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Absorption and Retention of Nickel from Drinking Water in Relation to Food Intake and Nickel Sensitivity

Gitte Dalsgaard Nielsen,*,† Ulla Søderberg,‡ Poul J. Jørgensen,§ Douglas M. Templeton,‖ Søren N. Rasmussen,¶ Klaus E. Andersen,‡ and Philippe Grandjean*,**

**Department of Environmental Medicine, Odense University, 5000 Odense, Denmark; †Department of Environmental Educations, Technical College Slagelse, Slagelse, Denmark; ‡Department of Dermatology, Odense University Hospital, 5000 Odense, Denmark; §Department of Clinical Biochemistry, Odense University Hospital, 5000 Odense, Denmark; ‖Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario M5G 1L5, Canada; ¶Department of Biological Sciences, The Royal Danish School of Pharmacy, 2100 Copenhagen, Denmark; and **Departments of Neurology and Environmental Health, Boston University Schools of Medicine and Public Health, Boston, Massachusetts 02118*

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Two studies were performed to examine the influence of fasting and food intake on the absorption and retention of nickel added to drinking water and to determine if nickel sensitization played any role in this regard. First, eight nonallergic male volunteers fasted overnight before being given nickel in drinking water (12 μg Ni/kg) and, at different time intervals, standardized 1400-kJ portions of scrambled eggs. When nickel was ingested in water 30 min or 1 h prior to the meal, peak nickel concentrations in serum occurred 1 h after the water intake, and the peak was 13-fold higher than the one seen 1 h after simultaneous intake of nickel-containing water and scrambled eggs. In the latter case, a smaller, delayed peak occurred 3 h after the meal. Median urinary nickel excretion half-times varied between 19.9 and 26.7 h. Within 3 days, the amount of nickel excreted corresponded to 2.5% of the nickel ingested when it was mixed into the scrambled eggs. Increasing amounts were excreted as the interval between the water and the meal increased, with 25.8% of the administered dose being excreted when the eggs were served 4 h prior to the nickel-containing drinking water. In the second experiment, a stable nickel isotope, ^{61}Ni, was given in drinking water to 20 nickel-sensitized women and 20 age-matched controls, both groups having vesicular hand eczema of the pompholyx type. Nine of 20 nickel allergic eczema patients experienced aggravation of hand eczema after nickel administration, and three also developed a maculopapular exanthema. No exacerbation was seen in the control group. The course of nickel absorption and excretion in the allergic groups did not differ and was similar to the pattern seen in the first study, although the absorption in the women was less. A sex-related difference in gastric emptying rates may play a role. Thus, food intake and gastric emptying are of substantial significance for the bioavailability of nickel from aqueous solutions.

Reliable data on nickel absorption and retention in humans are few and for the most part suggest an intestinal absorption of nickel contained in food of about 1% (Nielsen *et al.*, 1987; Grandjean *et al.*, 1988). Sunderman *et al.* (1989) studied eight healthy human volunteers who ingested nickel sulfate added to drinking water or food; absorbed nickel averaged 27 ± 17% (mean ± SD) of the dose ingested in water vs 0.7 ± 0.4% of the dose ingested in food, i.e., a 40-fold difference. The water was ingested after a 12-h fast and was then followed by an additional 3-h fasting period. Thus, food constituents, possibly phosphate, phytate, fibers, and similar metal-ion-binding components, may bind nickel and make it much less available for absorption than nickel dissolved in water and ingested on an empty stomach (Solomons *et al.*, 1982; Sunderman *et al.*, 1989).

In this regard, the gastric emptying rate and peristalsis of the intestine will be of importance. These patterns have been studied with radioactive tracers (Oester-Joergensen *et al.*, 1990). Following ingestion of water, the stomach almost immediately starts to release it to the duodenum; about one-half of a 150-ml water dose will be passed on within about 30 min, with the second half leaving the stomach somewhat more slowly. The presence of recently ingested food in the stomach will barely affect this pattern of gastric emptying, provided that the food has not yet become fully liquified. However, whether or not recently ingested food, not yet liquified, may liberate sufficient amounts of components that can prevent nickel absorption is unclear. On the other hand, it is likely that liquified food that has already remained, say, 30 min in the stomach, would more readily mix with subsequently ingested drinking water before passage into the intestine. In the case of a 1400-kJ standard breakfast of scrambled eggs, the lag-time before the beginning of gastric emptying is usually about 30 min, with about one-half being emptied within 90 min, and the rest being passed on within 4 h. Only if drinking water contains energy, e.g., due to a high sugar content, will the emptying pattern for the water approach the one seen with solid food. The gastric emptying varies with sex, as gastric emptying starts sooner in males compared to females.

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Previous studies have suggested differences in nickel kinetics between nickel-sensitized individuals and nonsensitized individuals (Nielsen *et al.*, 1987, 1990; Bonde *et al.*, 1990; Grandjean *et al.*, 1992). For example, one study (Bonde *et al.*, 1990) showed lower nickel concentrations in intercellular fluid obtained from the skin of nickel-allergic subjects as compared to controls. This difference could be due to adhesion of nickel to cells or serum proteins in the allergic patients, a possibility suggested by results showing that T-lymphocytes from nickel-sensitized patients may bind considerable amounts of nickel (from nickel subsulfide) to the cell membrane, while nickel binding was only observed with very few cells from nonsensitized persons (Hildebrand *et al.*, 1987). Such nickel binding could potentially affect the toxicokinetic patterns. Thus, in an experimental study, Chevin *et al.* (1988) found that urinary elimination of ^{63}Ni was significantly diminished in nickel-sensitized guinea pigs as compared to controls, and significantly more nickel was retained in the dermis of the sensitized animals than in controls.

