

Experimental systemic contact dermatitis from nickel: a dose–response study

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Systemic contact dermatitis is usually seen as flare-up of previous dermatitis or *de novo* dermatitis similar to allergic contact dermatitis. Although systemic contact dermatitis from medicaments is a well-established entity, the existence of clinically relevant systemic reactions to oral nickel exposure, in particular systemic reactions to nickel in the daily diet, remains controversial. Several studies have shown that oral exposure to nickel can induce systemic contact dermatitis in nickel-sensitive individuals. In most of these studies, however, the exposure dose of nickel used has been considerably higher than the nickel content in the normal daily diet. The aim of the current investigation was to study dose–response dependency of oral exposure to nickel. In a double-blind, placebo-controlled oral exposure trial, 40 nickel-sensitive persons and 20 healthy (non-nickel-sensitive) controls were given nickel sulfate hexahydrate in doses similar to and greater than the amount of nickel ingested in the normal Danish daily diet. The nickel content in urine and serum before and after oral exposure was measured to determine nickel uptake and excretion. The influence of the amount of nickel ingested on the clinical reactions to oral exposure and on nickel concentrations in serum and urine was evaluated. Among nickel-sensitive individuals, a definite dose–response dependency was seen, following oral exposure to nickel. 7 of 10 nickel-sensitive individuals had cutaneous reactions to oral exposure to 4.0 mg nickel, an amount approximately 10 times greater than the estimated normal daily dietary intake of nickel. 4 of 10 nickel-sensitive individuals had cutaneous reactions to 1.0 mg nickel, a dose which is close to the estimated maximum amount of nickel contained in the daily diet. 4 of 10 nickel-sensitive individuals reacted to 0.3 mg nickel or to the amount equivalent to that contained in a normal daily diet, and 1 of 10 reacted to a placebo. None of the 20 healthy controls had cutaneous reactions to 4.0 mg nickel or to a placebo. Prior to oral exposure, there was no measurable difference in the amount of nickel in the urine or serum of nickel-sensitive persons and healthy controls. Following the oral challenge, the nickel content in the urine and serum of both nickel-sensitive and healthy control individuals was directly related to the dose of nickel ingested.

Key words: dose–response; nickel allergy; nickel in serum; oral challenge; systemic contact dermatitis; urinary nickel. © Blackwell Munksgaard, 2003.

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Systemic contact dermatitis is seen when sensitized individuals are exposed to a hapten systemically, that is orally, transcutaneously, intravenously, subcutaneously, intramuscularly or by inhalation (1). The number of patients with systemic contact dermatitis is low compared to the number of patients with other type of dermatitis (2).

The most specific cutaneous reactions of systemic contact dermatitis include flare-up reactions in previously positive patch test sites and/or flares of previous sites of contact dermatitis. Other clinically characteristic cutaneous features are vesicular hand eczema, eruptions on previously unaffected skin such as flexural dermatitis, maculopapular

rash and vasculitis-like lesions. Itching and general symptoms, such as headache, nausea and malaise, have been reported after the oral nickel exposure of nickel-sensitive individuals.

Although systemic contact dermatitis from medicaments is a well-established entity, the existence of systemic reactions to oral nickel exposure, in particular systemic reactions to nickel exposure in the normal daily diet, remains controversial. Several studies have shown that oral intake of nickel can elicit systemic contact dermatitis in nickel-sensitive individuals (1). Flare-up reactions of previous nickel patch test sites after systemic oral exposure appear to be hapten specific, and

there appears to be a dose-response dependency (3–12). In most of these studies, the oral nickel challenge doses used were much greater than the nickel content of the normal daily diet. The doses used in the studies vary from 0.6 to 5.6 mg nickel, whereas normal daily dietary nickel intake has been found to vary from 0.1 to 0.5 mg nickel (13–21). The maximum amount of nickel in the normal daily diet has been estimated to be 0.9 mg (13). Individual variation in the type and severity of the reactions seen in the above studies makes it difficult to relate the elicitation of systemic contact dermatitis in nickel-sensitive individuals to nickel exposure in the daily diet. Furthermore, in most of the studies, because it is difficult to quantify and control the daily dietary nickel exposure of the subjects, it has not been possible to estimate the additional normal exposure to the ubiquitous hapten nickel.

The aim of this study was to investigate the dose-response dependency of cutaneous nickel allergic reactions in nickel-sensitive individuals exposed to a high dose of nickel and to oral nickel doses within the range of nickel exposure from the normal daily diet in a double-blind, placebo-controlled trial. Nickel content in urine and serum measured before and after oral nickel exposure were used to determine nickel uptake and excretion. The association between the doses of nickel ingested, the cutaneous reactions to the oral exposures and the measured nickel content in serum and urine was evaluated.

Subjects and Methods

Subjects

60 volunteers participated in a double-blind, oral nickel exposure study. 40 of the volunteers were nickel-sensitive individuals (39 women and 1 man, mean age 39.55 years, range 22–68 years) with a history of nickel contact dermatitis, who had previously had positive patch tests to nickel, and 20 were healthy (non-nickel-sensitive, who were negative in patch tests to nickel) controls (19 women and 1 man, mean age 44.2 years, range 29–56 years). An additional 12 individuals, 10 nickel-sensitive volunteers and 2 healthy controls were originally enrolled in the study but were excluded for various reasons. 8 nickel-sensitive volunteers were excluded, because they did not react to the nickel patches, 2 nickel-sensitive volunteers were excluded, because they reacted to all the patches, including the vehicle and the tape and 2 controls chose not to participate after the patch test. All enrolled volunteers were patch tested with 3 concentrations of nickel.

