ELEMENTS IN PLACENTA AND PREGNANCY OUTCOME IN ARCTIC AND SUBARCTIC AREAS

ABSTRACT

Objectives. This paper describes a comprehensive assessment of the association of concentrations of essential and toxic elements in maternal and neonatal body fluids and the placenta as predictors of birth weight and newborn body mass index (BMIC) for deliveries in northern Norway and Russia.

Study design. A prospective cross-sectional study of delivering women and their outcomes from different locations in Russian and Norwegian arctic and sub-arctic areas.

Methods. Life-style information, blood, urine and placenta specimens were collected for 50 consecutive mother-infant pairs from hospital delivery departments in a total of six communities located in Finnmark, Norway, or the western arctic/subarctic regions of Russia. Questionnaire information was collected by individual interviews performed by trained health personnel. Pregnancy outcomes were verified by consulting medical records. Cadmium, copper, iron (as ferritin), nickel, lead, selenium and zinc were measured in maternal blood, serum or maternal urine; and in cord blood, or neonatal urine and placental tissue. Univariate and multivariate linear regression analysis and ANOVA were employed to explore associations between these clinical chemistry outcomes and birth weight and BMIC.

Results. A number of significant relationships were evident between: placental and maternal blood cadmium (p < 0.005); cord and maternal blood lead (p < 0.001); placental and maternal blood lead (p < 0.001); placental and cord-blood lead (p < 0.001); placental and maternal serum, or blood, selenium (p < 0.001); and placental and maternal serum copper (p < 0.001). Maternal body mass index (BMI), maternal age, placental lead, or maternal blood lead, and smoking were retained as predictors of birth weight and BMIC in the multivariate modelling. Birth weights in both countries were normally distributed.
Conclusions. Maternal age and BMI as positive predictors of birth weight, and cigarette smoking and lead exposure as negative determinants, are discussed in terms of established evidence and recognized confounders, including maternal genetic factors, socio-economic status, socio-political change, life-style issues, prenatal care and nutrition. It is recommended that future work in societies undergoing socio-economic transition might best focus on preventive measures to improve neonatal health and development. (Int J Circumpolar Health 2004;63(2):169-187)

Key Words: Elemental composition; Placenta; Pregnancy Outcome

INTRODUCTION

In two recent papers, the authors have discussed the composition of toxic and essential elements of placentas collected at delivery departments in the Norwegian/Russian border area (1,2). Principal component analysis (PCA), also referred to as factor analysis (3), was introduced for the grouping and assessment of the elements. In the first paper, concentrations in human placenta of 16 elements (P, Ca, Mg, Cu, S, Na, Fe, Zn, K, Se, Mn, Ba, Sr, Pb, Ni, Cd) were compared for each of two arctic communities in Eastern Norway and Western Russia, and for another location in each country located at more southern latitudes (1). Only Fe, Mg, P and K were present in lower concentrations in Russia. The observed inter-element correlations were reflected in the four major factors identified in the factor analysis. The total variation explained was 67.3 %, of which more than half (35.3 %) was contributed by Factor 1. P, Ca, Mg, Ba, Sr, Pb, and Ni were major contributors to this factor. The placental concentrations of these elements depended strongly on gestational age, increasing from about week 35 and peaking near weeks 39 and 40; they also exhibited skewed frequency distributions and a dependence on maternal smoking. The gestational-dependent mineralization of the placenta was interpreted to reflect the deposition of phosphates coinciding with smoking-induced tissue damage. The loadings of the remaining three factors were associated with common absorption mechanisms, similar biochemistries and unique transport pathways.
In the subsequent paper, the total number of placentas considered in the factor analysis were nearly doubled (from 263 to 571) by including births with mothers for whom personal, life-style and morphometric information was not available (2). The prominence of Factor 1 was confirmed, which again grouped those elements known to form insoluble phosphate complexes and whose concentrations showed a dependence on gestational age and maternal smoking in the earlier study. PCA analysis at the community level showed promise in identifying intercommunity and temporal variations, study limitations and quality assurance shortfalls. It was concluded that factor analysis is a powerful statistical tool for exploring and identifying fundamental pathways and processes involved in governing the elemental composition of placental tissue.