In the human studies mentioned above, total nickel was measured. As the administered nickel was not labeled, the nickel kinetics derived from the data will be sensitive to interferences, e.g., due to variations in nickel intake or differences in enterohepatic circulation. This obstacle can be overcome by administering specific nickel isotopes. Radioactive nickel may not be suitable for studies in human volunteers, but a stable nickel isotope can be used, and determination of nickel isotopic ratios in body fluids may then be performed by inductively coupled plasma mass spectrometry (ICP-MS) (Templeton *et al.*, 1994).

The first aim of the present study was to examine the influence of fasting and food intake on the absorption of nickel added to drinking water. A second aim was to examine possible differences in nickel absorption between nickel-allergic patients with vesicular hand eczema and a control group of patients with a similar type of eczema without nickel allergy.

MATERIALS AND METHODS

Identification and Selection of Volunteers

Healthy men without known nickel sensitization were recruited for Study 1 on the effects of food intake on nickel absorption. The eight volunteers were nonsmokers who did not use any medication or alcohol at least 2 days prior to or during the study period. During the first nickel-administration schedule, one volunteer developed migraine and had to be replaced. The new subject then started with schedule 2 and went through the rest of the schedules with the group, after which schedule 1 was carried out. The age ranged from 21 to 30 years (median, 27 years), and the body weight varied between 66 and 81 kg (median, 75 kg). The volunteers were tested for nickel sensitization after termination of the study using patch tests with nickel sulfate 5% in petrolatum (Hermal, Reinbek, Germany) applied for 2 days in Finn Chambers (Epitest Ltd., Helsinki, Finland) on Scanpor (Norgesplaster A/S, Oslo, Norway) and read on the third day according to ICDRG recommendations (Wahlberg, 1995). No reactions were recorded in any of the volunteers.

In Study 2 on the effects of nickel sensitization, we examined two age-matched (±5 years) groups, each consisting of 20 women with current vesicular hand eczema (pompholyx type). They had no history or clinical signs of atopic dermatitis. The nickel-sensitized subjects had a history of dermatitis from skin contact with white metal objects and a positive patch test to 5% nickel sulfate in petrolatum within the last 2 years. The controls had no history of dermatitis from skin contact with white metal objects and had a negative patch test to nickel. The nickel-sensitized group varied in age from 20 to 65 years (median, 39.5 years), while controls were 19–69 years (median, 36.5 years). Body weights were similar in the two groups and varied between 50 and 84 kg (median, 67 kg). If a previous patch testing had not been performed within the last 2 years, retesting for confirmation was performed after termination of the experimental study as described above. Reactions graded + to +++ were considered nickel-sensitized.

This investigation was carried out in accordance with the Helsinki Convention and was approved by the regional ethical review committee.

TABLE 1

Six Nickel Intake Schedules Used for Male Volunteers to Examine Absorption and Retention Patterns in Relation to Food Intake, and Schedule 7 Used for Comparison of Nickel-Allergic Women with Control Subjects

Schedule number	Meal	Time interval	Nickel intake	Time interval	Meal	Samples: Serum	Samples: Urine
1	+	4 h	Water				+
2	+	1.5 h	Water				+
3			Water	0 h	+	+	+
4			Water	0.5 h	+	+	+
5			Water	1 h	+	+	+
6			Meal				+
7			Water	4 h	+	+	+

Note. The nickel dose was 12 μg/kg body wt, and the meal was a 1400-kJ portion of scrambled eggs.

Experimental Procedures

Study 1. Two days before and during each schedule, all volunteers avoided the following food items naturally high in nickel content: cocoa, soya beans and other dried legumes, nuts, oat meal, and sweets. Further, water from the tap was allowed to run for 1 min before consumption (Andersen *et al.*, 1983). Before each nickel intake, the volunteers fasted for 12 h (overnight). The nickel dose from the prepared drinking water was 12 μg/kg body wt; with a nickel concentration of 3 μg/ml, the required water intake of each individual was 4 ml/kg body wt.

For the eight male volunteers, the food intake consisted of a standardized 1400-kJ portion of scrambled eggs prepared in a microwave oven for 2.5 min from two eggs, 50 ml water, 2.5 g wheat flour, and 15 g butter. Six different time intervals between the nickel-supplemented water intake and the intake of food were chosen (Table 1). The nickel in either water or scrambled eggs was taken on a Tuesday morning at 9 a.m. In each schedule, 12-h urine samples were collected from Monday at 9 p.m. until nickel intake at 9 a.m. the next morning. The following four urine samples were at 3-h intervals and were followed by five 12-h urine samples, the last one completed at 9 a.m. Friday morning. Blood samples were taken (schedules 3, 4, and 5) 24 h before nickel ingestion and at times 0, 1, 3, 8, 24, 48, and 72 h afterward. The samples were collected under proper precautions against nickel contamination as previously described (Grandjean *et al.*, 1992; Nielsen *et al.*, 1990).

Study 2. Prior to the nickel ingestion and daily during the study, all patients were examined clinically by the same dermatologist who was blinded regarding nickel allergy. The status of hand eczema was evaluated according to the following clinical parameters: vesicles, erythema, scaling, and fissures; the number of vesicles was counted on the lateral surfaces of the fingers and on the palm of the hand with the highest number of vesicles. The grading

system used the following categories: slight (0–30 vesicles), moderate (31–70 vesicles), or severe (more than 70 vesicles). During the study period, none of the patients took systemic drugs that might influence the eczema. The patients were advised to continue using their skin care product and to increase topical steroid treatment if a flare-up of eczema was noted.