Informed consent was obtained from each volunteer. The study was approved by the ethics committees in Copenhagen and in the counties of Viborg and North Jutland.

Patch testing

The volunteers were patch tested with a ready-to-apply TRUE-Test (Mekos Laboratories AS, Hillerød, Denmark) specifically made with 4 squares: 3 with different concentrations of nickel sulfate hexahydrate (0.20, 0.066 and 0.022 mg cm⁻² equivalent to 5, 1.66 and 0.55% nickel) and 1 with a vehicle as a control. The patch tests were applied for 2 days, after which the volunteers removed them. The results were for practical reasons, only evaluated at 3 days after application by the investigator, according to International Contact Dermatitis Research Group (ICDRG) guidelines; however, to avoid the loss of volunteers reacting later, all participants were instructed to report back, if they happened to react within the next 7 days. Nobody did. A positive reaction was defined as at least homogeneous redness and infiltration in the test area. Reactions that did not fulfil these criteria, e.g. follicular reactions, faint erythema, or typical irritant reactions, were classified as non-allergic reactions.

Oral exposures

1 month after patch testing, the 40 nickel-sensitive individuals were randomly divided into 4 groups and orally exposed in a double-blind fashion to 0.3, 1.0 or 4.0 mg nickel (given as nickel sulfate hexahydrate) in a lactose capsule or just lactose as placebo. The selection of the oral exposure doses used in this study was based on the literature. A nickel exposure dose of 0.3 mg is close to the estimated average nickel intake from the normal daily diet. A 1.0 mg nickel dose was chosen, because this has been estimated to be close to the possible maximum nickel intake from the daily diet (12). Exposure to 4.0 mg nickel was expected to result in flare-up reactions in most of the nickel-sensitive individuals and was therefore included as a positive control. The 20 healthy controls were randomly divided into 2 groups and exposed orally in a double-blind fashion to 4.0 mg nickel or a placebo. No steps were taken to avoid recognizing nickel-sensitive individuals from healthy controls when the results of the oral exposures were evaluated. The oral exposures were given as a single bolus dose in the morning to individuals who had been fasting for 12 h. No other dietary intervention was conducted, hence each individual was exposed to nickel or placebo, in addition to the nickel exposure from the normal dietary intake. The additional nickel

exposure from the diet was not estimated. 1D after the oral exposure, the status of the skin area previously exposed to patch testing with nickel was scored according to ICDRG guidelines, and objective clinical skin responses to the oral exposures were recorded as positive cutaneous reactions. The positive cutaneous reactions were subdivided into the following categories:

- (1) Widespread clinical reactions, included any large or small clinical eruptions on previously unaffected skin such as flexural dermatitis, a maculopapular rash and vasculitis-like lesions.
- (2) Flare-up reactions in previous sites of contact dermatitis.
- (3) Flare-up reactions of previously positive nickel patch tests.

Additionally, itching and general symptoms, such as headache, dizziness, nausea and metallic taste, were recorded.

Nickel analyses of serum and urine before and after oral exposure

Urine samples and samples of peripheral blood were collected before and 1D after oral nickel exposure to determine nickel uptake and excretion, and to compare these values to the nickel doses ingested and the cutaneous reactions to the oral provocations. For practical reasons, the urine samples were collected as morning urine (prior to consuming food or beverage). Precautions were taken to avoid contamination of the samples, as described in the International Union of Pure and Applied Chemistry (IUPAC) guidelines, and the creatinine content of the urine was measured and used to standardize the sample volume for diuresis (22). Atomic absorption spectrometry (Perkin-Elmer Z-5100 or Perkin-Elmer SIMAA 6000, Shelton, USA) was used to determine nickel in serum and urine (23, 24). Spiked urine and serum were used as internal control samples. The between-run coefficient of variation was 10 and 13% for determination of nickel in serum at the concentrations 6.4 and 2.2 µg/l, respectively, and 6.5 and 9.4% at 5.0 and 2.4 µg/l for determination in urine. In more than half the samples taken before the oral exposures and in samples taken from the volunteers after they had been given a placebo, nickel concentration in serum and urine were lower than the detection limit (<0.12 µg/l) of the analytical method. In order to have values for statistical analyses from these individuals, the nickel concentrations in these samples was set at 1/3 of the detection limit (approximately 0.04 µg/l).

Statistical analysis

Logistic regression analysis was used to test the overall dose-response dependency of the cutaneous reactions to the different oral nickel exposure doses and placebo. Fisher's exact test was used to test the association between the oral exposure doses and the frequencies of the various cutaneous reactions and symptoms in different pairs of groups of volunteers.

Nickel concentrations in serum and urine samples taken before and after the oral exposure were transformed logarithmically and compared by both analysis of variance (ANOVA) and pair-wise *t*-tests. Logarithmic transformation of the data was carried out to stabilize the variations and allow application of ANOVA and pair-wise *t*-tests.

To test dose-dependency differences between volunteers with clinical reactions and volunteers with no reactions to the oral exposures, the logarithmically transformed data from serum and urine samples were tested in a 2-way analysis of variance. The model describes how the logarithm of the nickel concentration could depend upon the possible effects of 2 factors:

- (1) The nickel dose ingested (0, 0.3, 1.0 and 4.0 mg nickel).
- (2) Clinical reaction (yes or no).

