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Atomic Absorption Spectrometry of Nickel, Copper, Zinc, and Lead in Sweat Collected from Healthy Subjects during Sauna Bathing[1,2]

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Ni, Cu, Zn, and Pb were measured by atomic absorption spectrometry in sweat samples obtained by the arm-bag technique from 48 healthy adult subjects (33 ♂, 15 ♀) during sauna bathing (15 min at 93 °C, dry heat). The men sweated more profusely than the women (volume, in milliliters, of sweat collected: mean, SD, and range: 23 ± 12 (3–55) and 7 ± 3 (2–13), respectively. The concentrations, in μg/liter, (mean, SD, and range) of trace metals in sweat of men and women, respectively, were: *nickel*, 52 ± 36 (7–180) and 131 ± 65 (39–270); *copper*, 550 ± 350 (30–1440) and 1480 ± 610 (590–2280); *zinc*, 500 ± 480 (130–1460) and 1250 ± 770 (530–2620); and *lead*, 51 ± 42 (8–184) and 118 ± 72 (49–283). In sweat samples from 11 women on oral contraceptives, concentrations of Ni, Cu, Zn, and Pb did not differ significantly from the values in the 15 control women. Sweating is a demonstrably significant route for excretion of trace metals, and sweating may play a role in trace-metal homeostasis. Essential trace metals could conceivably be depleted during prolonged exposure to heat; conversely, sauna bathing might provide a therapeutic method to increase elimination of toxic trace metals.

Additional Keyphrases: *trace metals* • *normal values* • *oral contraceptives*

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Previous investigators have noted that appreciable quantities of trace metals—including aluminum (*1*), chromium (*1*), cobalt (*1*), copper (*1, 2*), iron (*2–8*), lead (*1, 9, 10*), manganese (*1, 2*), molybdenum (*1*), nickel (*1*), tin (*1*), and zinc (*1, 3*)—are excreted in sweat under conditions that produce profuse sweating. Thermally induced sweating has been used as therapy for mercury poisoning, to increase the excretion of mercury (*11*). With the exception of a study by Prasad et al. (*3*), previous measurements of trace metals in sweat have been based on sweat collections from very few subjects and have been obtained by relatively imprecise and insensitive colorimetric or spectrographic techniques. In the present investigation, atomic absorption spectrometry was used to obtain accurate measurements of the concentrations of nickel, copper, zinc, and lead in sweat samples from 48 healthy subjects.

Materials and Methods

The subjects were 48 healthy adult volunteers (33 ♂, 15 ♀) who had resided in central Connecticut for more than one year. In addition, 11 healthy women were studied who had been taking oral contraceptive medications (estrogen–progestogen combinations) for more than four months before the study began. The ages of the entire group of 59 subjects averaged 31 years (SD, 10; range, 19 to 52 years). The subjects were all white, except for 1 Negro male and 1 Oriental male. None of the subjects had been occupationally exposed to the metals under study. Sweat was collected by the armbag technique of Prasad et al. (*3*)., with stringent precautions to minimize contamination by trace metals and evaporative losses. The fingernails were carefully cleansed and trimmed, and each arm was scrubbed for 5 min with "Ivory" brand soap. The arms were rinsed copiously with distilled, demineralized water, and were dried by evaporation. Both arms were encased in polyethylene disposable shoulder-length gloves (Cat. No. BB-564; Bolab, Inc., Derry, N. H. 03038), which were secured beneath the axillae with rubber bands. Sweat was collected during a 15-min exposure to "dry" heat in a sauna bath 93 °C, <5% relative humidity). The sweat samples were removed from the gloves by puncturing each finger sheath with the tip of an acid-washed Pasteur pipet. The sweat collected from both arms was pooled and was centrifuged at 900 × *g* for 15 min in order to sediment the dermal detritus. The cell-free supernatant samples of sweat were removed and stored at 4 °C until the analyses were performed. Blood samples were obtained from 45 of the subjects immediately after the sauna bath. Specimens of blood were collected in acid-washed "Vacutainer" tubes, with and without heparin (Becton, Dickinson Co., Rutherford, N. J. 07070). Heparinized blood was used for lead analyses; heparinized plasma was used for zinc analyses, and sera were used for analyses for copper and nickel. Atomic absorption spectrometry was performed by means of a Model 403 atomic absorption spectrometer with three-slot "high-solids" burner and acetylene–air flame (Perkin-Elmer Corp., Norwalk, Conn. 06852). The following procedures were used for analyses of sweat, serum, plasma, or blood: *nickel*: method of Nomoto and Sunderman (*12*); *copper*: method of Sunderman and Roszel (*13*); *zinc:* method of Prasad et al. (*14*); and *lead:* method of Murphy et al. (*15*).