To study nickel kinetics in these patients, we used the stable isotope ^{61}Ni (Oak Ridge National Laboratory, Nashville, TN). The isotope was dissolved and diluted to a concentration of 500 μg/ml and then further diluted to the final concentration of 3 μg/ml just before use. This concentration was verified by atomic absorption measurements. According to schedule 7 (Table 1), the nickel solution (12 μg/kg body wt) was ingested on a Tuesday morning at 9 a.m., and fasting was then maintained for another 4 h. A baseline 12-h urine sample was collected from 9 p.m. on Monday evening. Subsequent to nickel ingestion, four 3-h urine samples, followed by five 12-h urine samples, were collected, the last one being finished by Friday morning at 9 a.m. Blood samples were taken 24 h before, and 0, 1, 2, 5, 8, 24, 48, and 72 h after nickel ingestion. Blood specimens for lymphocyte isolation were obtained twice from each volunteer, right before nickel intake and 5 h later. Intercellular fluid was collected during 1.5 h before administration of nickel and was initiated again 4 h after nickel intake.

Collection of blood and urine samples was carried out as previously described (Grandjean *et al.*, 1992; Nielsen *et al.*, 1990). Intercellular fluid was collected by the suction-blister technique (Kiistela, 1968; Bonde *et al.*, 1990). To isolate mononuclear cells, we added a 10% dextran solution 1:10 to heparinized venous blood (30 ml), and the erythrocytes were allowed to sediment at room temperature in four 10-ml vials. The cell-rich plasma was transferred to two 10-ml vials and centrifuged at 2500 cpm for 7 min. The supernatant was discarded. Hemolysis was induced by adding 2 ml of distilled water under mild agitation, followed after 30 s by 2 ml of 1.8% NaCl. After centrifugation at 2200 cpm for 7 min, the supernatant was discarded, and the cells were resuspended in 5.0 ml 0.9% NaCl. The cell count was assessed on a Sysmex hematology analyzer NE-8000 (Toa Medical Electronics, Kobe, Japan). For nickel analysis, a pellet was produced by centrifugation at 3000 cpm for 10 min.

Analytical Procedures

Urine samples were pretreated and analyzed by the method of Sunderman *et al.* (1986), serum samples as described by Andersen *et al.* (1986) and Grandjean *et al.* (1992). We used a Perkin-Elmer model 4100 ZL atomic absorption spectrometer with transverse-heated graphite atomizer, longitudinal Zeeman-effect background correction, and an AS-70 autosampler (Perkin Elmer, Norwalk, CT). Accuracy was ensured by using Seronorm Trace Elements Urine Batch 009024 (NycoMed, Oslo, Norway) as quality control material; we found an average nickel concentration of 41.2 μg/l ($n = 42$), compared to the assigned value of 39.92 μg/l. Accuracy of the serum analyses was ensured by using Seronorm Trace Elements in Serum batch 010017 (NycoMed) as quality control material; we found an average nickel concentration of 3.71 μg/l ($n = 25$), compared to an assigned value 3.581 μg/l.

Mononuclear cell pellets and control solutions were dried at 100°C for 3–4 h and then digested overnight with 200 μl of concentrated HNO_3 at room temperature before adding 800 μl of modifier solution (2% Triton X-100). After 0.5–1 h the samples were centrifuged and the supernatant was transferred to a sampling cup for atomic absorption spectrometry. The electrothermal atomic absorption spectroscopy procedure was similar to the one used for serum samples (Andersen *et al.*, 1986; Grandjean *et al.*, 1992). The mass of nickel in each pellet was calculated and then divided by the number of cells in the pellet to determine the nickel concentration per cell. Nickel in intercellular fluid was analyzed by the method described by Sunderman *et al.* (1984) for serum. The accuracy was ensured by again using Seronorm Trace Element batch 010017 (Nycomed) as quality control material; in these analytical series, we found an average nickel concentration of 3.50 μg/l ($n = 9$), compared to an assigned value of 3.581 μg/l.

Urine creatinine was determined by the conventional two-point kinetic Jaffé-reaction on a RA 1000, random-access analyser (Technicon, Tarryton, NY).

Nickel isotope measurements were made with an Elan 250 ICP-MS (Perkin Elmer-Sciex, Thornhill, Ontario, Canada) as previously described (Xu *et al.*, 1993; Templeton *et al.*, 1994). For measurement of ^{61}Ni in urine, calcium was precipitated with oxalic acid (Xu *et al.*, 1993). Calibration standards were prepared from a stock solution of isotopically enriched ^{61}Ni (found, 87%) and checked against standards prepared from a 1000 μg/l standard nickel solution assuming a natural isotopic abundance of ^{61}Ni of 1.25%. A similar method was used for serum samples. Serum (1.0 ml) was mixed with 1.0 ml H_2O, made to 15 mM in oxalic acid, and let stand at 4°C overnight, and, after centrifugation 1.0 ml of supernatant was transferred to a second tube for protein precipitation. While vortex mixing for 60 s, 50 μl of concentrated HNO_3 (Suprapur; Merck, Darmstadt, Germany) was added and the mixture incubated at 70°C for 5 min. After centrifugation, rhodium was added to 0.60 ml of supernatant to a final concentration of 100 μg/l in a final volume of 3.00 ml of 0.1% HNO_3. However, the oxalate precipitation method gave blank values of 0.7 μg/l, which is above the expected baseline value for total nickel in serum. Therefore, when a serum sample was found to have a ^{61}Ni concentration below 5 μg/l, the concentration was calculated by principal components analysis to correct for polyatomic interferences arising from sodium, potassium, and residual calcium, as previously reported (Vaughan and Templeton, 1990; Templeton *et al.*, 1994). Measurements were made at m/z = 58, 60, 61, and 62, and aqueous solutions of nickel, calcium, and sodium plus potassium were used to construct the target vectors. Within-run and between-run imprecision with serum spiked with nickel to 1.5 μg/l were 6% and 1%, respectively, by this method.

