

Serum and urine nickel in nickel-sensitized women: effects of oral challenge with the metal

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15 women with a positive patch test only to nickel (Ni) and without atopy and 10 control women were selected for the study. Blood and urine specimens were collected with a standard procedure either before (at 8 a.m.) or 4 and 24 h after the ingestion of 10 mg of Ni (as Ni sulfate). 7 of the Ni-sensitized patients showed a flare-up of eczema and/or urticaria during the test, while the other women were non-symptomatic. Serum and urine Ni of controls and Ni-sensitized women did not significantly differ. Serum and urine Ni levels determined before the oral Ni challenge were in the range of reference values recently reported by other authors (0.2–2.0 µg/l of serum or urine). Ni was greatly augmented in urine and serum 4 h after the challenge (25th–75th percentiles: 43–264 µg/l urine Ni and 15–52 µg/l serum Ni). 24 h after Ni ingestion, urine Ni was 41–153 µg/l and serum Ni 4–17 µg/l. Our study confirms a previous investigation showing similar levels of serum and urine Ni following ingestion of the metal in control and Ni-sensitized women without atopy.

Key words: allergic contact dermatitis; serum nickel; urine nickel; oral intake; oral provocation.
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Nickel (Ni) is an essential element for both animals and humans (1). Ni deficiency symptoms, however, have not been found in humans since Ni intake generally exceeds its requirements. About 10–15% of women and 1–3% of men living in industrialized countries are sensitized to nickel (Ni). Sources of the metal are coins and jewellery, as well as consumer goods and foods (1). Moreover, Ni exposure may occur during working activities (2, 3).

Ni-sensitized persons may suffer from allergic contact dermatitis (ACD); some of them may show, following ingestion of foods with high Ni content, a flare-up of eczema and/or urticaria associated with abdominal disorders (4). On the other hand, Ni-induced respiratory allergy (asthma with or without rhinitis) is not frequent and is found mainly in Ni-exposed workers (2). In a few cases, respiratory symptoms occur together with ACD and urticaria (3).

Treatment with Ni chelating agents, capable of reducing the Ni body burden, has been reported to decrease allergic symptoms in Ni-sensitized pa-

tients (5). On the other hand, 5 mg of Ni sulfate, taken orally 1× a week for 6 weeks, was found to attenuate the patch test reaction (6). Moreover, progressive decrease of intestinal absorption of Ni was found in sensitized patients treated with increasing doses of the metal (7).

Studies performed before 1990 report contradictory levels of Ni in biological fluids: this was mainly related to specimen contamination (10). Recently, correct procedures for collection, storage and determination of Ni and other trace elements in human blood and urine have been described (8–11). Moreover, improved atomic absorption spectrophotometers with a low detection limit for analysis of Ni in biological specimens have been utilized. For these reasons, accurate reference values for serum and urine Ni in normal populations were reported (9–11).

During a study on reference values for trace elements in humans, serum Ni was found to be lower in sensitized women than in controls (10). In another investigation, serum Ni was found to be

higher in patients with psoriasis than in healthy subjects (12), while in another study, urine Ni, following oral ingestion of the metal, was found to be higher in patients with atopy than in Ni-sensitized patients without atopy or in control subjects (13).

The purpose of this study was further to determine, using correct analytical procedures, both serum and urine Ni levels in Ni-sensitized women and in those without allergy, both before and following oral Ni challenge.

Materials and Methods

10 women with negative patch tests (Lofarma, Milano) to all allergens and another 15 with a positive patch test only to Ni, not suffering from atopy, were selected for the study. 2 of the Ni-sensitized women showed ACD during their working activities. All the women selected for the study were not suffering from diseases (except Ni allergy) and not recovering from recent infections. Both groups of women were not occupationally exposed to toxic agents and were living in areas with low environmental exposure to toxic agents (8). Only 1 woman in each group was smoker. Women were investigated between the 6th and the 25th day of the menstrual period. Mean age of the control group was (mean $\pm$ S.D) 28.6 $\pm$ 5.5 years (range 23–40) and that of the Ni-sensitized group 32.2 $\pm$ 7.4 years (range 20–44).

Patients were recommended to observe a diet with a low Ni content for the 4 days preceding the test.

Thereafter, with informed consent, they received 10 mg of Ni sulfate (Lofarma, Milan) orally at 8 a.m. in a fasting condition. Blood and urine samples were collected with a standardized procedure in order to avoid Ni contamination (8); in particular, a Teflon cannula was used for collecting

blood and plastic cryovials (Nalgene, PBI internationals, Milano) were utilised as containers of blood and urine. Biological specimens were collected at 8 a.m. (before Ni ingestion), at 12 a.m. (4 h after Ni intake) and at 8 a.m. on the following day in the same fasting condition (24 h after Ni ingestion).

