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Dietary chromium intake of lactating Finnish mothers: effect on the Cr content of their breast milk

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1. The chromium in the diets of lactating Finnish mothers and of their breast milk was determined by graphite furnace atomic absorption.

2. The average maternal diet was estimated to provide approximately 30 μg Cr/d. Breast milk samples had a mean Cr content of 0·4 ng/ml, with a range of 0·19–0·69 ng/ml.

3. Cr intake of the lactating mothers did not correlate with the corresponding breast milk Cr concentration.

4. The diets and the breast milk of these Finnish mothers were lower in Cr than those of any other population studied in the world.

Results of recent Cr supplementation studies with human subjects present new evidence concerning the role of Cr in human health (Liu & Morris, 1978; Nath *et al.* 1979; Riales, 1979). These studies indicate that dietary Cr has a positive effect not only on glucose but also on lipid metabolism. On the basis of these studies one may enquire if the dietary Cr intake in industrialized countries is adequate.

The risk of insufficient Cr intake may be especially high in pregnant and lactating women. Schroeder *et al.* (1970) have shown that the concentration of Cr is highest in the organs of the newborn and declines rapidly during the first years of life. Thus, the accumulation of Cr in the foetus may deplete Cr stores in the mother unless the dietary Cr intake is sufficient. This is further supported by the studies of Hambidge & Rodgerson (1969) and Mahalko & Bennion (1976) who showed that the hair Cr concentration of nulliparous women was higher compared with that of parous women. An additional loss of Cr in lactating mothers is due to the Cr secreted into human milk.

Special attention should be paid to the adequacy of Cr intake in lactating women in Finland, where the mean per capita Cr intake of the entire population has recently been reported to be only 29 μg/d (Varo & Koivistoinen, 1980). This is the lowest known Cr intake of any population in the world, and is considerably lower than the lower limit of the range of 50–200 μg/d recently recommended by the Committee on Recommended Dietary Allowances (RDA) for a provisional Cr allowance (Mertz, 1980).

In order to study the dietary Cr intake of lactating Finnish mothers, the concentrations of Cr in thirty individual diet samples of fifteen mothers were analysed during lactation. A total of ten milk samples from ten mothers in the previously-mentioned group as collected

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and analysed for Cr to study the possible effect of dietary Cr intake on the Cr content of breast milk.

The results showed that the maternal diets, otherwise nutritionally adequate, were very low in Cr, providing only 31 μg/d on average. However, no correlation was found between the maternal Cr intake and the Cr content of their milk.

SUBJECTS AND METHODS

Fifteen breast-feeding mothers who volunteered for a study on trace elements in human milk kept two 7 d food records according to instructions (Pekkarinen, 1970) during the course of lactation (Vuori *et al.* 1980). The 1st survey week ranged from the sixth to the eighth week post partum and the 2nd survey week from the seventeenth to the twenty-second week post partum. A detailed description of the mothers is available elsewhere (Vuori *et al.* 1980).

Milk samples (8 ml) were collected at the beginning and end of each feed during a period of 24 h and pooled to compose one mother's sample. The method of collecting milk samples has been described elsewhere in detail (Vuori & Kuitunen, 1979).

After thawing, the milk samples were heated to 40° and carefully mixed before analysis. Samples of 0·5 ml were pipetted into 0·5 ml porcelain crucibles (Haldenwanger, Berlin). The milk samples were dried slowly on a hot plate in a class 100 clean air hood (LIV 6016, Kojair, Tampere, Finland), and were then treated for 5 h in a low temperature asher (LTA) (LTA 600, Tracerlab, Richmond, Ca). Samples were then cooled and after the addition of 100 μl 30 % hydrogen peroxide (Merck, reagent grade) were dried slowly on a hot plate in a clean air hood. After this procedure the ash was usually white throughout. The LTA treatment was repeated if needed. The ash was dissolved in 0·5 ml of 1 M-hydrochloric acid, prepared from 30 % acid (Merck, Suprapur). After 30 min, 20 μl samples were injected into the pyrolytic graphite tube.

The Perkin-Elmer 703 atomic absorption spectrometer used was equipped with an HGA 500 graphite furnace, a deuterium background corrector, and a Model 56 recorder. The instrumental settings were: 357·9 nm Cr line; 0·7 nm slit width; peak height mode; read-out signal on concentration; 10 × scale expansion; 4 s integration time.

