation carriers, whereas 1 was a compound heterozygous mutation carrier. There was no correlation between nickel on the skin surface or nickel penetrating into the SC at any time points and *FLG* mutation status, self-reported hand dermatitis, or a history of atopic dermatitis. No trend could be found between patch test reactivity and nickel on the skin surface or penetration into the SC. In normal skin of nickel-allergic participants, there was a significant negative correlation between PCA concentration and nickel penetration immediately after exposure ($r_s = -0.54$) ($P = .03$).

4 | DISCUSSION

4.1 | Principal findings

This is the first study to quantify nickel penetration into the SC after a relatively short duration of skin contact with metallic nickel. A large amount of nickel was deposited onto the skin, and penetration into the SC took place within 1 hour. Nickel was recovered from the skin surface and the outer layers of the SC up to 72 hours after exposure, emphasizing sustained exposure from nickel on the skin surface. Unexpectedly, higher amounts of nickel were found on the skin surface and in the SC in normal skin of nickel-allergic participants than in controls, both immediately and 24 hours after exposure. There was a large variation within the group of nickel-allergic participants; some individuals had a much higher level of nickel on the skin surface,

resulting in higher penetration into the SC. An interesting negative correlation between nickel penetration into the SC and PCA concentration was found in nickel-allergic participants.

4.2 | Interpretation

Studies of nickel on the skin surface and subsequent penetration into the SC, following real-life exposure to nickel-releasing metallic items, are very limited. Although it has been claimed that short contact with metallic items is harmless in the context of nickel allergy, studies have shown rapid deposition of nickel onto the skin following contact, for example, coin handling.[5] On the basis of these findings, it has been suggested that short and repeated skin contact with metallic items may lead to considerable nickel build-up in the skin. In a recent study, in which fingertip skin was stroked against metallic nickel for 3 seconds, 4.7 μg/cm^2 nickel could be detected on the skin surface with the acid wipe method. In the same study, the amount of nickel deposited on the skin from nickel alloys and pure nickel was more dependent on contacts with newly abraded surfaces than on the actual number of repeated contacts with the same surface.[3] We found higher but comparable nickel skin doses (mean: 10.1–23.5 μg/cm^2) after 3 × 10 minutes of exposure with newly abraded metallic nickel discs. In our previous questionnaire study of 342 nickel-allergic individuals, a large proportion reported allergic nickel dermatitis after relatively short contact with metallic items (21.4% with ≤ 10 minutes of contact, and 30.7% with ≤ 30 minutes of contact).[13]

The large variation in nickel skin deposition observed in nickel-allergic participants is interesting. Considerable interindividual variation in nickel skin deposition after skin contact with nickel-containing metallic items has previously been shown in 2 studies that included individuals without nickel allergy.[3,14] The present study is the first to show a difference in skin deposition between persons with and without nickel allergy in a controlled set-up. One possible explanation could be differences in the amount and composition of sweat, which was not measured in this study, as sweat may affect nickel release and accumulation of nickel in the SC. "Rusters" in industry were described in the 1960s as workers with a tendency to cause corrosion on metal surfaces, owing to a high chloride content in sweat.[15,16] The skin topography, affecting friction and contact area, may also be important, but has rarely been studied in this context.[17]

Estimating the penetration of a contact allergen in the skin (μg/cm^2) is of importance, as the penetration is a requirement for sensitization, or for elicitation of allergic contact dermatitis. Penetration of nickel into the SC has previously been studied with different nickel salts in aqueous solutions after hours of contact, mostly in vitro.[7,18,19] If an aqueous solution with a nickel salt is used, the actual applied nickel ion dose and counter-ion are known, so the skin absorption can be calculated. These studies provide important information on the kinetics of nickel ion penetration and associated rate-determining factors. In addition, SC penetration has been studied after application of nickel powder, where both particles and released nickel ions were involved.[20–22] However, it is important to keep in mind that most short and daily contacts with nickel occur with metallic nickel items. The mechanism of nickel transfer to the skin surface in these contacts is also governed by pressure and friction.

The primary rate-limiting factor for nickel skin absorption is the SC.[19,23] A limited amount of the applied nickel dose is supposed to penetrate through the SC; 1 study found that <1% of nickel chloride penetrated the SC within 96 hours of exposure.[18] In the present study, the nickel amount was highest in the first tape and decreased in tapes from deeper layers. This is consistent with the observation that nickel accumulates superficially in the SC after exposure to nickel salts.[6,7,19,24,25] One study is of particular interest regarding comparison of the results of nickel penetration. Hostynék et al used tape stripping to measure the penetration of nickel in forearm skin in vivo after 30 minutes to 24 hours of exposure to an aqueous solution of different nickel salts.[7] Important differences from the present study were occlusion of the test areas, surface decontamination prior to stripping, and the use of another type of tape. However, similarly to our results, they found that the main nickel dose was located on the skin surface, and that the nickel concentration decreased with the number of tapes within 24 hours after exposure. If we exclude the first 2 tapes in our study, the amount of nickel in the following tapes were similar to those found 30 minutes after open application of a single liquid dose of nickel chloride (concentration: 19.8 μg/cm^2). Whereas there is little insight into nickel skin penetration, it is likely that the continuous proliferation and shedding of corneocytes will help to remove nickel that has been bound in the upper layers, in turn limiting the tendency of nickel ions to reach the viable layers. However, excessive exposure, either prolonged or repeated, will lead to high nickel concentrations and, in turn, allergic nickel dermatitis.