Urine Ni was determined by the atomic absorption spectrophotometers Perkin Elmer 4100 ZL and Varian 300Z (10) in 2 different laboratories. Detection limit for urine Ni was 0.2 μ g/l. Serum Ni was determined by a Perkin Elmer SIMM 6000 instrument. Accuracy and analytical recovery were monitored with a sero-norm reference serum sample, spiked with a known concentration of Ni. Detection limit for serum Ni was 0.2 μ g/l. Analytical quality control of urine and serum Ni was carried out on 10% of the samples by neutron activation analysis (8, 11). Statistical analysis of the data was made using SYSTAT software.

Results

10 control subjects, and 8 of the 15 women sensitized to Ni, did not suffer from symptoms following Ni ingestion. This group of 8 Ni-sensitized women was called “non-symptomatic”, while the other was called “symptomatic”. The reactions to ingested Ni appeared between the 6th and the 24th h after challenge. 4 patients experienced very pruriginous erythematovesicular, manifestations on the hands and wrists, 1 showed eczema reactions and pruritus on the face, neck and chest, and 2 generalised urticaria.

In all cases the skin region where the Ni patch test had been performed flared-up.

The levels of metals in morning urine may be related either to the volume of urine or to creatinine (14). In this study, we related urine Ni content

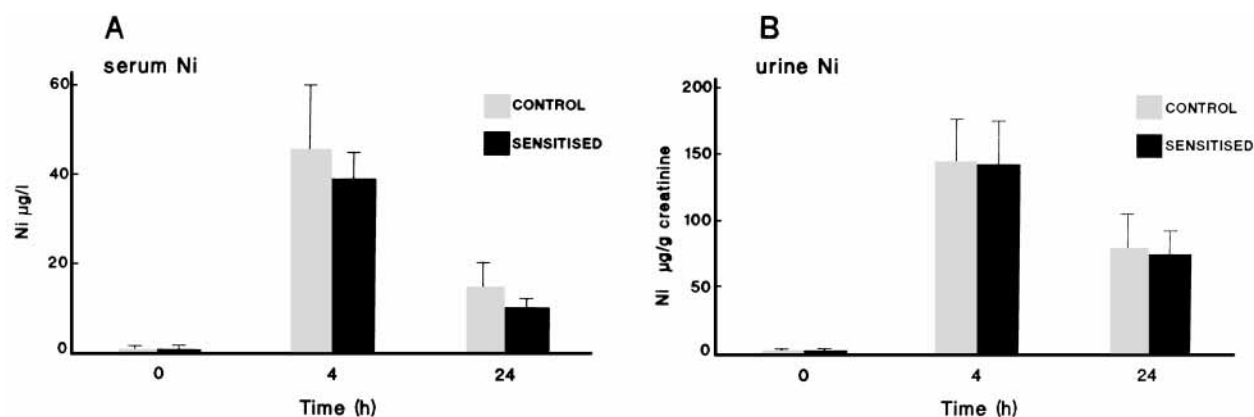


Fig. 1. Urine (A) and serum (B) Ni concentrations in 15 Ni-sensitized and 10 control women before (time 0), after 4 and 24 h ingestion of 10 mg of Ni (as Ni-sulfate). Values are expressed in means $\pm$ SEM.

Table 1. Urine and serum nickel in control ($n=10$), Ni-sensitized non-symptomatic ($n=8$) or symptomatic ($n=7$) women before, after 4 and 24 h Ni ingestion

	Control		Non-symptomatic		Symptomatic	
	median	25th–75th percent.I	median	25th–75th percent.I	median	25th–75th percent.I
<i>Urine Ni ($\mu\text{g/l}$)</i>						
basal value	1.3	0.9–1.8	1.0	0.6–1.5	1.2	1.0–1.4
after 4 h	154.2	86.3–264.7	57.3	43.5–130.1	115.8	95.5–164.6
after 24 h	87.0	46.7–153.0	68.2	41.6–87.7	63.0	38.4–148.7
<i>Urine Ni ($\mu\text{g/g creatinine}$)</i>						
basal value	0.8	0.6–1.2	0.6	0.5–1.3	0.7	0.5–1.1
after 4 h	114.0	79.7–222.1	70.5	40.1–131.7	95.8	66.8–265.0
after 24 h	50.5	30.5–88.6	66.7	27.5–104.9	47.2	37.8–121.0
<i>Serum Ni ($\mu\text{g/l}$)</i>						
basal value	1.0	0.8–1.1	1.1	0.8–1.2	0.9	0.8–1.0
after 4 h	30.3	15.7–45.6	24.2	17.0–38.9	35.1	28.2–52.7
after 24 h	8.2	7.4–13.6	7.4	4.6–10.4	9.7	6.3–17.1