Results

Recovery of trace metals added to biological fluids. Table 1 lists measurements of the recovery of nickel, copper, zinc, and lead added to seven samples of cell-free sweat and to five samples of serum, plasma, or whole blood. As shown in the Table, there was quantitative recovery of these added trace metals.

Tests for trace metal contamination from gloves. Table 2 summarizes tests for trace-metal contamination from the polyethylene gloves used to collect the sweat. Sweat samples from five healthy subjects were immediately removed from collection gloves and were each divided into two portions. One aliquot ("A") of each sample was stored in an acid-washed polystyrene test tube at 4 °C. The second aliquot ("B") was placed in a fresh polyethylene collection glove. The interior of the glove was thoroughly rinsed with the sweat sample, and the sweat was allowed to remain in the glove for 24 h at 4 °C. Aliquots "A" and "B" of each sample were analyzed concurrently for nickel, copper, zinc, and lead. (The volume of sweat in one of the samples was insufficient for anal-

Table 1. Recovery of Trace Metals Added to Biological Fluids

Sample	No. samples	Recovery of added metals (%)[a] Nickel[b]	Copper[c]	Zinc[c]	Lead[d]
Sweat	7	97 *98(90–104)*	101 *101(96–106)*	100 *101(93–107)*	101 *100(98–106)*
Serum	5	101 *104(97–107)*	101 *104(94–112)*	—	—
Plasma	5	—	—	98 *97(91–106)*	— —
Whole blood	5	—	—	—	100 *100(97–103)*

[a] The first line of each entry gives the mean value; the second line gives the median and range.
[b] 25 μg/liter.
[c] 1500 μg/liter.
[d] 250 μg/liter.

Table 2. Tests for Trace Metal Contamination from Gloves

Sweat sample no.	Nickel A	Nickel B	Nickel Δ	Copper A	Copper B	Copper Δ	Zinc A	Zinc B	Zinc Δ	Lead A	Lead B	Lead Δ
	Metal concentrations (μg/liter)											
1	25	25	0	910	920	+10	480	480	0	92	92	0
2	24	26	+2	210	210	0	230	230	0	36	36	0
3	12	12	0	290	290	0	220	220	0	29	29	0
4	5	6	+1	630	630	0	195	250	+60	19	26	+7
5				510	500	−10				25	26	+1
Mean	16	17	+1	510	510	0	280	295	+15	40	42	+2

A = Sweat sample removed immediately from collection glove.
B = Sweat transferred to a fresh collection glove and stored for 24 h at 4 °C.
Δ = B − A.

Table 3. Trace Metal Concentrations in Serial Arm Rinses in Nine Healthy Men

Sample	Volume (ml)	Metal concentrations (μg/liter)[a]			
		Nickel	Copper	Zinc	Lead
A(H_2O rinse)	100	6.3	<10	70	4
		4.6(<0.4–17)	*<10*	*30(<10–200)*	*<1(<1–30)*
B(EDTA rinse)[b]	100	2.2	10	180	62
		<0.4(<0.4–4.0)	*<10*	*150(<10–520)*	*52(<1–118)*
C(H_2O rinse)	100	1.4	<10	10	4
		<0.4(<0.4–5.0)	*<10*	*<10(<10–60)*	*1(<1–23)*
D(Sweat)	20	48.1	540	400	36
		31(17–97)	*460(300–1120)*	*360(140–1180)*	*21(8–110)*

[a] The first line of each entry gives the mean value; the second line, the median and range.
[b] Na_2EDTA, 0.1 mmol/liter.

yses for zinc and nickel.) As shown in Table 1, there was insignificant contamination of the sweat samples by trace metals from the collecting gloves.

