

Influence of dietary factors, age and nickel contact dermatitis on nickel excretion

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Summary

Background. Nickel is a frequently detected cause of allergic contact dermatitis. Ingestion of nickel may lead to flares of nickel contact dermatitis.

Methods. We examined nickel excretion in the urine of 164 female patients with and without nickel contact dermatitis. The associations between age, atopic dermatitis, nickel contact dermatitis and nickel exposure through nutrition (e.g. dietary supplements) and by patch tests were investigated prospectively. Nickel was measured with atomic absorption spectrometry with two different standardized methods.

Results. A nickel detection limit of 0.2 µg/l was exceeded by all samples. The 95th percentiles of urine nickel concentration were 3.77 µg/l (age 18–30 years) and 3.98 µg/l (age 31–46 years). Bivariate analyses pointed to significantly increased nickel excretion with increasing age, ingestion of dietary supplements, drinking of stagnant tap water, and consumption of nickel-rich food. In the multivariate analysis, age and dietary supplements remained significant predictors of high nickel excretion. A non-significant increase in the median concentration of nickel was observed after the administration of conventional nickel patch tests. Patients with atopic eczema showed urine nickel concentrations similar to those in non-atopic controls.

Conclusions. The 95th percentile of nickel excretion in our study population markedly exceeded the actual reference value of 3 µg/l. Age and consumption of dietary supplements are the most important predictors. The use of stagnant tap water and consumption of nickel-rich food contribute to the total load. These factors should be explicitly mentioned when allergic patients on a low-nickel diet are counselled. In contrast, existing nickel contact sensitization was not more frequent in subjects with higher nickel excretion. Nickel patch testing may cause transient minor systemic nickel exposure. The findings of this study extend our understanding and management of factors associated with nickel allergy.

Key words: age; dietary factors; nickel contact dermatitis; nickel excretion; nickel.

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Nickel is contained in many metal alloys, and is the most frequent elicitor of contact allergy. The exposure to this essential trace element occurs mainly through ingestion, and, to a lesser degree, through water in beverages (1–5). In higher concentrations ($> 50 \mu\text{g/g}$), nickel shows toxic effects and carcinogenicity (5, 6). The German reference value for internal nickel exposure of the population measured by renal nickel excretion and published by the Human Biomonitoring Committee of the 'Umweltbundesamt' (Federal Environment Agency) is $3 \mu\text{g/l}$ (5).

Only a few studies have used human biomonitoring methodology to track contact allergens by comparing individual patch test reactivity. Hypothetically, the internal load of reactive chemicals might modify the immune response to haptens and the propensity for sensitization and elicitation of allergic contact dermatitis or the development of tolerance (7). For a comprehensive recent review on the available studies, see Thyssen et al. (7).

Examples of nickel-rich foods include tree nuts, various cereals (oat, wheat), soy, chocolate, salads, broccoli, and animal liver and kidneys (3). Drinking stagnant water (i.e. water from infrequently used tap sources) has been shown to be associated with higher nickel intake (6). The role of other sources of nickel (regular use of dietary supplements, and nickel from cookware and from active and passive smoking (5, 8)) is under investigation. Female volunteers show higher renal nickel excretion than men (8). The influence of age on nickel exposure is controversial (9–12). This study was not aimed at answering the question of whether nickel ingestion leads to a direct flare of eczema. We investigated possible associations between nickel exposure (as measured by renal nickel excretion), nickel ingestion and nickel contact dermatitis in female patients attending a university allergy department. The significance of different lifestyle factors was evaluated with comprehensive questionnaires. The influence of conducting actual nickel patch testing on nickel excretion was also investigated. In addition, the 95th percentile of the nickel level in urine in the investigated group was compared with the national reference for human nickel biomonitoring; a European reference is not available to date.