The model also includes an interaction term between the effects of the 2 factors, describing how the effect of the dose factor could depend on the effect of the clinical reaction and vice versa. In the 2-way analysis of variance, the statistical significance of the interaction term was tested first. If it was not significant, each of the effects of the 2 factors was tested separately for significance. The statistical analyses were performed according to the FREQ (statistical analysis of frequency and crosstabulation data) and GLM (statistical methods for general linear models, including analysis of variance and regression) procedures of SAS[®] version 6.12, SAS Institute, Cary, USA, 1990.

Results

The relationship between the cutaneous reactions and oral exposures

Table 1 summarizes the distribution of the volunteers who had objective clinical cutaneous reactions and/or other symptoms from oral exposure to nickel or to a placebo. Figure 1 shows the distribution of volunteers with specifically nickel-related cutaneous reactions, as well as itching and general symptoms. 7 of 10 nickel-sensitive individuals had cutaneous reactions to the oral exposure to 4.0 mg nickel, 4 reacted to 1.0 mg, 4 had a reaction to 0.3 mg and 1 nickel-sensitive

Table 1. Reactions to placebo-controlled oral exposure to nickel among 40 nickel-sensitive persons and 20 healthy controls

	Oral provocation dose (mg Ni)	Clinically cutaneous reactions	Widespread cutaneous reactions	Flare-up of previous sites of dermatitis	Flare-up of previous patch test	Flare-up of former hand eczema	Itching	General symptoms
Nickel-sensitive persons	4	7/10	6/10	6/10	6/10	3/5	3/10	5/10
	1	4/10	0/10	4/10	1/10	2/8	4/10	2/10
	0.3	4/10	0/10	4/10	1/10	2/7	1/10	3/10
Healthy controls	Placebo	1/10	0/10	1/10	0/10	1/5	1/10	0/10
	4	0/10	0/10	0/0	0/10	0/0	0/10	2/10
	Placebo	0/10	0/10	0/0	0/10	0/0	0/10	2/10

Data shown are the number of reacting individuals of the total number of individuals in each subgroup.

individual reacted to a placebo. The result of the logistic regression analysis showed that the nickel-related reaction pattern seen among the nickel-sensitive volunteers was dose dependent ($P=0.021$). No one in the control group had a cutaneous reaction to any of the oral exposures. The frequency of reactions among nickel-sensitive volunteers exposed to 4.0 mg nickel was statistically significantly different from the frequencies in the group given a placebo ($P=0.0099$) and from the equivalent control group ($P=0.0015$). None of the other pair-wise comparisons showed a statistically significant difference ($P>0.05$).

Statistically significant widespread cutaneous reactions were seen only in the group of nickel-sensitive volunteers exposed to 4.0 mg compared to placebo (6 of 10 versus 0 of 10, $P=0.0054$).

6 individuals in the group of nickel-sensitive volunteers exposed to 4.0 mg nickel reacted with flares at previous sites of dermatitis. 4 in each of the groups exposed to 1.0 and 0.3 mg nickel reacted with flares at previous sites of dermatitis and 1 nickel-sensitive volunteer given a placebo had a flare-up. The frequency of flares among nickel-sensitive individuals given 4.0 mg nickel was significantly different from the frequencies in the placebo group ($P=0.029$) and both healthy control groups ($P=0.0054$). None of the other comparisons showed a significant difference ($P>0.05$).

6 individuals in the group exposed to 4.0 mg nickel experienced flares at sites of previously positive nickel patch tests. 1 in each of the groups exposed to 1.0 and 0.3 mg had such flares, whereas none in the placebo group reacted with this kind of flare. Again, only the nickel-sensitive individuals in the group exposed to 4.0 mg nickel had a significantly different response frequency compared to the other groups ($P=0.029$ and 0.0054).

More nickel-sensitive individuals in the 2 groups exposed to the highest nickel doses complained of itching: 3 in the 4.0 mg group and 4 in the 1.0 mg group, compared to only 1 in each of the other 2 groups of nickel-sensitive individuals. None in the control groups experienced itching. No significant differences were demonstrated in the pair-wise comparisons of the various groups.

4 of 10 volunteers had general symptoms, such as headache, dizziness, nausea and metallic taste, in the group of nickel-sensitive volunteers exposed to 4.0 mg nickel. 2 in the 1.0 mg group, 3 in the 0.3 mg group and none in the placebo group experienced general symptoms, and 2 individuals in each of the healthy control groups had such symptoms. A significant difference