Our findings also provided encouragement that placental concentrations of toxic elements can serve as an index to exposure and of nutritional intake for selected essential microelements. Iyengar and Rapp (4-6) have discussed the human placenta as a biomarker for monitoring fetal and maternal environments, with special reference to potentially toxic trace elements. In Part 1, they discuss the issues and problems of sampling of placental tissue in some detail (4). It is important to state that the placental collection procedure employed by us is consistent with their recommendations, as outlined earlier (1); originally, it was adapted from (7). In Parts 2 and 3, these authors have tried to establish reference values for minor and trace elements (both essential and toxic) (5,6). The placental concentrations reported by us are in good agreement with the tabulations provided for 10 of the 16 elements, with our values being lower for Ni, Na and Mn and higher for S. Our results for Ba and Sr appear to be the first to have been determined and reported.

The objectives of this paper are to explore associations between the placental concentrations of the different elements to those in biological fluids reported previously by us (8-10) for the mother and corresponding neonate, as well as to the pregnancy outcomes of birth weight and child’s body mass index at birth (BMIC).

The study was approved by The Regional Ethical Committee, University of Tromsø, Norway, the Norwegian Data Inspectorate and the Regional Health Administrations of Murmansk and Arkhangelsk Counties.
MATERIALS AND METHODS

The collection of questionnaire information, specimen collection and storage of placental tissue, maternal blood and urine, and child’s blood and urine are described in detail in previous papers by the authors (1-3,8-10).

The women were asked to join the study by means of completing a consent form and, in an interview format, personal and morphometric information were obtained through a questionnaire. The questionnaire was created in the Norwegian language, translated into English and Russian, and tested out in all areas under the guidance of the study coordinator before the study started. The questionnaire administration during the study was performed by experienced health personnel who participated in the prestudy testing. Even so, information on alcohol consumption was, unfortunately, very scarce and impossible to assess in the statistical model. None of the delivering women refused to join the study. Data of birth, gestational age, weight of the placenta and length of the baby were taken from the delivery records. In most communities, placentas and blood, serum and urine specimens were collected from 50 consecutive patients presenting themselves to the hospital delivery departments. The registration and sampling were conducted in the following communities and time periods: Arkhangelsk in April - May 1993; Kirkenes, Hammerfest, Bergen, Nikel and Monchegorsk from November 1993 to June 1994. Since blood lead is an important independent risk factor for low birth weight, the availability of maternal and cord blood concentrations determined the eligibility of the study group for the present paper. The selected cohort corresponded to that examined in (10). The sample preparation and instrumental analysis of blood, urine and placenta samples are described in (1) and (11).

For the univariate and multivariate linear regression analyses and the ANOVA, Epi Info 6 software program for personal computers, Version 6.04a, July 1996 (World Health Organization Geneva, Switzerland) was used. An association was accepted when the 95 % confidence interval (CI) of the regression coefficient did not include zero, although in selecting variables for the stepwise multiple regression carried out up to 10 % statistical significance in the univariate regression was considered. The non-
parametric Wilcoxon rank sum test was employed for those elements with skewed frequency distributions. Concentrations below the DL were arbitrarily assigned the value of 1/2 the detection limit (DL).

RESULTS

Analytical results
The observed concentrations of essential and toxic elements in maternal serum (Cu, Zn, Se and Fe as ferritin), maternal whole blood (Cd, Ni, Pb, Se), cord blood (Cd, Pb, Se), maternal and neonatal urine (Ni and creatinine) and placental tissue (Cd, Cu, Fe, Ni, Pb, Se, Zn) have been published previously (1,8-11). A summary of the medians and ranges are provided in Table I. For only 56 Russian and 25 Norwegian women, mercury levels in whole blood were measured (11). They were generally low and provided no impact when put into a statistical model using only these subgroups.

Pregnancy outcome
As pointed out in our previous papers (8-10), the mean birth weight of the Russian babies was significantly lower (p < 0.001), with or without adjustment for gestational age and gender. BMIC was also lower in Russia (p < 0.001), the babies were longer (p = 0.003) and the gestational age shorter (p < 0.001) (Table II). By contrast, there was no difference in placental weights between the two countries.