Methods

Subjects and study design

One hundred and sixty-four female patients attending the allergy unit, Department of Dermatology and Allergy Biederstein, Technische Universität München, Munich,

Table 1. Description of relevant dermatological conditions, demographic data, and smoking habits

	Total (n = 164), n %	Group I (n = 42), Ni patch test-positive, n %	Group II (n = 122), Ni patch test-negative, n %
Dermatological diagnosis*			
No data	2 (1.2)	—	2 (1.6)
Nickel contact dermatitis	42 (25.6)	42 (100)	0 (0)
Another contact dermatitis	24 (10.4)	7 (16.6)	17 (13.9)
Dermatitis/eczema	42 (25.6)	2 (4.8)	40 (32.8)
Other eczemas	12 (7.3)	3 (7.1)	9 (7.4)
Atopic eczema	27 (16.6)	8 (19)	19 (15.6)
Rhinoconjunctivitis	5 (3.0)	—	5 (4.1)
Allergic asthma	4 (2.4)	3 (7.1)	1 (0.8)
Other skin diseases	20 (12.1)	8 (19)	12 (9.8)
Urticaria, angioedema, food or drug allergy	29 (17.7)	12 (28.6)	17 (13.9)
Age			
Minimum	18	19	18
Maximum	46	42	46
Mean	30	28	31
Standard deviation	7.25	4.34	7.83
Age 18–30 years	99 (60.4)	33 (78.6)	66 (54.1)
Age 31–46 years	65 (39.7)	9 (21.4)	56 (45.9)
Nationality			
German nationality	129 (78.7)	35 (83.3)	94 (77.0)
Other nationality	35 (21.3)	7 (16.7)	28 (23.0)
Smoking			
Non-smoker	120 (73.2)	34 (81.0)	86 (70.5)
Smoker	44 (26.8)	8 (19.0)	36 (29.5)

*Dermatologists differentiated the diagnosis of dermatitis into presumed nickel or other contact dermatitis, atopic eczema or other eczema (e.g. seborrhoeic dermatitis). If no initial diagnosis of eczema type was possible, the category "Dermatitis/eczema" was chosen.

were enrolled in the study after informed written consent and approval by the ethics committee of the Bavarian Chamber of Physicians had been obtained. Inclusion criteria were: nickel patch test performed, living in the Munich area, absence of severe (i.e. chronic) internal diseases (e.g. kidney or gastrointestinal tract), and age 18–46 years. This age group is expected to have the highest incidence of nickel contact dermatitis (for demographics, see Table 1). Patients with sensitizations to other contact allergens were not excluded from the study. These patients were selected from a standardized allergy unit database and contacted with invitation letters.

The participants completed a comprehensive questionnaire (see statistical evaluation).

According to the results of the nickel patch test, 42 patients (26%) were allocated to the patch test-positive

(i.e. nickel contact dermatitis) group, and 122 (74%) to the patch test-negative group (for demographics, see Table 1).

The age range of the study participants was 18–46 years, with a mean of 30 years. Forty-four patients (27%) were smokers, 12 of them with a cigarette consumption of 20 or more per day. Twenty-seven patients (19%) had a history of atopic eczema.

Nickel patch tests

The standardized application and reading of patch tests was performed according to the guidelines of the German Dermatological Society (DDG) and the German Society of Allergology and Clinical Immunology (DGAKI) (13). Series of contact allergens with 5% Ni(II) sulfate (Hermal, Reinbek, Germany) were applied with 6-mm Finn Chambers® on Scanpor® tape (Epitest Ltd, Oy, Finland). Tests were read after 48 and 72 hr according to DDG and DGAKI guidelines, which correspond to the International Contact Dermatitis Research Group rules (13). A positive reaction required erythema and infiltration to be graded as +.

Nickel and creatinine analysis in urine

Morning urine of patients enrolled from June 2005 to May 2006 was collected. Acidified urine was used for nickel analysis, following a method of atomic absorption spectrometry modified from (14). Analyses were performed blinded in duplicate, with reagent controls and precision control urine samples in every series of measurements. After electrothermal atomization in graphite tubes with Zeeman background compensation, atomic absorption spectrometry was used for quantitative measurement (wavelength 232 nm; gap 0.2 nm). Calibration standards of defined nickel concentrations (0.25–5.00 µg/l) in aqueous glutathione solution with identical processing were used. Reliability was controlled with the criteria of a statistical quality management program. We used two different atomic absorption spectrometry methods in parallel in this study. In the first method, we used hexamethylene ammonium–hexamethylene dithiocarbamate as a chelating agent. In the second method, we used ammonium pyrrolidine dithiocarbamate (APDC), which also served as an enriching substance. The lowest limit of detection was 0.5 µg/l in the first method and 0.2 µg/l in the second method. There was good concordance between the two methods; the second method was more sensitive. Therefore, in the further analysis, the results obtained with this method (APDC) were evaluated. The urine creatinine concentration was measured photometrically

with a wavelength of 546 nm, according to the Jaffé method.