Some samples of serum and urine from Study 2 were also analyzed for total nickel by graphite furnace atomic absorption spectrometry as described previously (Templeton *et al.*, 1994). Assuming ^{61}Ni to account for 87% of the total nickel, agreement between atomic absorption and ICP-MS results was always within 5%. Seronorm Trace Elements serum (Nycomed; recommended Ni content, 5.0 $\pm$ 0.5 μg/l) was analyzed daily for quality control.

Statistical Analysis

Medians and ranges are given for all nickel concentrations. Since the distributions differed from Gaussian and could not be normalized with simple transformations, the nonparametric Wilcoxon signed ranks test was used to test possible differences in the paired samples from the eight males, and the Mann-Whitney U-test to test possible differences between the two matched groups of female volunteers. One-tailed p values are given.

Pharmacokinetic Calculations

Due to biological variability of the data, a short observation period may not allow characterization of more than one compartment. As a one-compartment model fits the data well, and since addition of more compartments would result in only limited changes, the former was used for calculations. The terminal rate constant (k_e) was calculated by log-linear regression on the last two to three concentrations, and the biological half life ($t_{1/2}$) was then calculated as $\ln 2/k_e$. The area under the curve from the time of ingestion to the last measured serum concentration ($AUC_{0\text{-}72}$) was calculated by the linear trapezoidal method.

The urinary excretion data were treated by the method Wagner (1980) developed for estimation of systemic availability of drugs. Using the urinary nickel excretion measured in six 12-h periods following ingestion and assuming a one-compartmental model with first-order transfer, linear regression was performed on the excretion at the time t, $X(t)$, vs $X(t + \Delta t) - X(t)$, and the intercept was recorded as X_∞, the total amount excreted. To estimate $t_{1/2}$, the following equation was used:

$$X_\infty - X(t) = X_\infty \cdot e^{-k_e t}.$$

The amount remaining to be excreted (ARE) can then be calculated as

$$\ln(\text{ARE}) = -k_e \cdot t + \ln(X_\infty).$$

A graph of ln(ARE) vs t allows the calculation of k_e from the slope of the regression line and thus of $t_{1/2}$. The renal clearance of Ni (Cl_{Ni}) was calculated as

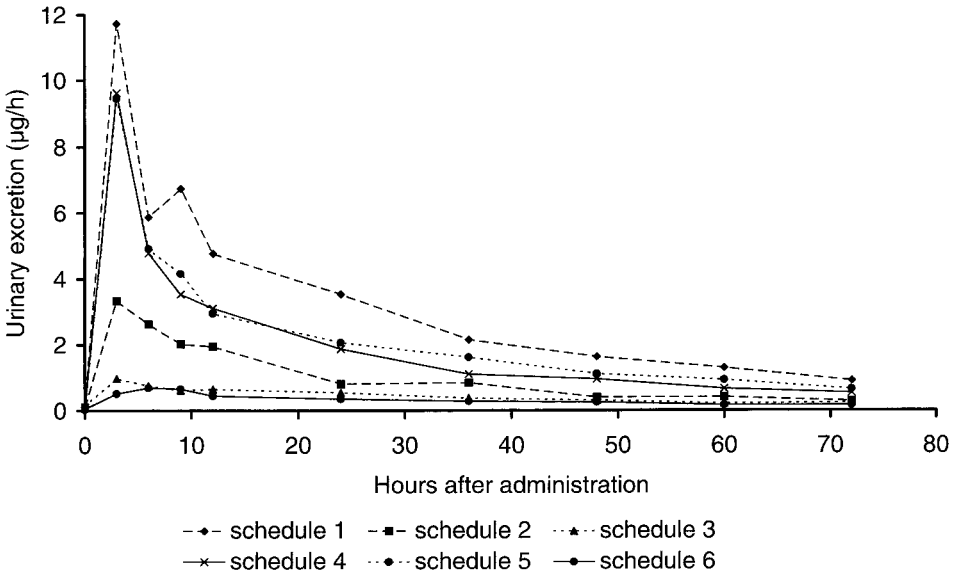

FIG. 1. Median urinary nickel excretion (μg/h) of eight subjects at specified intervals after ingestion of 12 μg nickel per kg body wt according to the schedules described in Table 1.

$$Cl_{Ni} = \frac{X_{0\text{-}72}}{AUC_{0\text{-}72}}.$$

The creatinine clearance Cl_{crea} was calculated from the total amount excreted during 84 h (12 h before nickel intake and the 72 h after) and assuming that the serum creatinine concentration was contant.

RESULTS

Influence of Meals on Nickel Absorption

During the predose periods of Study 1, the total range of baseline nickel levels in the 12-h urine samples was 0.07–3.48 μg Ni/g creatinine, with medians varying between 0.36 (schedule 5) and 2.09 (schedule 3). Baseline nickel concentrations in serum ranged from 0.07 μg/l to 1.45 μg/l, with medians varying between 0.26 μg/l and 0.62 μg/l. These nickel concentrations are all within the reference values for nonexposed healthy volunteers previously reported by our laboratory (Grandjean *et al.,* 1992), and the serum results are below the limit of 20 nmol Ni/l (1.17 μg/l) proposed by IUPAC for identification of samples with external contamination (Templeton, 1994).

The median urinary nickel excretion rates in micrograms per hour after the various schedules of nickel ingestion (Table 1) are shown in Fig. 1. When nickel was ingested in water 4 and 1.5 h after intake of eggs (schedules 1 and 2), the peak nickel excretion rate in urine was 12.2 times and 3.5 times, respectively, above the corresponding value when nickel in water and eggs were taken simultaneously. Likewise, when the nickel-containing water was taken 0.5 and 1 h prior to the eggs (schedules 4 and 5), the peak nickel excretion levels were 10.0 times and 9.8 times, respectively, above the corresponding values when they were taken together. When the nickel-containing water was taken together with the eggs (schedule 3), the urinary nickel excretion was significantly higher during the interval from 12 to 60 h after intake compared to when the nickel was mixed into the eggs during the meal preparation (schedule 6), but the difference corresponded to a factor of only 1.3–1.5.

