Acta Derm Venereol (Stockh) 1990; 70: 300–303

Nickel in Intercellular Fluid

Comparison between Nickel-allergic Patients and Controls

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Twelve non-atopic women with nickel allergy and allergic contact dermatitis (pompholyx type) were compared with matched controls without any known allergy. Intercellular fluid was obtained by the suction blister technique, and sufficient material for analysis was available from 10 women from each group. The median nickel concentration was 16 nmol/l in the patient group and 39 nmol/l in the controls ($p<0.02$). Calculated dietary intake of nickel during the week before examination was similar in the two groups, but the fluid intake was larger in the control group. Urine was collected in the morning before examination by 11 women in each group; the nickel concentrations showed no difference between the two groups. These observations suggest that cellular uptake of nickel in sensitized patients may be sufficient to influence the kinetics of nickel in the body. ***Key words: Allergic contact dermatitis; Hand eczema, Toxicokinetics.***

(Accepted December 20, 1989.)

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Initial nickel-sensitization is due to cutaneous contact with nickel (1). Subsequent development of hand eczema of the pompholyx type in nickel-allergic patients may in some cases be due to increased oral intake of nickel followed by a systemic exposure to the allergen, as indicated by several recent reports (2). An experimental study with radioactive nickel showed that percutaneously absorbed nickel was retained in the stratum granulosum and in the stratum spinosum where the Langerhans' cells are located (3). These cells play an important role in the pathogenesis of allergic contact dermatitis; they trap and concentrate antigens on the cell surface before presentation to T-lymphocytes (4, 5). Considerable amounts of nickel would bind to the cell membrane of T-lymphocytes from nickel-sensitized patients, while nickel binding was only seen with very few cells from non-sensitized persons (6). Thus, the presence of nickel in the intercellular fluid (IF) of the skin is probably crucial for the early stages in the primary sensitization and later eruptions of dermatitis in nickel-sensitive individuals.

The purpose of the present study was to examine the nickel content of IF in nickel-allergic patients with vesicular hand eczema, as compared with normal controls free from nickel allergy.

MATERIALS AND METHODS

Twelve women with nickel allergy and hand eczema of the pompholyx type were selected from among consecutive nickel-sensitive patients at the Department of Dermatology, Odense University Hospital. Nickel allergy was verified according to the ICDRG recommendations (7) by positive patch test to 5% nickel sulfate in petrolatum; the patches were applied for 48 h and read at 48 and 72 h. Patients with positive patch tests to other metals and patients with a history or clinical signs of atopic dermatitis were excluded. The median age of the 12 patients selected was 28.5 (range, 21–61) years. A comparison group consisted of 12 age-matched (within 5 years) women. None of these women had eczema or any known allergy; the median age was 32 (range, 21–62) years.

The participants answered a questionnaire concerning external cutaneous exposure to nickel, with regard to occupational hazards, kitchen utensils, water pipes and faucets at home. To estimate the oral intake of nickel, the two groups were instructed to keep a detailed account of their total diet during 7 days. Electronic scales and printed forms were supplied. Based on this information, the mean dietary intake of nickel, energy and water was calculated from published data (8, 9). At the end of the 7-day period, i.e. the day of examination, the two groups collected a sample of morning urine in pre-washed 500-ml polyethylene bottles (Kartell, Milan, Italy), and each participant was examined at the Department of Dermatology, Odense University Hospital.

IF was collected as previously described (10) with special precautions to prevent contamination with nickel. After a 3-min hand washing, the examiner put on disposable PVC gloves. The patient's abdominal skin was washed twice in

Table I. *Dietary intake of nickel, energy and fluids during a 7-day period for 12 nickel-allergic women and 12 age-matched women without know allergies*

	Patients		Controls	
	Median	Range	Median	Range
Total nickel intake (μmol/d)	2.18	1.53-3.81	2.93	1.93-5.81
Energy intake (kJ/d)	8278	5412-10011	9144	5537-11639
Relative nickle intake (μmol/1000 kJ)	0.34	0.20-0.49	0.39	0.26-0.49
Fluid intake (ml/d)	1462*	782-2664	2097	1361-3206

*$p<0.02$ when compared with controls (Mann-Whitney U-test).