Correlations between the compartments
Relationships between different body compartments for the selected elements are summarized in Table III, adjusted for country and based on linear regression analysis. There is a significant relationship between P-Cd and MB-Cd (p < 0.005). No other significant association for Cd could be demonstrated. Multiple significant correlations (p < 0.001) are evident for Pb: P-Pb/MB-Pb; P-Pb/CB-Pb; and CB-Pb/MB-Pb. At the community level (data not shown), the relationship between P-Pb/MB-Pb remains valid: the strongest were found in Nikel [r = 0.34, CI(95%) 0.02-0.92, p-value < 0.05 and N = 50] and Monchegorsk [r = 0.56, CI (95%) 0.21-0.92, p-value < 0.005 and N = 50]. No significant correlations between the different compartments were observed for Ni, but the-
Table I. Summary of elemental concentrations in blood, serum, urine and placental tissue.

| Element | Media         | Total Group Median(Range) | Russia Median (Range) | Norway Median (Range) | Number of Data Points | p-Value$^a$ | Source Reference |
|---------|---------------|---------------------------|-----------------------|-----------------------|-----------------------|-------------|-----------------|
| Ni      | Maternal urine (nmol/L) | 47.0 (4.3-2108) | 84.2 (4.3-2108) | 13.6 (4.3-96.9) | 252 | < 0.001 | 8 |
|         | Neonatal urine (nmol/L) | 18.7 (4.3-561) | 34.0 (4.3-561) | 4.3 (4.3-37.0) | 227 | < 0.001 | 8 |
|         | Placenta (mg/g) | 0.017 (0.005-0.377) | 0.023 (0.005-0.119) | 0.012 (0.005-0.377) | 220 | < 0.001 | 1 |
| Cd      | Maternal blood (nmol/L) | 2.0 (0.5-35.2) | 2.2 (0.5-35.2) | 1.8 (0.5-26.9) | 262 | 0.55 | 10 |
|         | Neonatal blood (nmol/L) | 0.5 (0.5-4.8) | 0.5 (0.5-4.8) | 0.5 (0.5-3.4) | 208 | 89.6 % at DL$^b$ | 10 |
|         | Placenta (mg/g) | 0.032 (0.011-0.201) | 0.035 (0.013-0.118) | 0.029 (0.011-0.210) | 221 | 0.03 | 1 |
| Pb      | Maternal blood (umol/L) | 0.1 (0.02-0.65) | 0.14 (0.04-0.65) | 0.06 (0.02-0.19) | 262 | < 0.001 | 10 |
|         | Neonatal blood (umol/L) | 0.07 (0.02-0.53) | 0.10 (0.03-0.53) | 0.05 (0.02-0.18) | 208 | < 0.001 | 10 |
|         | Placenta (mg/g) | 0.09 (0.03-0.57) | 0.11 (0.03-0.57) | 0.06 (0.03-0.53) | 221 | < 0.001 | 1 |
| Se      | Maternal blood (umol/L) | 1.42 (0.77-2.08) | 1.42 (0.80-2.08) | 1.41 (0.77-1.95) | 262 | 0.13 | 9 |
|         | Maternal serum (umol/L) | 1.05 (0.51-1.99) | 1.02 (0.60-1.63) | 1.08 (0.51-1.99) | 220 | 0.03 | 9 |
|         | Neonatal blood (umol/L) | 1.36 (0.86-1.98) | 1.34 (0.86-1.98) | 1.40 (0.87-1.93) | 208 | 0.01 | 9 |
|         | Placenta (mg/g) | 0.97 (0.63-1.90) | 0.94 (0.71-1.34) | 0.98 (0.63-1.90) | 221 | 0.06 | 1 |
| Cu      | Maternal serum (umol/L) | 35.80 (16.17-68.61) | 35.17 (18.53-56.99) | 36.58 (16.17-68.61) | 223 | 0.045 | 9 |
|         | Placenta (mg/g) | 5.21 (2.83-8.74) | 5.68 (4.29-8.74) | 4.86 (2.83-6.91) | 221 | < 0.001 | 1 |
| Zn      | Maternal serum (umol/L) | 8.26 (3.21-16.52) | 8.11 (4.90-14.38) | 8.42 (3.21-16.52) | 220 | 0.10 | 9 |
|         | Placenta (mg/g) | 56.0 (34.7-161.0) | 56.0 (38.0-161.0) | 56.3 (34.7-98.7) | 221 | 0.41 | 1 |
| Fe      | Maternal serum (umol/L) | 15.0 (3.0-245.0) | 13.0 (3.0-245.0) | 19.0 (5.0-157.0) | 262 | 0.004 | 9 |
|         | Ferritin (umol/L) | 0.62 (0.31-1.06) | 0.58 (0.31-0.96) | 0.67 (0.35-1.06) | 221 | < 0.001 | 1 |

$^a$ Comparison of country means by non-parametric Wilcoxon rank sum test

$^b$ Detection Limit
There was a strong relationship between MU-Ni and maternal urinary creatinine, with \( r = 0.19, \text{CI (95%)} 0.20-0.58, p < 0.001 \) or without \( r = 0.31, \text{CI (95%)} 0.36-0.75, p < 0.001 \) adjustment for country.