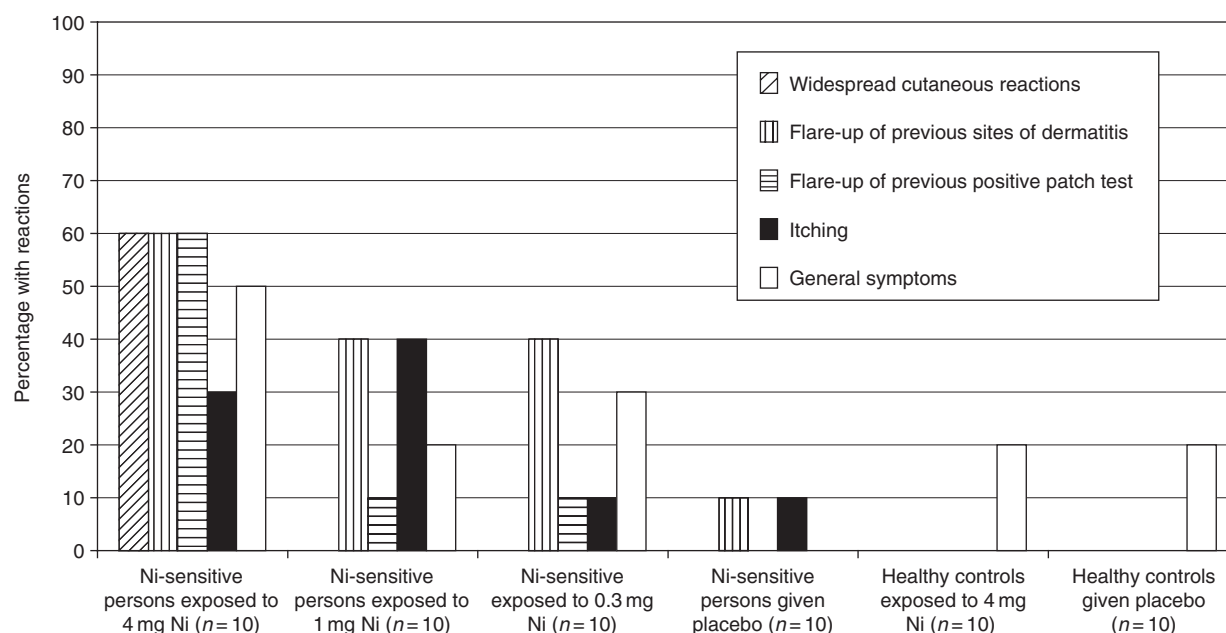


Fig. 1. Cutaneous reactions and other symptoms of nickel-sensitive individuals and healthy controls to oral exposure to nickel or placebo.

($P=0.016$) was seen only when the nickel-sensitive individuals exposed to 4 mg nickel were compared with the nickel-sensitive individuals given a placebo.

Analyses of nickel concentrations in serum and urine samples before and after oral exposure

Very low levels of nickel were seen in the serum and urine samples taken from both nickel-sensitive and healthy control volunteers before the oral exposure. The nickel content in 35 of 60 serum samples and in 46 of 60 urine samples taken before oral exposure was below the detection limit ($<0.12 \mu\text{g/l}$). In both groups given a placebo, for 6 of 10 nickel content in serum samples taken after exposure was below detection level. Nickel content in the urine samples was below detection level in 8 of 10 samples from nickel-sensitive volunteers and in 5 of 10 samples from healthy controls. The ANOVA of the nickel contents of serum and urine samples taken before the exposure showed that variations among the groups were not significant ($P > 0.05$).

Definite dose dependency was found in nickel content of both serum and urine after oral nickel exposure (Fig. 2). Higher oral exposure doses correlated with higher levels of nickel in serum and urine. The ANOVA showed highly significant differences between the different dose groups ($P < 0.0001$). When the nickel levels were compared in pair-wise t -tests, only the difference in nickel contents in serum of nickel-sensitive

individuals exposed to 0.3 mg nickel and nickel-sensitive individuals given a placebo was not statistically significant ($P=0.081$). All other pair-wise comparisons of different doses were found to be significantly different (P values ranging from $P=0.017$ to $P < 0.0001$). The mean nickel concentration in the serum and urine of nickel-sensitive volunteers before and after exposure to 4.0 mg nickel and the placebo was higher than the mean nickel concentration of the healthy controls given the same challenges. However, the difference was not statistically significant ($P > 0.05$).

The relationship between the cutaneous reactions and the nickel concentrations in serum and urine

The level of nickel in both serum and urine, following the oral exposures, showed a definite dose dependency for both clinically cutaneous reacting and non-reacting nickel-sensitive individuals (Fig. 3). The ANOVA of the logarithm of the nickel concentrations in the urine showed no statistically significant interaction between the 2 factors: nickel dose and clinical reaction ($P=0.96$). However, the dose dependency was highly significant ($P < 0.0001$). Furthermore, the differences between the nickel content in the urine of clinically cutaneous reacting and non-reacting nickel-sensitive individuals were also significant ($P=0.037$).

The 2-way ANOVA of the logarithm of the nickel content in serum showed a significant interaction