Influence of patch tests

The application of patch tests in 14 female adult patients (age 35 ± 6.6 years) was performed according to the guidelines of the DDG and the DGAKI (13). A standard series of contact allergens with 5% Ni(II) sulfate was applied with 6-mm Finn Chambers® on Scanpor® tape. Tests were read after 48 and 72 hr, according to DDG and DGAKI guidelines (13).

Urine samples of these 14 patients were evaluated before patch testing, after 2 days, and after 11 days.

Statistical evaluation

Only data from patients with completed questionnaires and available urine samples were used in this study. According to the results of the patch test, the patients were allocated to two different groups (nickel-positive and nickel-negative). Nickel concentrations were expressed as natural logarithm-transformed data, adjusted to the creatinine levels. Questionnaires provided information on dermatological history (atopic diseases), occupational history, smoking habits, and drug intake, as well as information regarding factors relevant to nickel contact sensitization, exposure, and factors influencing nickel turnover (food and drinking habits and dietary supplements).

Consumption of 14 nickel-rich foods was separately recorded in the questionnaires. Frequent ingestion (at least twice a week) or infrequent ingestion (less than once per week) were evaluated for the different foods. The number of frequently ingested foods was summarized, resulting in a score from 0 to 14. Similar frequency parameters were used for the ingestion of certain food additives (e.g. vitamin C and blue-green algae products), the use of nickel-releasing cookware, and the habits of tap water use and smoking.

The influence of age was evaluated by comparing two age groups, 18–30 and 31–46 years. In the first step, the influence of the investigated factors on nickel excretion in urine was analysed with bivariate analysis, using the logarithm of creatinine-adjusted nickel concentrations. Dichotomous factors were analysed by comparing means of logarithmic data by *t*-tests. A linear regression analysis was used, comparing the multicategorical factor of nickel-rich nutrition with nickel content of the urine samples. Eventually, all variables were evaluated in a multivariate regression analysis investigating the observed associations of the bivariate analysis for their

independence from each other. spss™ version 13.0.1 was used for statistical calculations.

Results

Nickel excretion

Data of 163 patients were available for per protocol analysis. In all samples, measured renal nickel excretion was above the cutoff, varying from 0.2 to 10.1 $\mu\text{g}/\text{l}$; the median was 1.2 $\mu\text{g}/\text{l}$, with a creatinine value of 1.2 $\mu\text{g}/\text{g}$. Ninety-five per cent of samples had a nickel level under 3.9 $\mu\text{g}/\text{l}$ (3.1 $\mu\text{g}/\text{g}$ creatinine). The reference of the German Human Biomonitoring Committee of 3 $\mu\text{g}/\text{l}$ was exceeded in 13 patients (7.9%). The distribution of nickel concentrations and creatinine-adjusted nickel contents in urine showed a normal distribution after logarithmic transformation, as shown in Fig. 1.

Influence of nickel in diet, food supplements, and beverages

The questionnaires provided data on the consumption of 14 nickel-rich foods. Linear analysis showed a significant ($p < 0.01$) increase in renal nickel excretion with increased ingestion of nickel-rich food, mainly influenced by the high nickel concentrations in patients with frequent consumption of more than five nickel-rich foods, such as