The cumulative urinary nickel elimination during the first 72 h after nickel ingestion, expressed in percentage of the administered dose, is shown in Fig. 2. When the eggs were taken 4 h prior to nickel in drinking water (schedule 1), a total of 23.2% of the administered dose appeared in the urine collected, whereas only 7.1% was found if the eggs were taken 1.5 h prior to the nickel intake (schedule 2). When the eggs were taken 0.5 or 1 h after the nickel in water (schedules 4 and 5), 12.8% and 16.7%, respectively, were detected in the urine, and a significant difference between the two was observed from 9 h after the nickel intake. When water and eggs were taken together or if they were mixed together, cumulative amounts of 3.4% and 2.3%, respectively, were detected in the urine. The two latter schedules differed significantly from 12 h after the administration.

Serum nickel concentrations show a peak 1 h after administration (Fig. 3) when nickel was ingested in water 0.5 and 1 h prior to the intake of eggs (schedules 4 and 5), and in both cases it corresponded to 12.8 times the value at the same time when nickel in water and eggs were taken simultaneously (schedule 3), where a delayed peak was observed 3 h after the administration.

Table 2 shows the nickel clearance (Cl_{Ni}) calculated from $Ni_{0\text{-}72}$ and $AUC_{0\text{-}72}$ for the three schedules where nickel was measured in serum. The nickel clearances showed some variation but did not differ between the schedules. However, the

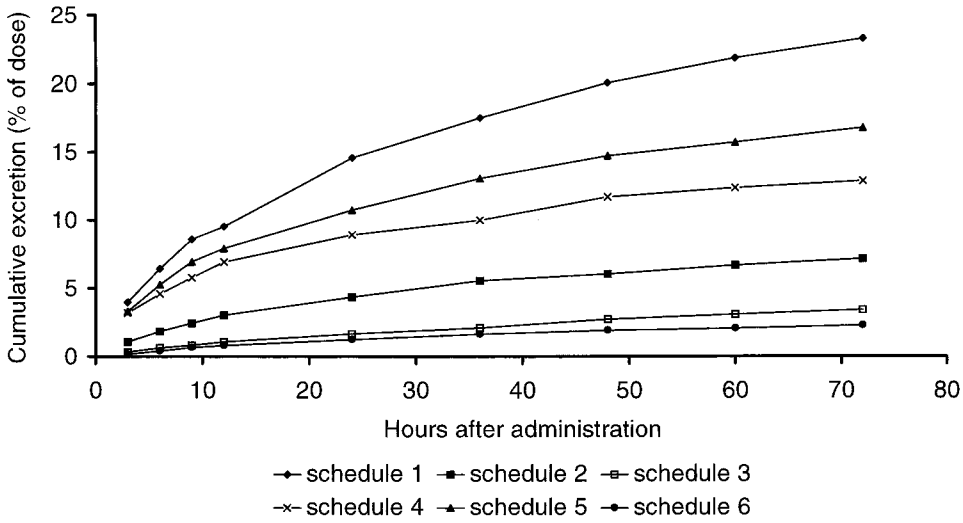

FIG. 2. Median cumulative nickel excretion (% of given dose) in urine of the same eight subjects as in Fig. 1.

creatinine clearance varied much less (Table 3). The half-times for the urinary nickel excretion based upon the ARE calculations are shown in Table 3. The range of median half-times of the six schedules was 19.92–26.65 h, with a range of individual means of 21.00–35.78 h. When the eggs were taken 4 h prior to nickel in drinking water (schedule 1), a cumulative median amount of 25.81% (25.00 ± 11.02) of the administered dose was excreted, while 2.51% (2.95 ± 1.32) was excreted when the nickel was mixed into the eggs (schedule 6).

In the second study, 17 controls had a slight hand eczema, three had a moderate eczema, and all remained unchanged during the study period. In the nickel-sensitized group, 9 showed a flare-up of symptoms after nickel intake, and they also reported increased use of topical steroids. Two of these subjects developed a maculopapular rash, and a baboon syndrome (Andersen *et al.*, 1984) was observed in another. All excacerbations started within 12 h after nickel administration. The patient with the baboon syndrome was treated with systemic prednisolone. The clinical symptoms were unrelated to the magnitude of nickel concentrations found in serum and urine samples.

The first serum sample from the female volunteers and the first 24-h urine sample before nickel administration showed nickel concentrations very similar to the first study. The serum analyses (Table 4) then showed a steep increase during the first couple of hours after nickel ingestion, followed by a decline. By 72 h, the nickel concentrations had not yet returned to the level before nickel ingestion. Considerable variation occurred,