For the essential elements, the associations between the variables P-Se/MS-Se is highly significant \( p < 0.001 \), as well as for CB-Se/MS-Se \( p < 0.001 \), while no relationship was seen for P-Se/CB-Se. Similarly, the relationship between P-Cu/MS-Cu was highly significant \( p < 0.001 \), but, for Zn and Fe, no comparable correlations could be demonstrated.

### Table II. Selected maternal characteristics and pregnancy outcomes by countrya.

|                          | Russia n=148 | Norway n=114 | p-value   |
|--------------------------|--------------|--------------|-----------|
| **Maternal characteristics** |              |              |           |
| Mean maternal age, years | 25.0 (5.9)   | 28.2 (5.1)   | < 0.001*  |
| Mean number of deliveries| 1.2 (4.6)    | 1.5 (4.8)    | 0.004*    |
| Mean body mass index (BMI; kg/m²) | 26.8 (4.6)  | 26.9 (4.8)   | 0.9*      |
| Smoking habits (%)       |              |              |           |
| Non-smokers              | 76.4 (6.4)   | 63.2 (5.1)   | < 0.01†   |
| 1-10 cigarettes/day      | 20.9 (20.2)  | 20.2 (20.2)  |           |
| >10 cigarettes/day       | 2.7 (14.0)   | 14.0 (14.0)  |           |
| **Pregnancy outcomes**   |              |              |           |
| Mean birth weight (g)    | 3178 (616)   | 3571 (488)   | < 0.001*  |
| Mean length of baby (cm) | 51.6 (3.4)   | 50.5 (2.2)   | 0.003*    |
| Mean body mass index of child (BMIC; kg/m²) | 11.9 (1.7)  | 13.9 (1.3)   | < 0.001*  |
| Mean placenta weight (g) | 584 (142)    | 589 (144)    | 0.8*      |
| Mean gestational age (weeks) | 38.6 (2.0)  | 39.7 (1.4)   | < 0.001*  |

*a All information was derived from medical records.

* Comparing Russian and Norwegian populations, t-test.

† Comparing Russian and Norwegian populations, chi-squared test.

Univariate linear regression of birth weight and BMIC

The birth weight distributions for both countries are depicted in Figures 1 a and b; both appear normal. In Table IV, the weight changes associated with unit concentration changes of the different placental elements are shown, adjusted for country (and gestational age). The on-
ly significant variable with negative impact on birth weight is P-Ni (p < 0.005). However, it just loses significance at the 5 % level when adjusted for gestational age. P-Pb and, perhaps, P-Cu are both close to having a significant negative contribution to the birth weights, with or without adjustment for gestational age. P-Fe, P-Se and P-Zn are relatively weak and non-significant positive contributors to birth weight and do exhibit some sensitivity to gestational age adjustment.

The regression of BMIC on placental element concentrations demonstrated no significant contributions at the 5 % level (Table V). However, as for birth weight, the contributions of P-Pb and P-Ni are negative and close to significance. Again P-Fe, P-Se and P-Zn make positive, but relatively small contributions (p > 0.05), with or without adjustment for gestational age.
Multivariate models to predict influences on birth weight and BMI

In our previous papers maternal age, number of deliveries, maternal weight, height and body mass index (BMI), and serum concentrations of the essential elements Fe (as ferritin), Se and Zn were found to be positively associated with birth weight, while maternal smo-
king frequency, maternal urinary creatinine, and MB-Pb have been found negatively associated (8-10). The only factor that significantly reduced the country factor was MB-Pb (10). All these parameters were put into a multivariate linear regression model, together with the significant (p < 0.05) or, close to significant, variables (p < 0.10) found in the univariate linear reg-

### Table IV. Univariate regression analysis of birth weight. Weight change in g/unit (95 % CI) and p-value, adjusted for country and (gestational age).