Measurements of trace metals in arm rinses. The results of analyses of trace metals in washings of the arms are given in Table 3. Nine healthy men washed and rinsed their arms as described in "Materials and Methods." They then encased their arms sequentially for three 15-min periods in three polyethylene collection gloves that contained successively: (*a*) 100 ml of distilled water; (*b*) 100 ml of disodium ethylenediaminetetraacetate (Na_2EDTA) solution, 0.1 mmol/liter; and (*c*) 100 ml of distilled water. The subjects sat in a comfortably air-conditioned room during the three 15-min periods, and no attempt was made to inhibit normal insensible perspiration. Finally, (*d*) the subjects entered the sauna bath for 15 min, and arm sweat was collected and analyzed. As shown in Table 3, there was no detectable elution of copper with the water or Na_2EDTA rinses. Appreciable quantities of nickel, zinc, and lead were eluted into each of the rinses, and particularly high concentrations of zinc and lead were found in the Na_2EDTA rinse. It should be pointed out that insensible perspiration may have been partially responsible for the observed elution of these trace metals. However, it appears that zinc and lead can be released from the skin by soaking the arms in a chelating solution.

Trace metals in sweat during successive collections. Sweat collections from 14 healthy men were repeated after an interval of seven days. Based on a paired-sample *t*-test, there were no significant differences between the mean concentrations of nickel, copper, zinc, and lead in the two successive samples of sweat (Table 4).

Trace metals in sweat from normal subjects and from women receiving oral contraceptives. Table 5 lists the concentrations of trace metals observed in sweat from 48 normal men and women, and in sweat from 11 healthy women who were on oral contraceptive medications at the time. About three times as much sweat was collected from the men as from the women. There appeared to be an inverse relationship between the volumes of the sweat samples and the concentrations of trace metals, inasmuch as the concentrations of nickel, copper, zinc, and lead in sweat from the women were about two to three times those for the men. No statistically significant differences were observed between the mean concentrations of nickel, copper, zinc, and lead in sweat of women who did or did not receive oral contraceptive pills.

Concentrations of trace metals in serum, plasma, or blood. Table 6 lists the concentrations of serum nickel and copper, plasma zinc, and blood lead in normal men and women, and in healthy women taking oral contraceptive medications. The mean concentration of blood lead in the men was slightly but significantly greater than in the women. Consistent with previous reports (*16–18*), the mean concentration of serum copper was significantly higher in women who received oral contraceptives than in those who did not. Contrary to previous reports (*18, 19*), no significant difference was found between the mean concentration of plasma zinc in women who

Table 4. Trace Metals in Collections of Sweat from Fourteen Healthy Men on Two Successive Weeks

Sweat collection	Volume (ml)	Metal concentrations (μg/liter)[a]			
		Nickel	Copper	Zinc	Lead
A	20 ± 11	63 ± 46	610 ± 370	670 ± 390	70 ± 55
	19(3–46)	*53(7–180)*	*500(180–1440)*	*570(150–1460)*	*46(26–184)*
B	27 ± 13[b]	81 ± 44	750 ± 450	520 ± 310	49 ± 28
	27(8–41)	*57(27–140)*	*570(290–1790)*	*420(190–1090)*	*45(19–96)*

[a] The first line of each entry gives the mean ± standard deviation; the second line, the median and range.
[b] $P < 0.05$ vs. collection A (paired-sample *t*-test).