70% ethanol. A perspex cup with a diaphragm perforated by 18 holes (diameter 5 mm) was then tightly attached with Scanpor (Norgesplaster A/S, Oslo, Norway) to an area of the lower abdominal skin unaffected by dermatitis. A steady negative pressure of 300–400 mmHg was established with a pump. Small vesicles usually appeared within 2 h and subsequently coalesced to form bullae corresponding to the entire 5 mm holes. The IF was drained with a micropipette (Socorex, Renens, Switzerland) with a polyethylene tip (Medical Laboratory Automation, Pleasantville, NY, USA) into a 3 ml polyethylene test tube. The test tube was carefully closed, labelled and stored at −80°C until analysis. To minimize the risk of contamination, all equipment (specimen containers, pipette and perspex cups) was disposable, and was kept in tightly sealed plastic bags and stored in closed boxes until use.

Electrothermal atomic absorption spectrometry was applied to determine nickel in the body fluids. We used a Perkin-Elmer model 5000 instrument with Zeeman background correction, HGA-500 graphite furnace, and an AS-40 autosampler (Perkin-Elmer, Norwalk, Conn., USA). For IF, the procedure recommended for serum was applied (11). The results for IF-nickel were read in duplicate against a serum-based standard curve. The total analytical imprecision was estimated to be 11% at a serum nickel concentration of 12 nmol/l. The accuracy was ensured by using Seronorm Trace Element batch 105 (Nycomed, Oslo, Norway) as quality control material; we found an average nickel concentration of 52.4 nmol/l in 15 determinations (assigned value, 54.5 nmol/l).

Urine nickel was determined by atomic absorption (12) and standard addition. The total analytical imprecision was estimated to be 24.3%, 11.7% and 7.6% at urine-nickel levels of 35, 57 and 126 nmol/l, respectively. The accuracy of the nickel determinations was ensured by using Lanonorm® Metalle 1 (Behringwerke AG, Marburg, FRG) as quality control material; measured nickel concentrations averaged 57 nmol/l in 12 determinations (assigned nickel value, 0.06 ± 0.01 μmol/l). The urinary nickel excretion was expressed in relation to the creatinine concentration to avoid possible variations due to changes in urinary flow. The concentration of creatinine in the urine was measured on an AutoAnalyzer II according to the recommendations by the manufacturer (Technicon, Tarrytown, NY, USA).

The data from the two groups were compared by a non-parametric Mann-Whitney U-test.

RESULTS

As expected, the nickel-sensitive patients made efforts to avoid cutaneous exposure to nickel. The questionnaires revealed, e.g. that one of the 12 nickel-allergic patients had installed a plastic-covered water faucet at home, while another used a towel to open faucets. Further, all the participants used wood or plastic utensils in the kitchen, and all pots and saucepans were made of stainless steel. However, none of the women in the two groups made any specific effort to avoid any food items due

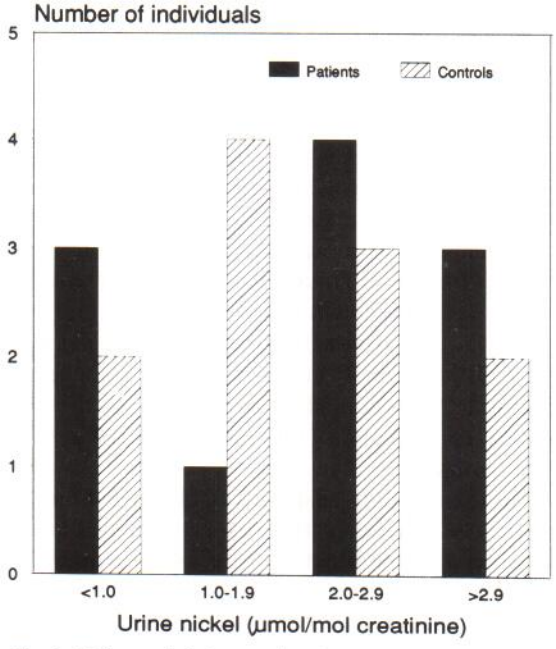

Fig. 1. Urinary nickel excretion in a morning urine in 11 nickel-allergic women and 11 women without known allergies.

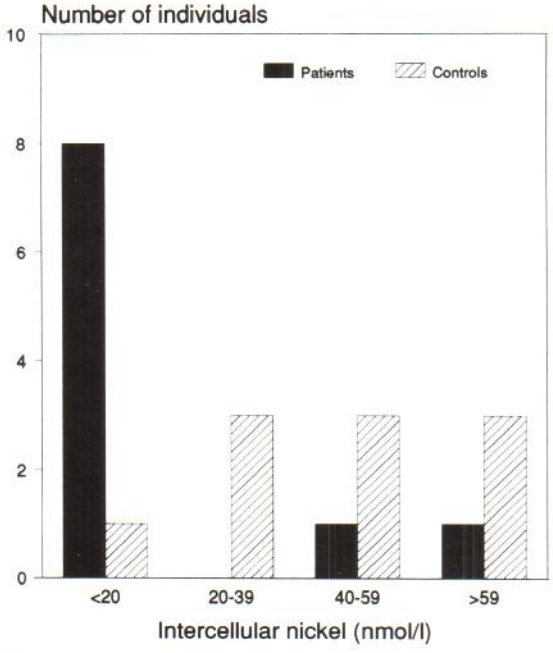

Fig. 2. Nickel concentration in intercellular fluid from 10 nickel-allergic women and 10 women without known allergies.

to its known or suspected nickel content. No occupational exposure to nickel was found.