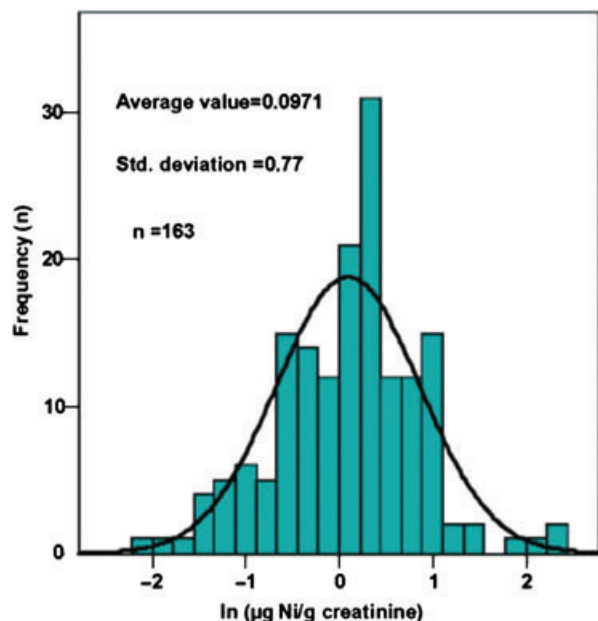


Fig. 1. Creatinine-adjusted urine nickel concentration ($\mu\text{g}/\text{g}$ creatinine) of 163 female volunteers. Logarithmic transformation shows a normal distribution.

cereals. In 15 patients without ingestion of nickel-rich food, the geometric average value of nickel was 0.96 $\mu\text{g}/\text{g}$. In 16 volunteers who regularly ate seven to nine nickel-rich foods, the creatinine-adjusted geometric average was 1.89 $\mu\text{g}/\text{g}$.

Bivariate analysis (Table 2; Fig. 2) showed an increased geometric mean in patients with frequent ingestion of dietary supplements and stagnant tap water. The mean nickel values in the group of patients with intake of food supplements ($n = 43$) and the group of patients without intake of food supplements ($n = 120$) were 1.48 and 0.99 $\mu\text{g}/\text{g}$, respectively. In patients with frequent ingestion of dietary supplements, the geometric mean was 41% higher (95% confidence interval 8–84%). Multivariate analysis showed a significant association between frequent use of dietary supplements and increased nickel concentration ($p = 0.01$).

The mean nickel values in the group of patients who drank stagnant water ($n = 91$) and in the group of patients who avoided stagnant water ($n = 72$) were 1.23 and 0.96 $\mu\text{g}/\text{g}$, respectively (Table 2, bivariate analysis, $p < 0.05$).

Influence of nickel sensitization

No significant differences in geometric means of nickel concentration were seen with regard to nickel contact dermatitis, the use of certain cookware, or smoking behaviour (Table 2).

Influence of age

Bivariate analysis (Table 2; Fig. 2) showed an increased geometric mean of nickel excretion in older patients. In the younger (18–30 years) age group ($n = 99$), the geometric mean creatinine-adjusted nickel value was 0.96 $\mu\text{g}/\text{g}$, and in the older (31–46 years) age group ($n = 64$), the median creatinine-adjusted nickel value was 1.37 $\mu\text{g}/\text{g}$. Multivariate analysis confirmed a statistically significant association between increasing age and increased nickel concentration. A 36% higher geometric mean was seen in women 31–46 years of age than in younger women (18–30 years) (95% confidence interval 7–73%).

Influence of actual application of nickel patch tests

In the context of the study, we examined the influence of nickel patch testing on nickel exposure in 14 patients before patch testing, after 2 days, and after 11 days. In this group, 2 patients had a positive nickel patch test reaction (i.e. nickel contact dermatitis) and 12 patients were negative. The mean nickel values in

Table 2. Influence of age, different factors of nickel ingestion and nickel dermatitis on nickel excretion in urine