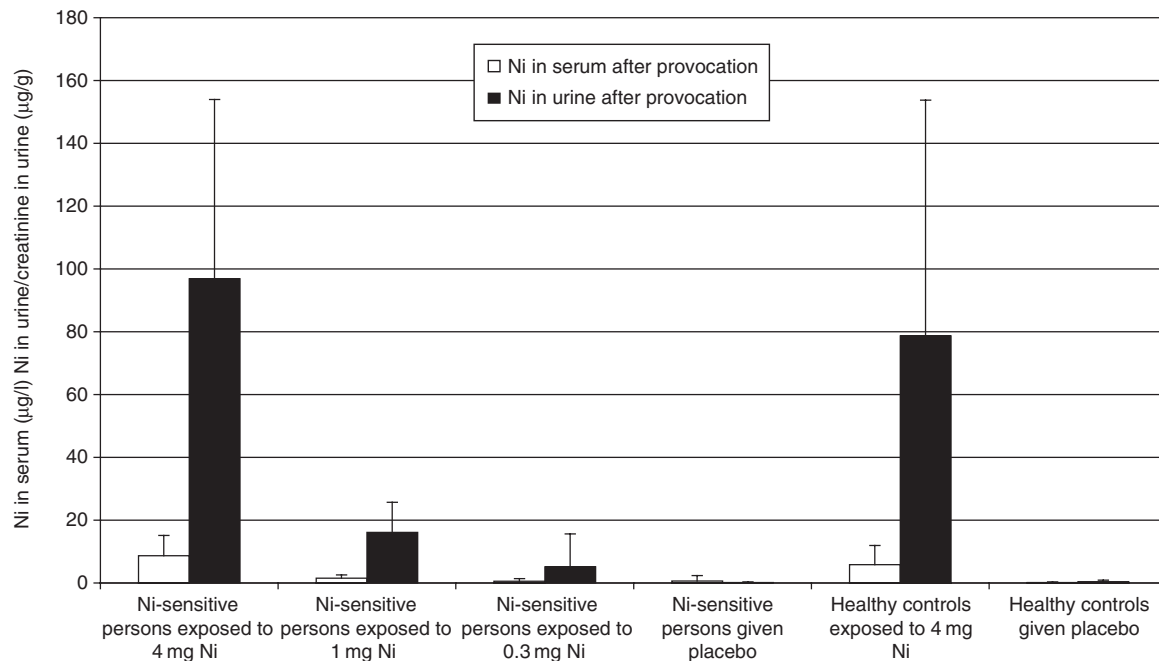


Fig. 2. Mean concentrations of nickel in serum and urine of nickel-sensitive persons and healthy controls after oral nickel exposure.

between the 2 factors: nickel dose and cutaneous reaction ($P=0.038$). As shown in Fig. 3, the nickel content in the serum of nickel-sensitive volunteers who reacted to the 4.0 or 1.0 mg nickel was higher than the content in serum of nickel-sensitive volunteers who did not react, whereas the opposite was true when 0.3 mg nickel or a

placebo was given. No such shift was observed in the nickel content of the urine.

Discussion

In a double-blind, placebo-controlled clinical study, we have demonstrated that oral nickel

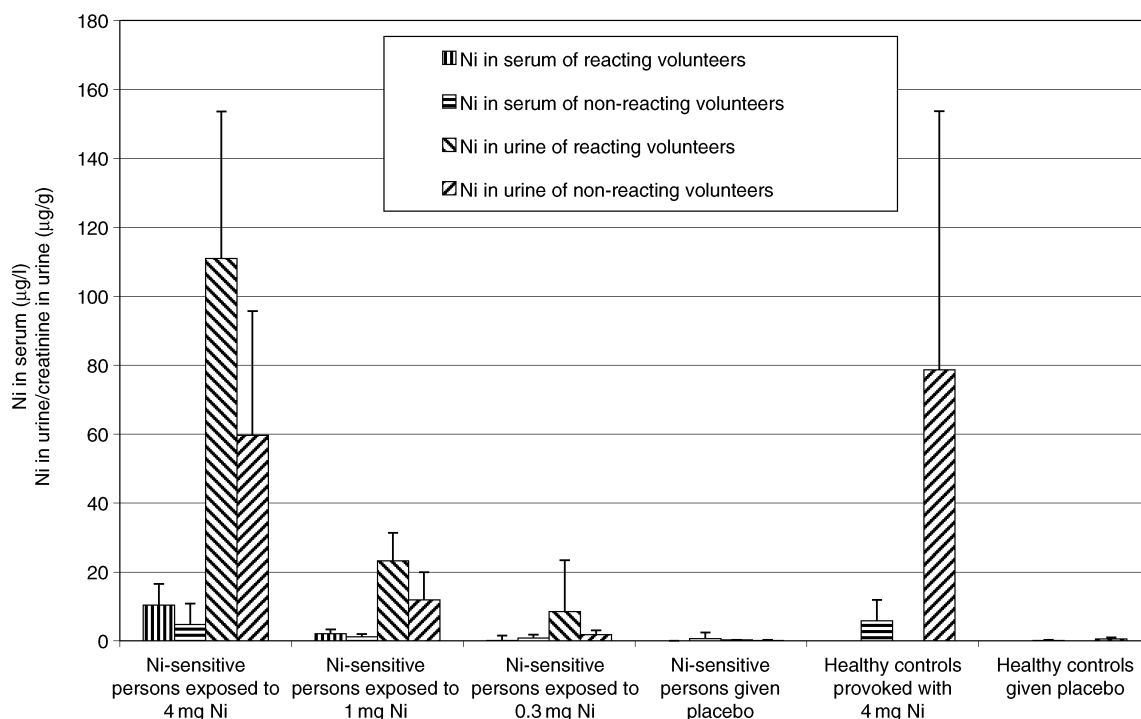


Fig. 3. Mean concentrations of nickel in serum and urine of cutaneous reacting and non-reacting nickel-sensitive volunteers and healthy controls after oral nickel exposure.