All patients recorded details concerning their diet during the week prior to examination (Table I). The energy intake of the two groups differed slightly, perhaps due to differences in recording. The calculated intake of nickel was quite similar in the two groups, especially when determined per 1000 kJ. In contrast, the fluid intakes differed significantly.

A sample of morning urine was obtained from 11 women from each group. Despite the difference in fluid intake between the groups during the previous week, the urinary nickel excretions were similar with medians of 24 nmol/l (1.4 μg/l); the same applies to the nickel excretion adjusted according to the creatinine concentration (1.9 μmol/mol or 1.0 μg/g in both groups) (Fig. 1).

Sufficient IF for analysis was obtained from 10 women in each group. The nickel content was found to differ ($p < 0.02$) between the two groups, with medians for the patients of 0.95 μg/l (16 nmol/l) and for the controls 2.3 μg/l (39 nmol/l) (Fig. 2).

DISCUSSION

In this study, a group of women with nickel allergy was compared with an age-matched group with no known allergy. They had similar dietary intakes of nickel, comparable energy intakes, and the same urinary nickel excretion. The median nickel intake was close to the average nickel content of the Danish diet of 18 μg/1000 kJ (0.31 μmol/1000 kJ) (9). The median energy intake approximates the recommended energy intake of 8400 kJ/d for adult women; this observation suggests that the participants delivered virtually complete lists of their diet. The urinary nickel excretion is well within the normal interval (13). The reported fluid intake was larger in the control group, but in view of the other similarities between the groups, this difference is of questionable relevance.

Most importantly, the nickel content in the IF was significantly lower in the group of nickel-allergic patients when compared with the control group. Before any implications of this finding are discussed, the validity of the results must be carefully assessed.

Increased nickel concentration in IF could potentially be due to contamination. Although extensive safeguards against all external contamination during collection and analysis were instituted, such sources of error cannot entirely be ruled out. However, a selective contamination of the control samples would be unlikely. In addition, nickel is excreted in sweat; nickel concentrations levels up to 20 μg/l (0.34 μmol/l) have been determined in sweat during sauna bathing (14). These concentrations are of the same magnitude as the IF concentrations found in the present study. Thus, in the unlikely event that leakage occurred, nickel from body sweat could perhaps contaminate the IF samples collected.

Although suction may marginally increase microvascular escape of some serum components, the suction fluid can generally be regarded similar to average IF (18). Compared with serum, some components occur in lower concentrations in suction IF; for example, the median ratio of IF/S was 0.21 for IgE and 0.32 for IgG in a group of patients without skin diseases (19). From available reference values (13), the serum nickel concentration in the individuals examined would be expected to be of the same order of magnitude as the IF concentrations found, or slightly below that level. Thus, the IF/S ratio for nickel would then be close to or above unity in non-allergic patients. Such differences in IF/S ratios

could be related to different rates of cellular uptake or passage of the capillary basement membrane. The influence of these processes deserves closer study to elucidate the fate of nickel in intercellular fluid.

The cellular aspects of nickel kinetics related to uptake, distribution and excretion are incompletely known (13, 15). No obvious difference between allergic and non-allergic individuals was observed in serum nickel concentrations after oral challenge (16). Thus, the general toxicokinetic pattern may be independent of nickel allergy. However, because of the uptake of nickel by activated T-lymphocytes (6) and the increased number of activated T-lymphocytes in allergic patients, the kinetics of nickel in intercellular fluid could conceivably differ considerably between allergic and non-allergic individuals. This notion is supported by the experimental evidence that nickel-sensitized guinea pigs tend to retain absorbed nickel in the skin, thus giving lower concentrations in serum and urine (17). However, whether nickel uptake by T-lymphocytes takes place primarily in the eczematous skin areas is presently unknown. Also, the present study has not examined whether excema status as such is related to changes in nickel kinetics.

ACKNOWLEDGEMENTS

The skilful technical assistance of Hanne Albæk, Brita Andersen and Ranja Bjerring is greatly appreciated. This study was supported by grants from the Danish Medical Research Council.

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