	n	Nickel concentration in urine, range $\mu\text{g/g}$ creatinine	Geometric mean $\mu\text{g/g}$ creatinine	Bivariate regression	Multivariate regression		
				t-test	Coefficient of the ln value (95% confidence interval)	Ratio of the geometric mean, non-transformed data	p-value
Age (years)							
18–30 years	99	0.1–8.7	1.0	$p = 0.004^*$	Reference	Reference	0.01*
31–46 years	64	0.2–10.5	1.4		0.31 (0.07–0.55)	1.36 (1.07–1.73)	
Ingestion of food supplements							
Rarely	120	0.1–6.0	1.0	$p = 0.006^*$	Reference	Reference	0.01*
Frequently	43	0.2–10.5	1.5		0.34 (0.08–0.61)	1.41 (1.08–1.84)	
Ingestion of nickel-rich food	NA	NA	NA	$p = 0.006^*$	NA	NA	0.08
Change per additional food							
Intake of stagnant tap water							
Rarely	72	0.2–9.9	1.0	$p = 0.04^*$	Reference	Reference	0.19
Frequently	91	0.1–10.5	1.2		0.16 (–0.08–0.40)	1.17 (0.92–1.49)	
Use of cookware containing nickel							
No	15	0.2–2.4	0.9	$p = 0.15$	Reference	Reference	0.15
Yes	116	0.2–10.5	1.2		–0.11 (–0.26–0.04)	0.90 (0.71–1.04)	
Use of electric kettle with open coil							
No	116	0.1–10.5	1.1	$p = 0.68$	Reference	Reference	0.84
Yes	43	0.2–3.9	1.2		0.01 (–0.12–0.14)	1.01 (0.89–1.15)	
Smoking							
Non-smoker	119	0.1–9.9	1.1	$p = 0.78$	Reference	Reference	0.84
Smoker	44	0.2–10.5	1.1		–0.03 (–0.29–0.24)	0.97 (0.75–1.27)	
Nickel dermatitis							
Positive Ni patch test	42	0.1–4.7	1.0	$p = 0.56$	Reference	Reference	0.62
Negative Ni patch test	121	0.2–10.5	1.1		–0.07 (–0.34–0.20)	0.93 (0.71–1.22)	

ln, natural logarithm; NA, not applicable; multicategorical factor. Rarely: one time a week or rarely. Frequently: two times a week or more.

*Significance, $p \leq 0.05$.

14 patients before nickel patch testing and 2 and 11 days after administration of the nickel patch were 1.51 $\mu\text{g/g}$ (median 1.03), 1.51 $\mu\text{g/g}$ (median 1.67), and 1.15 $\mu\text{g/g}$ (median 1.14), respectively. The results of the Wilcoxon test did not show statistically significant differences in the nickel concentration before and after patch testing.

Discussion

This study showed that the reference of the German Human Biomonitoring Committee of 3 $\mu\text{g/l}$ was exceeded in 13 patients (7.9%). Age and, especially, consumption of dietary supplements were the most significant positively correlated factors.

The creatinine-adjusted mean urinary nickel concentrations of the female patients in our study were relatively low as compared with studies from other countries (9, 11, 12, 15–18). Lower nickel concentrations have only been reported in the Norwegian general population (19) and in Danish patients with and without nickel contact

dermatitis (20). The differences may be explained by sampling time (morning urine versus 24-hr urine) and the age and sex distribution in different studies, as well as by sampling bias and a generally lower study size. Female volunteers show higher renal nickel excretion than men (8). Two reports on German female volunteers are available (8, 15), giving slightly higher nickel references in a similar age group. Currently, the individual internal nickel exposure of the German population measured by nickel concentration in urine is evaluated with regard to a 'reference' of the Human Biomonitoring Committee of the German 'Umweltbundesamt' (Federal Environment Agency): 3 $\mu\text{g/l}$ (5). However, this nickel level is not derived from a representative sample study. Oral provocation with 1 mg of nickel (nickel sulfate) in 2 volunteers led to 24-hr urine concentrations of 45 and 49 $\mu\text{g/l}$, respectively (9). In our study, the 95th percentile of nickel excretion in female patients markedly exceeded the reference value of the German Human Biomonitoring Committee.