exposure does elicit cutaneous reactions in nickel-sensitive individuals, and that the reactions are dose dependent for most of the clinical symptoms. The number of nickel-sensitive volunteers, who experienced cutaneous reactions following oral exposure to nickel, increased as the oral nickel dose increased. Nickel-sensitive individuals exposed to 4.0 mg nickel reacted statistically significantly more often than those given a placebo. This was not the case for nickel-sensitive individuals exposed to 1.0 and 0.3 mg nickel. However, unequivocal clinical reactions, such as flare-up of previously positive nickel patch test sites and flare-up of previous sites of contact dermatitis from nickel, were seen when nickel-sensitive individuals were exposed to 1.0 and 0.3 mg nickel and a placebo, in addition to the nickel exposure from the normal dietary intake. Hindsén et al. (12) made similar observations. The limited number of persons exposed in each group ($n=10$ per group) may explain why no statistically significant differences were found between the nickel-sensitive individuals exposed to 1.0 or 0.3 mg nickel and those given a placebo. Assuming the frequencies found in this study are correct, a power-calculation (power $\geq 80\%$, significance level $P=5\%$) found that each group had to include 36 volunteers to demonstrate statistically significant differences between the groups of nickel-sensitive volunteers exposed to 1.0 mg nickel or 0.3 mg nickel and the group given a placebo. This would have required a total of 144 nickel-sensitive volunteers. In order to demonstrate statistically significant differences between the reactions found in the 4.0 mg nickel group and the 1.0 and 0.3 mg groups, each group would have had to include 48 volunteers or a total of 192 nickel-sensitive volunteers. Of course, an extrapolation of the results found among a low number of individuals, as in this study, to a larger number would be statistically incorrect, and the outcome of a study involving the estimated number of individuals might still show no statistical significance.

Despite intensive research by others and us on the significance of orally ingested nickel in nickel-sensitive individuals, it is difficult to determine an oral nickel dose that would not represent a significant risk for the majority of nickel-sensitive individuals. Oral exposure studies (5, 9, 12), including the current investigation, show that under experimental conditions, an oral exposure to nickel given as nickel sulfate hexahydrate, in addition to the nickel in the normal diet, will elicit systemic contact dermatitis in most nickel-sensitive individuals, provided the oral nickel dose is sufficiently high. The fact that 4 of 10 nickel-sensitive

individuals did react with unequivocal cutaneous reactions to a nickel dose equivalent to the estimated daily dietary nickel exposure, in addition to the nickel in their normal diet, indicate that some hypersensitive individuals might react to ingestion of soluble nickel at that concentration. The fact that 1 nickel-sensitive individual reacted to a placebo, in addition to the nickel in the normal diet, with a flare of her hand eczema could probably be ascribed to a not unexpected placebo-effect or the recurrent flare-up of hand eczema observed in many patients with chronic nickel dermatitis (3, 5). The absorption of nickel given as nickel sulfate hexahydrate, as used in this and other oral exposure studies, is not necessarily comparable with the absorption of the nickel naturally contained in food (25). It should be noted that absorption of nickel from food is significantly less than that of soluble nickel exposure in a fasting state (26). Therefore, oral elicitation values derived in this and other studies, where nickel-sensitive individuals were exposed to nickel sulfate hexahydrate in a fasting state, cannot be directly extrapolated to nickel in food. In foods, various other constituents, possibly phosphate, phytate, fibers and similar metal-ion-binding components, may bind nickel and make it less available for absorption than nickel sulfate in a capsule or dissolved in water and taken on an empty stomach (26). In oral exposure studies, such as those in the current study, nickel is usually given as a bolus, and the absorption of nickel in the gut under oral exposure conditions may therefore not be comparable to nickel exposure from the normal diet. In the current study, as in most other studies, the additional nickel exposure from the normal dietary intake was not estimated, so it has not been possible to estimate the total nickel exposure. However, dietary intervention studies indicate that it is possible to reduce the activity of dermatitis in some patients who are allergic to nickel, if these patients maintain a diet, low in nickel (4, 27–29). It has also been demonstrated that a nickel-rich diet can aggravate hand eczema in nickel-allergic patients (30). The number of patients with systemic contact dermatitis is low compared to the number of patients with allergic contact dermatitis caused by external contact with nickel, but many in the former group have long-lasting dermatitis (2). For these patients, the question may still be whether their chronic dermatitis is caused by external nickel exposure or unknown endogenous factors or several factors acting simultaneously (31, 32). Individual differences in immunological cellular and humoral interactions (8–10, 32–37), in the interaction between nickel and drug treatment

(38–40) and/or genetic variation (41) may also be of significance. Immunological reactivity to nickel may change with time (12, 42), and it might be altered by sex hormones (43–45) or the development of tolerance (46–52). Nielsen et al. (32) found that, after the implementation of the nickel exposure regulation in Denmark, nickel allergy became no longer statistically associated with recurrent or chronic hand eczema. This suggests that direct cutaneous skin exposure to nickel may also play a role in chronic hand eczema, and that it may be possible to improve the prognosis of hand eczema in some patients by reducing cutaneous exposure to nickel.

Statistically significant differences were found in the nickel levels in both serum and urine samples taken after oral exposure to different doses. The higher the oral exposure dose, the higher were the levels of nickel in serum and urine. No statistically significant differences were seen in the nickel levels in serum and urine samples from nickel-sensitive and healthy control individuals exposed to the same nickel doses, indicating that nickel absorption and excretion of nickel are similar for the 2 groups. Andreassi et al. (53) reported similar findings. When the logarithmically transformed data from the nickel contents of serum and urine samples were tested in a 2-way ANOVA, it was found that the nickel-sensitive volunteers with clinical reactions to 4.0 and 1.0 mg nickel had higher levels of nickel in the serum than non-reacting nickel-sensitive volunteers given the same nickel doses. In contrast, the volunteers with clinical reactions after the ingestion of 0.3 mg nickel or a placebo had lower levels of nickel in the serum than the volunteers who did not react clinically (graph not shown). The data from the study by Andreassi et al. (53) showed a similar tendency. The analysis was only weakly statistically significant ($P=0.038$). The high nickel content in the serum could explain why nickel excretion in urine was almost twice as high among nickel-sensitive individuals who had clinical reactions than among nickel-sensitive individuals who had no clinical reaction.