There was no difference in immediate nickel skin deposition between normal and irritated skin of nickel-allergic participants, but more nickel was found in the SC of normal skin. This finding may indicate that nickel had already been absorbed into the viable epidermis of irritated skin 0 to 1 hours after exposure, which is also supported by the fact that most nickel-allergic participants (63%) developed allergic nickel dermatitis in irritated skin.[8] It is not known to what extent rapid shunting of nickel ions via sweat ducts, pores, etc. occurs, and this cannot be quantified by tape stripping.[23]

In this study, high SC nickel penetration correlated with a low PCA concentration in nickel-allergic participants. PCA is an important marker of the relative amounts of amino acids in the natural moisturizing factor (NMF). PCA and other amino acids originate from the decomposition of SC proteins. The best known is filaggrin, but many others participate. The active known nickel-chelating element in NMF is the amino acid histidine. It is known that this amino acid varies in parallel with PCA in the NMF.[10] In this study, the PCA concentration was used as an estimation of acquired filaggrin deficiency to supplement filaggrin genotyping.[26]

No correlation was found between atopic dermatitis, hand eczema or *FLG* mutations and deposition/penetration, although the power of these analyses was low, owing to a limited number or participants with these conditions. Previous epidemiological studies have suggested higher risks of nickel allergy in individuals with *FLG* mutations.[27–29] Our study indicates that histidine levels in the outer SC influence nickel penetration.

The duration of exposure to metallic discs of this study corresponded to the definition of "prolonged contact" in the EU nickel restriction ("10 minutes on three or more occasions within two weeks,

or 30 minutes on one or more occasions within two weeks").[30] From our results, we conclude that the duration of contact is not crucial for the deposition of high amounts of nickel onto the skin surface. Ten minutes 3 times is sufficient to result in nickel deposition onto the skin, penetration into the SC, and allergic nickel dermatitis.[8] In accordance with others, we found that nickel ion release in artificial sweat after a short duration of contact led to a considerable proportion of the total release after 168 hours of immersion.[2,3] It cannot be estimated from our experimental study how longer, shorter or no time intervals between nickel exposures may affect the dose of nickel in skin.

4.3 | Strengths and weaknesses

The study was carefully controlled in many respects; most importantly, the metallic discs had equal surface properties, and exposures with the metallic discs and the tape-stripping procedure were performed by the same investigator. Furthermore, the temperature during exposures was controlled, as it may influence nickel release and SC penetration.[19,31]

Owing to the set-up with skin exposure to metallic discs, a limiting aspect was that the exact nickel exposure dose remained unknown. Although the rotations of discs were performed in the same manner, pressure and rotation could not be fully standardized, and the friction of the skin differed according to skin texture and moisture. Furthermore, it is known that skin temperature and the amount and composition of sweat vary between body parts and individuals. The nickel exposure dose from nickel discs was estimated with a wiping procedure, although it may be overestimated, as the wipe was moistened in artificial sweat. Variations in measured nickel surface doses at later time points may partly be attributable to differences in compliance with restrictions during the study period (use of emollients and washing) and differences in clothing. Another weakness was that we used only pure nickel in this study. In real life, nickel alloys are more commonly used in metallic items, although pure nickel is used in nickel-plated items and some coins. Finally, we did not assess the possible influence of lipid bilayers or paracellular penetration via hair follicles and sweat glands.

To obtain a quantitative measurement of protein removed in each tape strip (an indirect measure of the mass of skin cells removed), infrared densitometry was used. However, interference between nickel and protein in tapes was noted; hence, the results are not presented. Previous research has indicated that a constant amount of SC is removed after the first 2 tape strips, for a given test person, tape, and skin site.[7]

5 | CONCLUSION

This study shows that relatively short skin contact with nickel, corresponding to what is covered by the current EU restriction on nickel, gives rise to substantial doses of nickel on the skin surface and penetration into the SC, in amounts capable of eliciting allergic nickel dermatitis.[8] In some nickel-allergic individuals, nickel was apparently "glued" onto the skin after exposure, which led to great differences in nickel penetration as compared with controls. The current inclusion of items intended for relatively short skin contact will likely result in more efficient prevention of nickel allergy in EU countries.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