The furnace program was: (*a*) 150° for 25 s (5 s ramp), (*b*) 1100° for 11 s (10 s ramp), (*c*) 2650° for 3 s (0 s ramp). Argon purge gas was used at an internal flow rate of 300 ml/min. The automatic remote baseline correction and start of integration time were programmed at 2 and 1 s prior to atomization.

Working standards in the range of 0·5–5 ng/ml in 1 M-HCl were prepared daily from a 1 mg potassium dichromate/l stock standard.

As no certified standard reference material for biological materials at the concentration level of 1 ppb exists, recovery studies on the LTA procedure were conducted by adding $^{51}CrCl_3.6H_2O$ into the crucibles before ashing. The recovery was 97·6 % (SD 2·4 %, *n* 10) and adsorption on the walls of the crucibles was 0·6 % (SD 0·1 %, *n* 7).

The precision of the method was tested by repeatedly analysing samples of pooled human milk. The day-to-day variation was 9·6 %. The mean (± SD) blank was 0·10 ± 0·05 ng Cr/ml and the detection limit 0·20 ng Cr/ml calculated from: mean + 2 × SD of the blanks.

The effectiveness of the deuterium background correction was tested with a sample of pooled human milk digested and dissolved as described above. The results of this experiment are presented in the form of a chart recorder tracing in Fig. 1. Moreover, the background correction capability of the very same instrumentation has been shown to be adequate for the determination of Cr in urine (Kumpulainen, 1980).

The 7 d food records were handled according to the computing system programed for a Burroughs 6700 computer at the Computing Centre of the University of Helsinki to give the

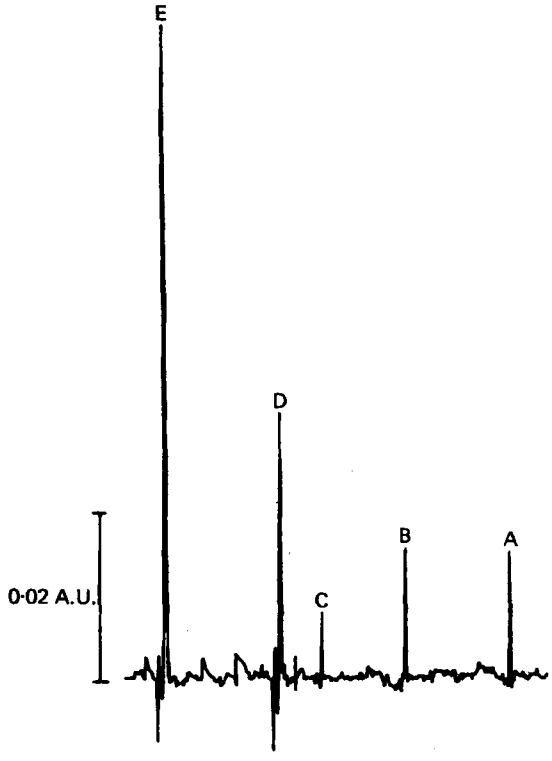

Fig. 1. Comparison of background only and background corrected peaks for Cr in ashed human milk. A and B, background corrected signals, sample volume 20 μl; C, background corrected signal, sample volume 10 μl; D, background only, sample volume 10 μl; E, background only, sample volume 20 μl.

average daily food consumption and nutrient intake of the mothers (Ahlström *et al.* 1972; Seppänen *et al.* 1976). A mixture representing the calculated average daily food consumption of the two survey weeks separately was prepared from foodstuffs bought in supermarkets in the Helsinki area. The fresh food mixtures were homogenized in a Kenwood Chef A701/A for 1 min at speed setting no. 3. Cr contamination by the steel blades of the homogenizer was tested with cooked meat in orange juice and with dried pieces of bovine liver (Fig. 2). The wet food sample was used as a model in estimating the amount of contamination in the diet samples.

Food mixture samples of 0·5 g (dry weight) were weighed in 20 ml quartz crucibles (Vitreosil, England) and ashed in a muffle furnace overnight at 500°. The procedure used for diet samples that were difficult to digest has been described in detail elsewhere (Kumpulainen, Anderson *et al.* 1979).

The volatility of Cr in biological materials during dry ashing at 500° has been extensively studied in yeast extracts high in biologically active Cr, with NBS standard reference materials No. 1577, 1569 and 1571 (Kumpulainen, Wolf *et al.* 1979; Kumpulainen, Anderson *et al.* 1979) and with a brewer's yeast, into which Cr was biologically incorporated during the growth by addition into the medium of $^{51}CrCl_3$ (Kumpulainen, 1977). No volatile Cr was found in any of the materials tested. In recent studies, other investigators have not found volatile Cr fractions during dry ashing at 500° (Cary & Olson, 1975; Koirtyohann & Hopkins, 1976; Christensen *et al.* 1976; Versieck *et al.* 1979).