In conclusion, it was found that oral nickel exposure elicits cutaneous nickel-allergic reactions in nickel-sensitive individuals in a dose-dependent manner. The nickel-sensitive individuals exposed orally to 4.0 mg nickel had a clinical reaction frequency that was statistically significantly different from the reaction frequency of the placebo group. This was not seen when we compared the reaction frequencies in the groups exposed to 1.0 and 0.3 mg nickel to the frequency in the placebo group. Nickel absorption and the excretion of

nickel appears to be similar in nickel-sensitive individuals and healthy controls.

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References

1. Menné T, Veien N K. Systemic contact dermatitis. In: *Textbook of Contact Dermatitis*, Rycroft R J G, Menné T, Frosch P J, Lepoittevin J-L (eds): Berlin, Heidelberg, New York, Springer Verlag, 2001: 355–366.
2. Veien N K, Hattel T, Justesen O, Nørholm A. Diagnostic procedures for eczema patients. *Contact Dermatitis* 1987; 17: 35–40.
3. Christensen O B, Möller H. Nickel allergy and hand eczema. *Contact Dermatitis* 1975; 1: 129–135.
4. Kaaber K, Veien N K, Tjell J C. Low nickel diet in the treatment of patients with chronic nickel dermatitis. *Br J Dermatol* 1978; 98: 197–200.
5. Kaaber K, Menné T, Tjell J C, Veien N K. Antabuse treatment of nickel dermatitis. Chelation – A new principle in the treatment of nickel dermatitis. *Contact Dermatitis* 1979; 5: 221–228.
6. Cronin E, Di Michiel A D, Brown S S. Oral challenge in nickel-sensitive women with hand eczema. In: *Nickel Toxicology*, Brown S S, Sunderman F W Jr (eds): New York, Academic Press, 1980: 149–152.
7. Burrows D, Creswell S, Merrett J D. Nickel, hands and hip prostheses. *Br J Dermatol* 1981; 105: 437–444.
8. Christensen O B, Lindström G C, Löfberg H, Möller H. Micromorphology and specificity of orally induced flare-up reactions in nickel-sensitive patients. *Acta Derm Venereol* 1981; 61: 505–510.
9. Gawkrödger D J, Cook S W, Fell G S, Hunter J A. Nickel dermatitis: the reaction to oral nickel challenge. *Br J Dermatol* 1986; 115: 33–38.
10. Veien N K, Hattel T, Justesen O, Nørholm A. Oral challenge with nickel and cobalt in patients with positive patch tests to nickel and/or cobalt. *Acta Derm Venereol* 1987; 67: 321–325.
11. Möller H, Ohlsson K, Linder C, Björkmer Bruze M. The flare-up reactions after systemic provocation in contact allergy to nickel and gold. *Contact Dermatitis* 1999; 40: 200–204.
12. Hindsén M, Bruze M, Christensen O B. Flare-up reactions after oral challenge with nickel in relation to challenge dose and intensity and time of previous patch test reactions. *J Am Acad Dermatol* 2001; 44: 616–623.
13. Nielsen G D, Flyvholm M. Risks of High Nickel Intake with Diet. *IARC Sci Publ* 1984; 53: 333–338.
14. Veien N K, Andersen M R. Nickel in Danish food. *Acta Derm Venereol* 1986; 66: 502–509.
15. Pennington J A, Jones J W. Molybdenum, nickel, cobalt, vanadium, and strontium in total diets. *J Am Diet Assoc* 1987; 87: 1644–1650.
16. Smart G A, Sherlock J C. Nickel in foods and the diet. *Food Addit Contam* 1987; 4: 61–71.
17. Barberá R, Farre R, Mesado D. Oral intake of cadmium, cobalt, copper, iron, lead, nickel, manganese and zinc in the University student's diet. *Nahrung* 1993; 37: 241–245.