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REFERENCES

1. Thyssen JP, Uter W, McFadden J, et al. The EU nickel directive revisited—future steps towards better protection against nickel allergy. *Contact Dermatitis*. 2011;64:121-125.
2. Julander A, Midander K, Herting G, et al. New UK nickel-plated steel coins constitute an increased allergy and eczema risk. *Contact Dermatitis*. 2013;68:323-330.
3. Erfani B, Lidén C, Midander K. Short and frequent skin contact with nickel. *Contact Dermatitis*. 2015;73:222-230.
4. Lidén C, Skare L, Vahter M. Release of nickel from coins and deposition onto skin from coin handling—comparing euro coins and SEK. *Contact Dermatitis*. 2008;59:31-37.
5. Fournier PG, Govers TR. Contamination by nickel, copper and zinc during the handling of euro coins. *Contact Dermatitis*. 2003;48:181-188.
6. Wells GC. Effects of nickel on the skin. *Br J Dermatol*. 1956;68:237-242.
7. Hostýnek JJ, Dreher F, Nakada T, et al. Human stratum corneum adsorption of nickel salts: Investigation of depth profiles by tape stripping in vivo. *Acta Derm Venereol*. 2001;11-18.
8. Ahlström MG, Thyssen JP, Menné T, et al. Short contact with nickel causes allergic contact dermatitis: an experimental study. *Br J Dermatol*. 2018. https://doi.org/10.1111/bjd.16935.
9. Meldgaard M, Szecsi PB, Carlsen BC, et al. A novel multiplex analysis of filaggrin polymorphisms: a universally applicable method for genotyping. *Clin Chim Acta*. 2012;413:1488-1492.
10. Kezic S, Kammeyer A, Calkoen F, Fluhr JW, Bos JD. Natural moisturizing factor components in the stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. *Br J Dermatol*. 2009;161:1098-1104.
11. Buist H, Craig P, Dewhurst I, et al. Guidance on dermal absorption. *EFSA J*. 2017;15:1-60.
12. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377-381.
13. Ahlström MG, Menné T, Thyssen JP, Johansen JD. Nickel allergy in a Danish population 25 years after the first nickel regulation. *Contact Dermatitis*. 2017;76:325-332.
14. Staton I, Ma R, Evans N, Hutchinson RW, McLeod CW, Gawkrodger DJ. Dermal nickel exposure associated with coin handling and in various occupational settings: assessment using a newly developed finger immersion method. *Br J Dermatol*. 2006;154:658-664.

15. Buckley WR, Lewis CE. The 'ruster' in industry. *J Occup Med*. 1960;2:23-31.
16. Burton JL, Pye RJ, Brookes DB. Metal corrosion by chloride in sweat. The problem of 'rusters' in industry. *Br J Dermatol*. 1976;95:417-422.
17. Kovalev AE, Dening K, Persson BNJ, Gorb SN. Surface topography and contact mechanics of dry and wet human skin. *Beilstein J Nanotechnol*. 2014;5:1341-1348.
18. Tanojo H, Hostýnek JJ, Mountford HS, Maibach HI. In vitro permeation of nickel salts through human stratum corneum. *Acta Derm Venereol*. 2001;19-23.
19. Fullerton A, Andersen JR, Hoelgaard A, Menné T. Permeation of nickel salts through human skin in vitro. *Contact Dermatitis*. 1986;15:173-177.
20. Hostýnek JJ, Dreher F, Pelosi A, et al. Human stratum corneum penetration by nickel—in vivo study of depth distribution after occlusive application of the metal as powder. *Acta Derm Venereol*. 2001: (suppl 212;5-10.
21. Larese F, Gianpietro A, Venier M, Maina G, Renzi N. In vitro percutaneous absorption of metal compounds. *Toxicol Lett*. 2007;170:49-56.
22. Filon FL, D'Agostin F, Crosera M, et al. In vitro absorption of metal powders through intact and damaged human skin. *Toxicol In Vitro*. 2009;23:574-579.
23. Hostýnek JJ. Factors determining percutaneous metal absorption. *Food Chem Toxicol*. 2003;41:327-345.
24. Kalimo K, Lammintausta K, Mäki J, Teuho J, Jansén CT. Nickel penetration in allergic individuals: bioavailability versus X-ray microanalysis detection. *Contact Dermatitis*. 1985;12:255-257.
25. Malmberg P, Guttenberg T, Ericson MB, Hagvall L. Imaging mass spectrometry for novel insights into contact allergy—a proof-of-concept study on nickel. *Contact Dermatitis*. 2018;78:109-116.
26. Thyssen JP, Kezic S. Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis. *J Allergy Clin Immunol*. 2014;134:792-799.
27. Thyssen JP, Johansen JD, Linneberg A, et al. The association between null mutations in the filaggrin gene and contact sensitization to nickel and other chemicals in the general population. *Br J Dermatol*. 2010;162:1278-1285.
28. Scharschmidt TC, Man M, Hatano Y, et al. Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. *J Allergy Clin Immunol*. 2009;124:496-506.
29. Novak N, Baurecht H, Schäfer T, et al. Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. *J Invest Dermatol*. 2008;128:1430-1435.
30. European Chemicals Agency (ECHA) (2014). Prolonged contact with the skin- Definition building for nickel. https://echa.europa.eu/documents/10162/13641/nickel_restriction_prolonged_contact_skin_en.pdf. Accessed August 30, 2018.
31. Menné T, Solgaard P. Temperature-dependent nickel release from nickel alloys. *Contact Dermatitis*. 1979;5:82-84.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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