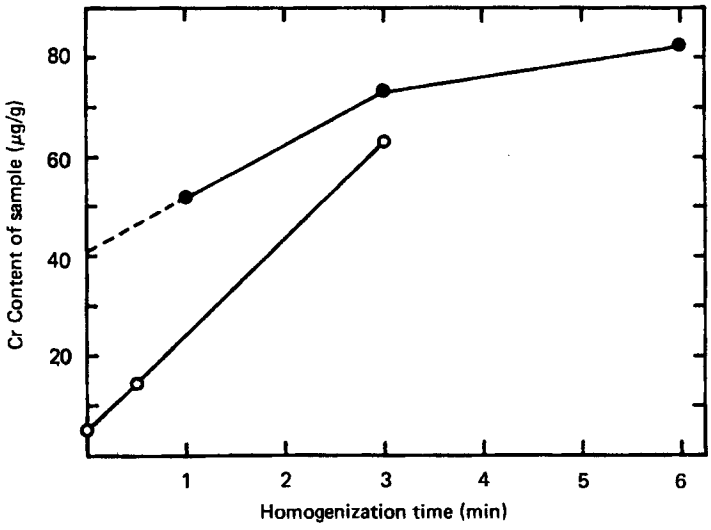

Fig. 2. Extent of Cr contamination as a function of homogenization time using a Kenwood Chef A701/A homogenizer. The wet food sample (●) consisted of 200 g freshly cooked meat together with 300 ml orange juice. Dried food sample (○) was 50 g dried bovine liver pieces. The speed setting used was no. 3 (scale 1–8). Extent of Cr contamination for diet samples was estimated by using wet food sample as a model. Assuming that the slope of the curve during the first min of homogenization (– – –) is similar to that during the second min of homogenization, the original Cr content of the model sample was 41 μg/g. This is 21·2 % lower than that after 1 min of homogenization. Accordingly the original Cr contents of the diets were estimated to be 21 % lower than the concentrations actually found.

Table 1. *Accuracy of the analytical method used tested with National Bureau of Standards' Standard Reference Material No.* 1577 (*bovine liver*) *three times during the experimental analyses*

(Certified value is 88 ± 12 ng/g dry weight)

No. of samples	Chromium content (ng/g dry wt)	
	Mean	SD
6	88	8
8	85	6
5	89	5

The ash was dissolved in 2·0 ml of 1 M-HCl (Suprapur Merck) and after 30 min 20 μl samples were injected into the graphite furnace.

The instrumental settings for the food samples were the same as those for the human milk samples described above. HCl was used for the dissolution of the ash because it is much easier to evaporate in the graphite tube than sulphuric acid. In addition the graphite tube lifetimes are much longer as HCl is less corrosive. Use of nitric acid is not recommended as it is less effective in dissolving Cr adsorbed on the walls of crucibles than HCl (Kumpulainen, 1977).

No volatility of Cr has been found in a graphite furnace at 1100° when dissolved in HCl (Routh, 1979, 1980). It is important that charring temperatures higher than 1200° are not used, because at 1300° volatilization of Cr occurs both in HCl and HNO_3. The working standards in the range of 5–30 ng/ml in 1 M-HCl were prepared every 2nd day from 1 mg $K_2Cr_2O_7$/l stock standard.

Table 2. *Chromium content of diet samples of lactating Finnish mothers and their estimated daily Cr intakes*

(Mean values with their standard errors. The first survey week ranged from 6th to 8th and the second survey week from 17th to 22nd week post partum)

	First survey week			Second survey week		
	Cr content (ng/g dry wt)		Cr intake (μg/d)	Cr content (ng/g dry wt)		Cr intake (μg/d)
Mother	Mean	SE		Mean	SE	
11	90	6	45	48	1	18
13	43	1	35	51	1	30
14	49	3	34	64	4	37
16	48	3	21	38	2	16
17	98	2	36	76	2	39
18	50	0·1	26	54	3	26
19	94	8	40	64	6	34
20	60	0·4	34	52	1	34
22	74	2	38	66	1	42
27	62	7	29	51	0·2	18
31	56	0·1	31	45	3	22
34	39	3	23	55	1	23
38	50	1	40	55	1	25
39	56	1	44	54	1	30
40	55	2	27	66	8	31
Mean	62		34	56		28

The diet samples were analysed in triplicate. The accuracy of the method was tested by analysing bovine liver (Standard Reference Material No. 1577, National Bureau of Standards (NBS), Washington, DC) at the beginning, in the middle and at the end of the analysis period (Table 1).