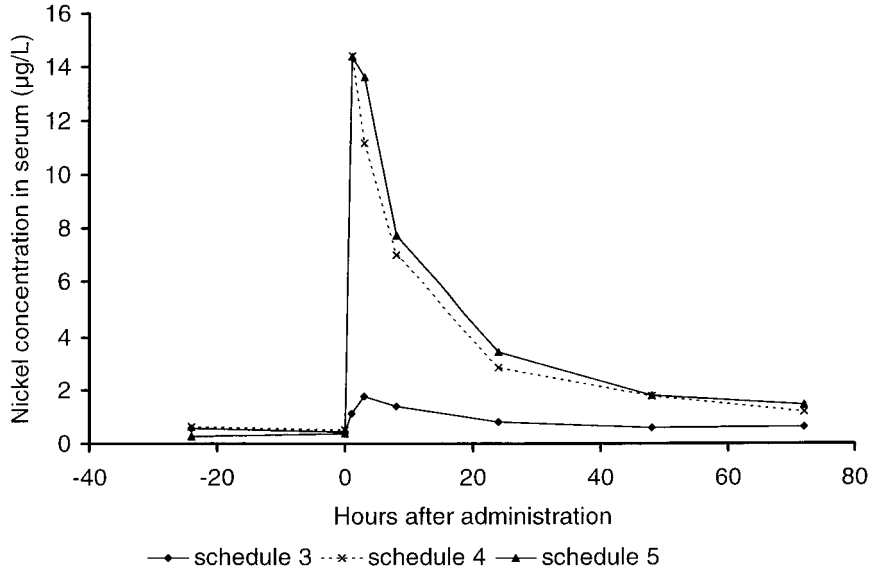

FIG. 3. Median nickel concentration in serum (μg/l) of the same eight subjects as in Figs. 1 and 2.

TABLE 2

Median Nickel Clearance (Cl_{Ni}) Calculated as the Ratio between the Nickel Excretion during the 72 h Following Oral Administration ($X_{0\text{-}72}$) and the Area under the Serum Curve during the same Time Period ($AUC_{0\text{-}72}$)

Schedule no.	$AUC_{0\text{-}72}$ (μg/l*h)	$X_{0\text{-}72}$ (μg)	Cl_{Ni} (ml/min)
3	57.58 (31.63–73.21)	28.27 (13.65–39.38)	8.15 (6.20–11.55)
4	249.4 (181.1–371.5)	118.2 (91.41–183.6)	8.40 (5.52–9.60)
5	275.0 (220.5–463.3)	134.5 (104.5–223.5)	8.15 (7.69–9.24)

Note. The median nickel dose was 893.4 μg (range, 795.6–969.6 μg). Total range shown in parentheses.

but overall the two groups showed essentially the same time course, and there was no significant difference at any time. The urinary elimination (Table 5, Fig. 4) confirmed the tendencies seen in the serum concentrations. The highest nickel excretion was seen during the first 3 h after nickel ingestion, and the elimination remained increased 72 h later. Again, much interindividual variation occurred, but the two groups did not significantly differ at any time.

Following nickel ingestion, intercellular nickel concentrations increased by a factor of about eight in both groups (Table 6). Again, much interindividual variation occurred. In this case, some of the variation can be ascribed to the fact that the intercellular fluid must be collected over a certain time period, and the time needed to obtain the required sampling volume will necessarily vary. Two individuals showed a slightly lower nickel concentration at the second sampling, but otherwise clear increases were found. The nickel concentration in the cell pellets (Table 6) did not seem to reflect the changes revealed by the other parameters.

DISCUSSION

This study replicates and extends the results previously obtained by Sunderman *et al.* (1989) who administered nickel in drinking water and nickel mixed into a meal. The present study demonstrates considerable differences between cumulative nickel excretion in urine following different nickel intake schedules. The highest amount excreted (25.8% of the administered dose) was observed when the scrambled eggs were taken 4 h prior to nickel in drinking water (schedule 1), and the lowest (2.5%) was observed when the nickel was mixed into the eggs (schedule 6), i.e., a 10-fold difference (Fig. 1). Other intake schedules resulted in cumulative excretions between those two extremes.

Peak nickel concentrations in serum were observed 1 h after administration of nickel 0.5 or 1 h prior to eggs (schedules 4 and 5), and the levels were about 13 times the value when nickel in water and eggs were taken simultaneously (schedule 3) (Fig. 2). The peak value for the latter was delayed and occurred at 3 h after administration. The range of median nickel half-times of the six schedules in the present study was 19.9–26.7 h (Table 3), i.e., similar to the 28 $\pm$ 9 h observed by Sunderman *et al.* (1989).

When interpreting the differences between the nickel intake schedules (Figs. 1–3), the known patterns of peristalsis should be taken into account (Oester-Joergensen *et al.*, 1990). The highest cumulative nickel excretion is found in the series in which nickel is ingested during fasting (schedules 4 and 5) and 4 h before meal ingestion (schedule 1). In the fasting state, gastric emptying of a noncaloric liquid depends upon the phase activity of the migrating motor complex at the time of ingestion. The median duration of the entire migrating motor complex cycle is about 90 min. The phase activity at the time of nickel ingestion of each subject is unknown and could be a major reason for the substantial variation in the results. For more precise results, future studies on nickel absorption in the fasting state should schedule nickel ingestion in relation to preselected phases of the migrating motor complex.

The difference between the results from schedule 1 (ingestion of nickel in water 4 h after eating eggs) and schedule 2 (ingestion of nickel in water 1.5 h after eating eggs) is more difficult to explain. The mechanism might be related to the change of motility and transit elicited by meal ingestion. Thus, ingestion of a meal induces increased gastrointestinal motility within a few minutes (Oester-Joergensen *et al.*, 1990). It is thus possible that ingestion of a meal 1.5 h prior to ingestion of nickel is followed by a faster transit of nickel present in the

TABLE 3

Median Creatinine Clearance (Cl_{Crea}), Nickel Half-life ($t_{1/2}$), and Calculated Amount of Total Nickel Excretion (X_∞) in μg and Relative to the Dose, as Compared to the Amount Excreted within 72 h ($X_{0\text{-}72}$)

Schedule no.	Cl_{Crea} (ml/min)	$t_{1/2}$ (h)	X_∞ (μg)	X_∞ (% of dose)	$X_{0\text{-}72}$ (% of dose)
1	94.62 (75.39–117.0)	22.35 (9.45–29.83)	226.0 (100.6–326.2)	25.81 (11.07–37.42)	23.18 (9.24–33.68)
2	93.93 (81.42–102.9)	19.92 (15.64–28.60)	74.54 (34.42–148.7)	8.82 (3.79–16.93)	7.12 (3.54–14.95)
3	94.55 (87.33–134.9)	25.60 (21.45–71.96)	40.85 (25.73–45.26)	4.31 (2.83–5.27)	3.39 (1.50–4.60)
4	89.34 (65.57–112.9)	21.81 (12.40–28.89)	126.7 (86.73–190.6)	14.43 (9.52–21.70)	12.83 (10.96–20.90)
5	95.23 (88.25–104.8)	23.73 (16.64–27.47)	154.0 (115.1–259.1)	19.14 (12.68–29.50)	16.72 (11.50–25.44)
6	91.84 (79.39–107.7)	26.65 (12.81–46.05)	21.90 (11.28–47.87)	2.51 (1.24–5.26)	2.26 (1.03–4.71)

Note. Total range shown in parentheses.