18. Dabeka R W, McKenzie A D. Survey of lead, cadmium, fluoride, nickel, and cobalt in food composites and estimation of dietary intakes of these elements by Canadians in 1986–88. *J AOAC Int* 1995; 78: 897–909.
19. Biego G H, Joyeux M, Hartemann P, Debry G. Daily intake of essential minerals and metallic micropollutants from foods in France. *Sci Total Environ* 1998; 217: 27–36.
20. Stelz et al. Nutritional content in the daily diet from selected nursing homes for the aged in State of Hessen Second Report—minerals. *Z Ernährungswiss/Eur J Nutr* 1998; 37: 269–278.
21. Ysart et al. 1997 UK Total Diet Study – dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. *Food Addit Contam* 2000; 17: 775–786.
22. Cornelis et al. Sample collection guidelines for trace elements in blood and urine. *Pure Appl Chem* 1995; 67: 1575–1608.
23. Christensen J M. Human exposure to toxic metals. Factors influencing interpretation of biomonitoring results. *Sci Total Environ* 1995; 166: 89–135.
24. Christensen J M, Kristiansen J, Nielsen N H, Menné T, Byrialsen K. Nickel concentrations in serum and urine of patients with nickel eczema. *Toxicol Lett* 1999; 108: 185–189.
25. Sunderman F W, Hopfer S M, Sweeney K R, Marcus A H, Most B M, Creason J. Nickel absorption and kinetics in human volunteers. *Proc Soc Exp Biol Med* 1989; 191: 5–11.
26. Nielsen et al. Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity. *Toxicol Appl Pharmacol* 1999; 154: 67–75.
27. Veien N K, Hattel T, Justesen O, Nørholm A. Dietary treatment of nickel dermatitis. *Acta Derm Venereol* 1985; 65: 138–142.
28. Veien N K, Hattel T, Laurberg G. Low nickel diet: an open, prospective trial. *J Am Acad Dermatol* 1993; 29: 1002–1007.
29. Antico A, Soana R. Chronic allergic-like dermatopathies in nickel-sensitive patients. Results of dietary restrictions and challenge with nickel salts. *Allergy Asthma Proc* 1999; 20: 235–242.
30. Nielsen G D, Jepsen L V, Jørgensen P J, Grandjean P, Brandrup F. Nickel-sensitive patients with vesicular hand eczema: oral challenge with a diet naturally high in nickel. *Br J Dermatol* 1990; 122: 299–308.
31. Veien N K. Ingested food in systemic allergic contact dermatitis. *Clin Dermatol* 1997; 15: 547–555.
32. Nielsen et al. The association between contact allergy and hand eczema in 2 cross-sectional surveys 8 years apart. *Contact Dermatitis* 2002; 47: 71–77.
33. Veien N K, Christiansen Ah, Svejgaard E, Kaaber K. Antibodies against nickel albumin in rabbits and man. *Contact Dermatitis* 1979; 5: 378–382.
34. Menné T, Veien N K, Sjølin K E, Maibach H I. Systemic contact dermatitis. *Am J Contact Dermat* 1994; 5: 1–12.
35. Silvennoinen-Kassinen S, Karvonen J, Ikaheimo I. Restricted and individual usage of T-cell receptor beta-gene variables in nickel-induced CD4+ and CD8+ cells. *Scand J Immunol* 1998; 48: 99–102.
36. Boscolo et al. Systemic effects of ingested nickel on the immune system of nickel sensitised women. *Life Sci* 1999; 64: 1485–1491.
37. Di Gioacchino et al. Lymphocyte subset changes in blood and gastrointestinal mucosa after oral nickel challenge in nickel-sensitized women. *Contact Dermatitis* 2000; 43: 206–211.
38. Menné T, Kaaber K. Treatment of pompholyx due to nickel allergy with chelating agents. *Contact Dermatitis* 1978; 4: 289–290.
39. Klein L R, Fowler J F Jr. Nickel dermatitis recall during disulfiram therapy for alcohol abuse. *J Am Acad Dermatol* 1992; 26: 645–646.
40. Gamboa P, Jauregui I, Urrutia I, Antepara I, Peralta C. Disulfiram-induced recall of nickel dermatitis in chronic alcoholism. *Contact Dermatitis* 1993; 28: 255.
41. Menné T, Holm N V. Hand eczema in nickel-sensitive female twins. Genetic predisposition and environmental factors. *Contact Dermatitis* 1983; 9: 289–296.
42. Hindsén M, Bruze M, Christensen O B. The significance of previous allergic contact dermatitis for elicitation of delayed hypersensitivity to nickel. *Contact Dermatitis* 1997; 37: 101–106.
43. Alexander S. Patch testing and menstruation. *Lancet* 1988; 2: 751.
44. McLelland J, Lawrence C M. Premenstrual exacerbation of nickel allergy. *Br J Dermatol* 1991; 125: 83.
45. Hindsén M, Bruze M, Christensen O B. Individual variation in nickel patch test reactivity. *Am J Contact Dermat* 1999; 10: 62–67.
46. Sjövall P, Christensen O B, Möller H. Oral hyposensitization in nickel allergy. *J Am Acad Dermatol* 1987; 17: 774–778.
47. Todd D J, Burrows D. Nickel allergy in relationship to previous oral and cutaneous nickel contact. *Ulster Med J* 1989; 58: 168–171.
48. van Hoogstraten et al. Reduced frequency of nickel allergy upon oral nickel contact at an early stage. *Clin Exp Immunol* 1991; 85: 441–445.
49. Panzani et al. Oral hyposensitization to nickel allergy: preliminary clinical results. *Int Arch Allergy Immunol* 1995; 107: 251–254.
50. Kerosuo H, Kullaa A, Kerosuo E, Hensten-Pettersen A. Nickel allergy in adolescents in relation to orthodontic treatment and piercing of ears. *Am J Orthod Dentofacial Orthop* 1996; 109: 148–154.
51. Morris D L. Intradermal testing and sublingual desensitization for nickel. *Cutis* 1998; 61: 129–132.
52. Mørtz C G, Lauritsen J M, Bindeslev-Jensen C, Andersen K E. Nickel sensitization in adolescents and association with ear piercing, use of dental braces and hand eczema. *Acta Derm Venereol* 2002; 82: 359–364.
53. Andreassi et al. Serum and urine nickel in nickel-sensitized women: effects of oral challenge with the metal. *Contact Dermatitis* 1998; 38: 5–8.

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