Table 2. The Pearson correlation coefficients between serum and urine Ni in control ($n=30$), Ni-sensitized non-symptomatic ($n=24$) or symptomatic ($n=21$) women in all the biological specimens collected before, after 4 and 24 h Ni-ingestion

	Control		Asymptomatic		Symptomatic	
	r	p	r	p	r	p
liter of urine	0.769	<0.001	0.443	<0.05	0.748	<0.001
creatinine	0.649	<0.001	0.551	<0.01	0.765	<0.001

with both these parameters. Both urine and serum Ni of control and Ni-sensitized women were similar in basal condition before the ingestion of Ni; there was no difference between non-symptomatic and symptomatic sensitized women (Fig. 1 and Table 1).

4 h after Ni ingestion (at 12 a.m.), urine and serum Ni were greatly augmented in both control and sensitized patients (Fig. 1). There was also no significant statistical difference in serum and urine Ni values between symptomatic and non-symptomatic sensitized patients (Table 1). However, this last group showed urinary Ni levels slightly lower and slightly more diluted (in relation to the creatinine) than the other groups (Table 1).

Urine and serum Ni of all women examined, 24 h after the ingestion of Ni (at 8 a.m. on the following day), was reduced in relation to the test performed at 12 a.m. on the day before. There was also no significant statistical difference between the serum and urine Ni of control and sensitized patients. However, serum Ni of non-symptomatic sensitized patients was slightly lower (Table 1 and Fig. 1).

The Pearson correlation coefficient showed that the serum Ni of all 25 patients (determined in all the 75 urinary samples collected either before or following ingestion of Ni) was closely correlated with urine Ni related both to the volume of urine ($r=0.699$, $p<0.001$) or to creatinine ($r=0.629$, $p<0.001$). The correlation coefficients between

serum and urine Ni of control and non-symptomatic or symptomatic sensitized patients were similar (Table 2).

Discussion

A review of the investigations concerning serum and urine Ni levels in the normal population was performed. Selection of persons for the investigation, procedures for specimen collection, analytical methods and statistical analyses were evaluated. In 5 of these studies, serum Ni values were $<0.3 \mu\text{g/l}$ (9). Serum Ni levels found in our study are slightly higher than those of Grandjean et al. (10) and slightly lower than those of Minoia et al. (11). Urinary Ni levels in the normal population are generally found to be suitable when they are less than or equal to $2.0 \mu\text{g/l}$ (9). The levels of urine Ni found in this study are similar to those reported by Roels et al. (15). Moreover, our unpublished data on a group of 20 healthy men show median reference values of $0.8 \mu\text{g/l}$ for serum Ni, $1.0 \mu\text{g/l}$ and $0.5\text{--}0.6 \mu\text{g/g}$ of creatinine for urine Ni. It is known that many factors, including diet, alcohol consumption, physical activity and possibly circadian and seasonal variations, may influence serum and urine Ni levels (9–11). Therefore, normal reference values for serum Ni levels may fall within a wide range. The slight differences between the values of serum and urine Ni of this study and those of other investigations, defined as suitable

for reference values (9–11), may thus not depend on methodological inaccuracy but rather on physiological and environmental factors. In a previous study, serum Ni was described as slightly reduced (without reporting the data) in individuals with eczema (10); the authors suggested that Ni-sensitized patients had avoided food items containing high Ni levels or other sources of the metal. In another study, 24-h urine Ni excretion after ingestion of 1 mg of Ni (as sulfate) was about 7–16 $\mu\text{g/l}$ in controls and Ni-sensitized patients without atopy and about 8–25 $\mu\text{g/l}$ in patients with atopy (13). Ni urinary excretion increased with age. When age differences were taken into account, the urine Ni levels were found to be higher in the patients with atopy. Some of these patients suffered from vesicular eruptions similar to those seen in some Ni-sensitized subjects.

This study demonstrates higher urine dilution in asymptomatic Ni-sensitized women 4 h following Ni ingestion. In these patients, serum protein complexes of Ni (17) or renal mechanisms of Ni excretion may be different from those of symptomatic patients. This may be evaluated in further studies.

The results of this study are in agreement with those of a recent study showing no difference in serum or urine Ni after oral nickel challenge, between Ni-sensitized and control women (13). This demonstrates the lack of important alterations of absorption, transport, tissue distribution and excretion in Ni-sensitized subjects. Therefore, in these patients, only a modification in the mechanisms underlying the response of the immune system to the metal can explain the allergic symptoms.

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