TABLE 4

Median Serum Nickel Concentrations (μg/l) in 20 Nickel-Allergic Patients and 20 Controls Matched by Sex and Age in Relation to the Time of Nickel Ingestion

Time (h)	Nickel-sensitized	Control
−24	0.19 (0.05–0.32)	0.17 (0.06–0.55)
0	0.22 (0.10–0.67)	0.30 (0.10–0.60)
1	11.12 (0.53–21.95)	9.16 (0.35–26.16)
3	14.93 (2.14–31.83)	15.11 (6.52–30.17)
5	11.16 (1.60–26.14)	12.32 (4.36–23.16)
8	7.72 (1.14–20.91)	9.45 (3.24–16.61)
24	3.31 (0.54–7.80)	3.71 (1.26–6.32)
48	2.42 (0.47–3.82)	2.23 (0.55–3.61)
72	1.31 (0.52–3.05)	1.34 (0.33–3.18)

Note. Total range shown in parentheses.

small intestine and therefore a lower absorption in schedule 2 compared to schedule 1.

The low cumulative nickel excretion in schedules 3 and 6 is most likely related to the lasting contact between the solid component of the meal and nickel, perhaps involving binding of nickel to proteins or other components of the meal. Still, the cumulative excretion observed is higher than the 0.7% reported by Sunderman *et al.* (1989) for nickel mixed into a breakfast meal. The lower excretion in schedule 4 (nickel in water 30 min before the eggs) than in schedule 5 (nickel 1 h before the eggs) may be partially due to differences in gastric emptying before meal ingestion. Further, proteolytic breakdown of meal components in the stomach may decrease the nickel-binding potential. Nickel binding to food components may also be pH-dependent and thereby depend on the time interval between food ingestion and ingestion of nickel. Thus, when nickel is ingested 1 h prior to meal ingestion (schedule 5), intragastric pH at the time of meal intake would be lower than when nickel is ingested 30 min prior to the meal (schedule 4).

The study of nickel-sensitized women was carried out because of several indications that nickel-allergic patients may eliminate nickel less efficiently (Nielsen *et al.,* 1987, 1990; Bonde *et al.,* 1990; Grandjean *et al.,* 1992), perhaps due to cellular binding of nickel (Hildebrand *et al.,* 1987). However, the differences seen in cross-sectional data could be related to a lower nickel intake among nickel-allergic subjects. Under the controlled circumstances of the present study, where nickel ingestion (schedule 7 in Table 1) was designed to obtain a maximal absorption, considerable interindividual variation was shown. As seen in Study 1, absorption mainly occurred within the first couple of hours, and elimination was quite rapid during the first day or so, with nickel concentrations approaching baseline levels after 72 h. However, at no point of time did any of the nickel concentrations suggest any difference in nickel toxicokinetics between nickel-allergic women and the age-matched controls (Tables 4 and 5). If any difference occurred, it is likely to have been minimal and much less than the differences seen in the nickel intake schedules in first study. The fact that the results for the two groups are so similar would therefore argue against more than a slight difference in average toxicokinetics, although small subgroups with greater differences obviously could be hidden within the groups examined. Still, nickel-allergic patients experiencing eczema aggravation did not differ in nickel concentrations from those without such reactions. These cutaneous reactions are in accordance with previous findings that peroral nickel provocation may exacerbate eczema in some nickel-allergic patients with hand eczema of the pompholyx type (Kaaber *et al.,* 1978; Veien *et al.,* 1985; Gawkrodger *et al.,* 1988).

The difference between comparable administration schedules in the two studies deserves additional comment. In schedule 5 of the first study, the urinary nickel excretion of the male volunteers during the first 3 h after nickel ingestion was about twice as high as in the women who ingested the stable nickel isotope according to the similar schedule 7 in the second study,

TABLE 5

Median Urinary Nickel Excretion per Hour (μg/h) and Cumulative Excretion (% of Dose Given) in 20 Nickel-Allergic Patients and 20 Controls Matched by Sex and Age in Relation to the Time of Nickel Ingestion

	Nickel-sensitized		Control	
Time (h)	Hourly	Cumulated	Hourly	Cumulated
−24–0	0.04 (0.01–0.14)	–	0.05 (0.01–0.09)	–
0–3	5.47 (1.12–12.48)	2.05 (0.19–4.91)	4.98 (1.12–12.48)	2.01 (0.64–4.58)
3–6	3.23 (0.80–11.00)	3.60 (0.49–9.17)	3.11 (0.93–7.53)	3.26 (1.19–7.25)
6–9	3.05 (0.69–8.03)	4.86 (0.49–11.16)	2.89 (0.57–6.88)	4.17 (1.42–9.82)
9–12	1.90 (0.47–6.17)	5.53 (0.74–13.54)	2.52 (0.62–6.37)	5.56 (1.68–12.20)
12–24	1.16 (0.33–3.67)	7.25 (1.24–19.23)	1.26 (0.34–2.82)	7.46 (2.24–16.40)
24–36	0.73 (0.17–3.44)	8.43 (1.48–24.57)	0.88 (0.31–2.23)	8.64 (2.75–19.73)
36–48	0.69 (0.10–1.58)	9.33 (1.64–26.19)	0.60 (0.08–1.49)	9.61 (3.27–21.96)
48–60	0.51 (0.06–1.19)	10.11 (1.73–28.04)	0.53 (0.21–1.29)	10.43 (3.61–23.89)
60–72	0.41 (0.04–0.92)	10.82 (1.79–29.46)	0.42 (0.20–0.84)	11.26 (4.03–25.14)

Note. Total range shown in parentheses.