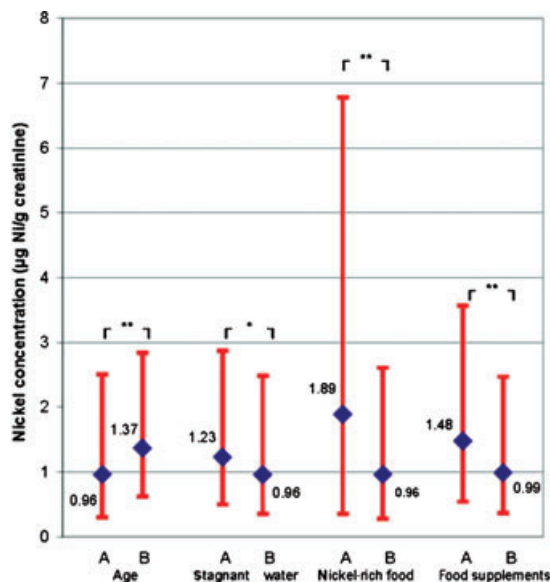


Fig. 2. Nickel in urine: geometric means, 10th and 90th percentiles. Age: (A) age group 18–30 years ($n = 99$); (B) age group 31–46 years ($n = 64$). Stagnant water: (A) group drinking stagnant water ($n = 91$); (B) group avoiding stagnant water ($n = 72$). Nickel-rich food (multicategorical factor, two categories shown): (A) group with regular ingestion of seven to nine nickel-rich foods ($n = 16$); (B) group without ingestion of nickel-rich food ($n = 15$). Food supplements: (A) group with regular intake of food supplements ($n = 43$); (B) group without intake of food supplements ($n = 120$). * $p < 0.05$; ** $p < 0.01$ (bivariate analysis).

Age, lifestyle and other factors influencing nickel excretion

The literature on the influence of age on nickel urine levels has shown inconsistent results to date. Whereas studies from Germany (8) and Japan (12) are in line with our finding of increased levels with age, other studies (11, 16, 21) did not support this age-dependency. The European Food Safety Authority has previously pointed to an additional effect of certain nutritional factors on nickel load. Our finding of an association between elevated renal nickel excretion and the frequent ingestion of dietary supplements seems to be relevant in the population, is corroborated by multivariate analysis, and deserves further investigation for the nickel sources. In many EU countries, multivitamin and mineral food supplements containing nickel sulfate are manufactured (22). It can be speculated that such products are consumed preferentially in the higher age groups. Other explanations may include different overnight water use habits (influencing the use of stagnant water), geographical differences in nickel intake, smoking, or unknown lifestyle factors.

In the multivariate analysis, we were not able to corroborate the results of other studies (1, 8) and our own bivariate analysis showing an independent influence of the ingestion of nickel-rich food and stagnant water on nickel excretion. Possibly because of sample size or the categorization of nutrition, statistical significance was not achieved. Thus, the role of dietary supplements in our study seems to be more prominent than that of the consumption of nickel-containing foods.

Our data show no evidence for an association between nickel sensitization and renal nickel excretion. The same has been observed previously in two small (35 and 32 volunteers) studies in female patients (9, 20). The influence of smoking behaviour (high nickel content in tobacco) may have been overrated in the past, perhaps because of the relatively low additional nickel load of $0.45 \mu\text{g/day}$ (8). Similar negative results with regard to smoking have been reported by several groups (8, 10, 11), whereas smoking as a risk factor for nickel sensitization has been proposed in two other well-designed studies (23, 24). In addition, we could not show a significant effect of the use of nickel-releasing cookware (Table 2), although a potential effect has been proposed previously (25, 26).

Our data also confirm the results of previous studies with regard to there being only a tendency for a higher risk of nickel sensitization in patients with atopic eczema (27).

In the interpretation of our results, we have to consider that only female patients from the allergy unit were enrolled. Whereas comparison with other populations may be difficult, the identification of covariates influencing nickel levels is possible. As long as no significant differences in nickel turnover are expected, the relevance of lifestyle factors should also hold true for other population groups. To date, no evaluated questionnaire for alimentary nickel ingestion allowing quantitative analysis is available. Therefore, only qualitative comparisons of higher or lower nickel exposure were made in this study.

Conclusions

The findings of this study extend our understanding and management of factors associated with nickel allergy. We showed that the 95th percentile of renal nickel excretion in our female patients markedly exceeded the $3 \mu\text{g/l}$ reference value of the German Human Biomonitoring Committee. Age and consumption of dietary supplements are the most significant positively correlated factors. Frequent ingestion of stagnant tap water and nickel-rich food can contribute to the total load. These factors should be explicitly mentioned when allergic patients on a low-nickel diet are counselled. The interpretation of individual results needs to take into account age and

many lifestyle factors. The role and kind of dietary supplements causing a higher nickel load deserve further investigation.

On the other hand, nickel sensitization status was not significantly associated with the current nickel concentration in the urine. This result may argue against a major epidemiological role of high internal nickel exposure for nickel sensitization rates in the population. Hypothetically, higher nickel intake may also result in

more nickel-tolerant individuals in a population through oral tolerance induction.

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