Table 5. Trace Metals in Sweat of Healthy Adults

Subjects	No.	Volume (ml)	Metal concentrations (μg/liter)[a] Nickel	Copper	Zinc	Lead
Men	33	23 ± 12	52 ± 36	550 ± 350	500 ± 480	51 ± 42
		21(3–55)	*44(7–180)*	*460(30–1440)*	*430(130–1460)*	*38(8–184)*
Women	15	7 ± 3	131 ± 65	1480 ± 610	1250 ± 770	118 ± 72
		6(2–13)	*110(39–270)*	*1550(590–2280)*	*880(530–2620)*	*79(49–283)*
Women (oral contraceptives)	11	8 ± 4	145 ± 123	1010 ± 700	1140 ± 620	101 ± 49
		7(5–20)	*93(33–386)*	*760(220–2270)*	*980(210–2200)*	*109(28–171)*

[a] The first line of each entry gives the mean ± standard deviation; the second line, the median and range.
[b] $P < 0.001$ vs. men (*t*-test).

Table 6. Trace Metals in Serum, Plasma, or Blood of Healthy Subjects

Subjects	No.	Metal concentrations (μg/liter)[a] Nickel	Copper	Zinc	Lead
Men	19	3.1 ± 1.0	1120 ± 100	950 ± 210	160 ± 49
		3.3(1.1–4.5)	*1120(950–1280)*	*930(740–1300)*	*152(111–295)*
Women	15	2.7 ± 1.2	1170 ± 150	860 ± 60	96 ± 25[b]
		2.9(0.4–4.6)	*1150(950–1420)*	*850(760–980)*	*96(76–142)*
Women (oral contraceptives)	11	3.4 ± 1.5	1940 ± 350[c]	920 ± 90	114 ± 29
		4.0(0.4–4.7)	*1960(1490–2430)*	*880(820–1090)*	*103(78–159)*

[a] The first line of each entry gives the mean ± standard deviation; the second line, the median and range.
[b] $P < 0.005$ vs. men (*t*-test).
[c] $P < 0.001$ vs. women without oral contraceptives (*t*-test).

did or did not receive oral contraceptives. Oral contraceptive medications did not cause any significant changes in the mean concentrations of serum nickel or blood lead.

Discussion

The present investigation has confirmed earlier reports (*1–10*) that substantial quantities of trace metals can be excreted in sweat. The only published data comparable to the results of the present study are the zinc analyses that were reported by Prasad et al. (*3*). These workers measured zinc concentrations in cell-free sweat that was collected by the arm-bag technique from eight healthy Egyptian men who were exposed for 2 to 3 h to the direct sun at an ambient temperature of about 40 °C. The mean concentration of zinc in their sweat samples was 930 ± 260 μg/liter, compared to a mean concentration of 500 ± 480 μg/liter in sweat from our 33 American men.

There is controversy whether or not sweat that is collected by the arm-bag technique is truly representative of total body sweat (*20–22*). Consolazio et al. (*22*) obtained reasonable correlations between the concentrations of nitrogen, calcium, and iodine in arm sweat and in total body sweat. They concluded that sweat obtained in arm-bags may serve as an index of the sweat produced by the total body surface, but that it is more reliable to measure the total body sweat, collected by the "wash-down" technique. Our efforts to use the "wash-down" technique for analyses of trace metals in sweat have been unsatisfactory, owing to problems of metal contamination. Despite the limitations of our method of sampling, it is apparent that sweating can be a significant route for the excretion of trace metals. Thus, the mean concentration of nickel in arm sweat of healthy men is 52 ± 36 (SD) μg/liter, whereas the mean concentration of nickel in urine of healthy men is 2.3 ± 1.4 μg/liter (*12*). Similarly, the mean concentration of copper in arm sweat of healthy men is 550 ± 350 μg/liter, whereas the mean concentration of copper in urine of healthy men is 16.8 ± 7.0 μg/liter (*13*).

From our results, we speculated that body stores of trace metals may be depleted during prolonged exposure to heat. This possibility is consistent with the observations of Szadkowski et al. (*23*), who reported that the mean concentration of nickel in serum was abnormally low in 35 healthy workmen who were chronically exposed to extreme heat in a steel plant.

We also speculated that sauna bathing may provide a therapeutic method to increase the excretion of toxic trace metals. To investigate this possibility, measurements of nickel in sweat from subjects with occupational exposures to nickel compounds, and measurements of copper in sweat from patients with Wilson's disease are currently being performed in our laboratory.

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