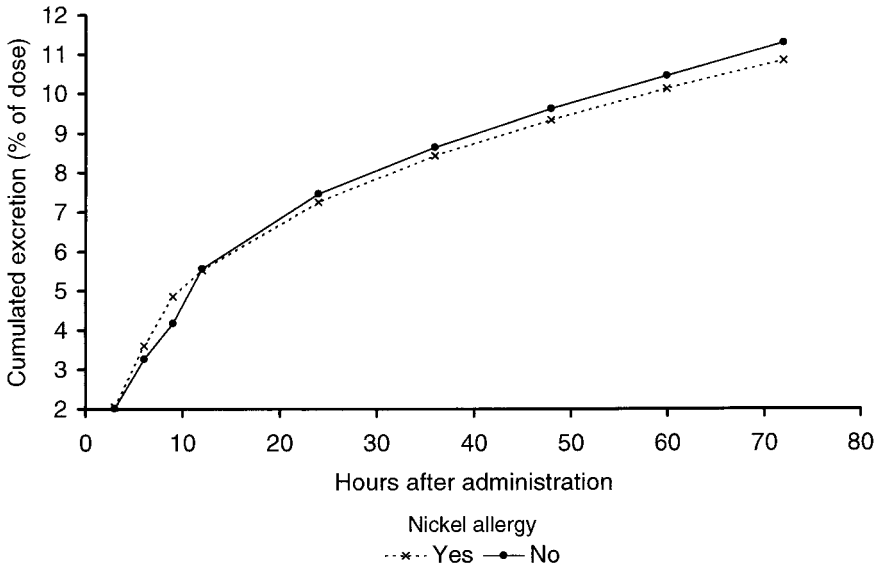

FIG. 4. Median cumulative urinary nickel excretion expressed in relation to the amount of nickel ingested by 20 fasting nickel-allergic patients and 20 controls matched by sex and age in relation to the time of nickel ingestion (schedule 7 in Table 1).

where the interval before meal intake was much longer. Also, the total amount excreted in the urine was less than 12% in the women, again much lower than in the eight men who participated in the first study. As nickel doses were given in relation to body weight, a gender difference could be postulated and may be related to gastric emptying patterns. Thus, men tend to show faster gastric emptying than women; in one study, men had a 40% faster emptying rate than women (Oester-Joergensen *et al.*, 1991). If nickel remains in the stomach for a longer period, the likelihood that food materials subsequently ingested can bind the nickel and prevent absorption increases. A slower gastric emptying rate in the women would therefore result in a decreased, and perhaps also slightly more protracted, nickel uptake.

The magnitude of the sex-related difference also provides a level of comparison. Thus, any slight toxicokinetic difference attributable to nickel allergy would be small in comparison with the one related to the sex difference. Further, differences in peristaltic phases are likely to result in substantial variation, also at an individual level, thus perhaps explaining some of the interindividual variation seen in each schedule.

The above considerations have been based on urine and serum data. The intercellular fluid may be a useful additional parameter (Bonde *et al.*, 1990). The increase in nickel concentrations during the first few hours after nickel ingestion appears not to be as steep as in serum, although the data obtained show an eight-fold increase in samples collected a couple of hours after the nickel ingestion. As the collection of intercellular fluid requires a procedure that may take a longer time in some subjects, the collection time is not as well defined as with other parameters. Nonetheless, the data show that the increases in nickel concentrations in serum may be mirrored in the intercellular compartment.

Much less clear are the data on nickel in mononuclear cell pellets from venous blood samples. First of all, the results show no increase in nickel concentrations after nickel ingestion. Second, the laborious preparation procedure may affect the nickel concentrations in the final pellet analyzed. Third, in nickel-allergic patients, nickel absorption could affect numbers of circulating immune-responsive cells. The relative importance of these factors is unclear at present. Given the results obtained, the nickel concentration in mononuclear cells does not warrant further exploration at the present time.

TABLE 6
Median Nickel Concentrations in Intercellular Fluid (in μg/l) and Mononuclear Cells (in pg/10^6 Cells) from 20 Nickel-Allergic Patients and 20 Age-Matched Controls before and after Nickel Ingestion

	Nickel-sensitized		Control	
Time	Intercellular fluid	Mononuclear cells	Intercellular fluid	Mononuclear cells
Before	0.73 (0.26–1.30)	37.2 (5.55–56.7)	0.56 (0.27–2.93)	34.5 (14.1–76.6)
After	4.00 (1.01–12.2)	27.5 (3.75–61.6)	4.55 (1.66–8.06)	29.3 (7.17–74.0)

Note. Total range in parentheses.

The importance of the substantially different nickel absorption levels demonstrated in this study is stressed by the development of eczema exacerbations in almost half of the nickel-allergic patients who, on an empty stomach, had ingested an increased, yet not unrealistic, amount of nickel in water. No change in nickel toxicokinetics was found in relation to nickel allergy, and future experimental studies should therefore exclude such subjects from increased nickel exposure. Further, the sensitivity to nickel would emphasize the need to control nickel contamination of drinking water, and nickel-allergic subjects should be aware of the increased absorption when drinking water on an empty stomach.

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