The linear correlation coefficient was calculated between the Cr content of maternal diet and corresponding concentration in breast milk.

RESULTS AND DISCUSSION

The results of the contamination experiments (Fig. 2) clearly demonstrate the importance of contamination control during homogenization. This is especially important in the instance of dried food samples, where homogenizer blades made of high-Cr containing steel are totally unacceptable. For wet food samples, the extent of contamination must be studied in each instance, but homogenization with low-Cr containing blades is always preferable (Kumpulainen, Wolf *et al.* 1979).

The results of Cr analyses of the diets of lactating Finnish mothers and of their corresponding breast milk samples are presented in Tables 2 and 3. Fig. 3 shows the daily Cr intake of the mothers.

The mean and median per capita Cr intake was 31 μg/d, with the upper quartile of 37 and the lower quartile of 25 μg/d.

The Cr intake of the lactating Finnish mothers studied seems to be very low. All the diets studied were lower in Cr than the lowest limit of Cr intake (50 μg/d) proposed for the RDA. The mean intake estimated in the present study, approximately 30 μg/d, agrees very well with that of 29 μg/d recently reported to be the mean per capita Cr intake in Finland (Varo & Koivistoinen, 1980) which according to the available values seems to be lower than that of any population in the world (Waslien, 1976).

Table 3. *Chromium in breast-milk of lactating Finnish mothers and their dietary Cr intakes*

	First survey week			Second survey week		
		Cr in milk (ng/ml)			Cr in milk (ng/ml)	
Mother	Cr intake (μg/d)	Mean	SE	Cr intake (μg/d)	Mean	SE
11	—	—	—	18	0·34	0·06
16	—	—	—	16	0·27	0·03
18	—	—	—	26	0·54	0·02
27	—	—	—	18	0·31	0·01
31	—	—	—	22	0·24	0·03
13	35	0·20	0·02			
17	36	0·35	0·03			
19	40	0·69	0·05			
20	34	0·19	0·03			
22	38	0·50	0·06			

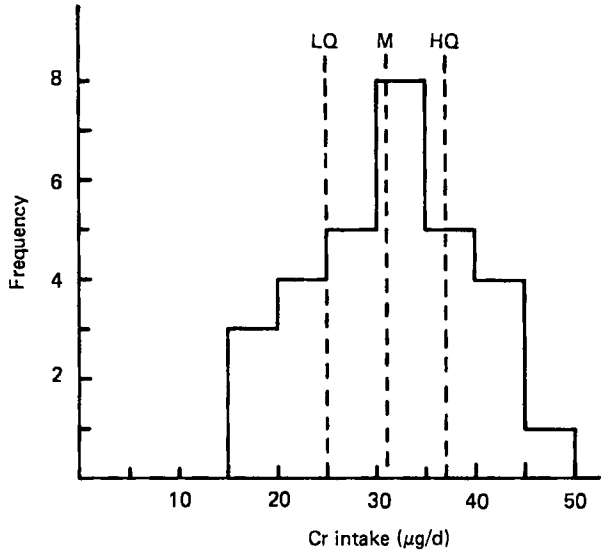

Fig. 3. Distribution frequencies of daily Cr intake of lactating Finnish mothers. Lower quartile (LQ) 25 μg/d, mean and median (M) 31 μg/d, higher quartile (HQ) 37 μg/d.

The analytical method used in the present study is essentially the same as that used in the determination of Cr in selected diets in the United States (Kumpulainen, Anderson *et al.* 1979). The mean Cr content of those diets was 78 μg, the same level of intake as that found by other investigators in the United States (Levine *et al.* 1968; Schroeder *et al.* 1962). In Western Europe, too, the Cr intake seems to be much higher than that in Finland (Schelenz, 1977; Abdulla & Svensson, 1979).

The diets analysed in the present study were nutritionally adequate in terms of other trace elements and energy, furnishing on average 9·6 MJ energy, 1·8 mg copper, 15·6 mg iron, 5·0 mg manganese and 13·3 mg zinc (Vuori *et al.* 1980).

The Cr concentration in human milk reported here agrees with that found in a study of pooled human milk (Kumpulainen, 1980) and is approximately one order of magnitude lower than previously reported in the literature.

Our results suggest that the Cr intake of lactating mothers does not have an effect on the Cr content of human milk at the level of intake studied.

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