| Variable   | Weight change g/unit | 95 % CI          | p-value |
|------------|----------------------|------------------|---------|
| P-Cd (μg/g) | 1694                 | -2038, 5426      | > 0.05  |
| (1081)   |                      | (-1887, 4048)    | (> 0.05) |
| P-Pb (μg/g) | -864                 | -1913, 185       | > 0.05  |
| (-784)   |                      | (-1603, 36)      | (> 0.05) |
| P-Ni (μg/g) | -2526                | -4659, -394      | < 0.005 |
| (-1510)  |                      | (-3191, 170)     | (> 0.05) |
| P-Cu (μg/g) | -88                  | -181, 48         | > 0.05  |
| (-38)    |                      | (-113, 37)       | (> 0.05) |
| P-Fe (mg/g) | 331                  | -155, 817        | > 0.05  |
| (131)    |                      | (-260, 522)      | (> 0.05) |
| P-Se (μg/g) | 194                  | -338, 725        | > 0.05  |
| (-72)    |                      | (-497, 352)      | (> 0.05) |
| P-Zn (μg/g) | 1.41                 | -3.5, 6.3        | > 0.05  |
| (0.19)   |                      | (-3.6, 4.0)      | (> 0.05) |

### Table V. Univariate regression analysis of BMI. Weight change per unit (95 % CI) and p-value, adjusted for country and (gestational age).

| Variable   | Weight change (kg/m² per unit) | 95 % CI          | p-value |
|------------|--------------------------------|------------------|---------|
| P-Cd (μg/g) | 6.20                           | -3.14, 15.54     | > 0.05  |
| (4.40)     |                                 | (-3.95, 12.75)   | (> 0.05) |
| P-Pb (μg/g) | -2.25                          | -4.88, 0.37      | > 0.05  |
| (-2.08)    |                                 | (-4.39, 0.23)    | (> 0.05) |
| P-Ni (μg/g) | -4.90                          | -10.27, 0.47     | > 0.05  |
| (-2.73)    |                                 | (-7.49, 2.02)    | (> 0.05) |
| P-Cu (μg/g) | -0.04                          | -0.28, 0.19      | > 0.05  |
| (0.05)     |                                 | (-0.16, 0.26)    | (> 0.05) |
| P-Fe (mg/g) | 0.97                           | -0.25, 2.18      | > 0.05  |
| (0.69)     |                                 | (-0.41, 1.79)    | (> 0.05) |
| P-Se (μg/g) | 0.37                           | -0.97, 1.70      | > 0.05  |
| (-0.15)    |                                 | (-1.35, 1.05)    | (> 0.05) |
| P-Zn (μg/g) | 0.007                          | -0.005, 0.02     | > 0.05  |
| (0.005)    |                                 | (-0.006, 0.02)   | (> 0.05) |
**Table VI.** Multivariate linear regression analysis models to predict birth weight and BMIc. Backward stepwise regression was employed including all non-complementary variables that showed significant (p<0.05) or near significant associations in the univariate linear regression analyses.

| Variable | Change in: | 95% CI | p-Value |
|----------|------------|--------|---------|
|          | weight (g/unit; models 1 to 3); BMIc (kg/m² per unit; models 4 to 6) |        |         |
| **Model 1** |            |        |         |
| BMI      | (Russia/Norway)a,b | 17.96  | 5.38, 30.54 | < 0.005 |
| P-Pb     | -736       | -1527, 55 | ≥ 0.05 |
| Country  | 89         | -34, 214 | > 0.05 |
| **Model 2** |            |        |         |
| Age      | (Russia)d,e | 14.3   | 0.84, 27.7 | < 0.05 |
| MB-Pb    | -730       | -1470, 8.4 | ≥ 0.05 |
| **Model 3** |            |        |         |
| Smoking  | (Norway)g,h | -106   | -212, -0.8 | < 0.05 |
| BMI      | 24.8       | 7.4, 42.3 | < 0.005 |
| **Model 4** |            |        |         |
| BMI      | (Russia/Norway)i | 0.05   | 0.009, 0.08 | < 0.025 |
| MB-Pb    | -1.94      | -4.21, 0.33 | ≥ 0.05 |
| Country  | 1.32       | 0.31, 0.49 | < 0.001 |
| **Model 5** |            |        |         |
| Age      | (Russia)j,m | 0.04   | -0.0001, 0.09 | ≥ 0.05 |
| MB-Pb    | -2.6       | -5.0, -0.19 | < 0.05 |
| **Model 6** |            |        |         |
| Smoking  | (Norway)k,p | -0.04  | -0.7, -0.1 | < 0.005 |
| BMI      | 0.05       | -0.00004, 0.095 | ≥ 0.05 |

a Variables considered: maternal age, maternal smoking frequency, maternal BMI, maternal height, number of deliveries, P-Pb, P-Ni, P-Cu, maternal urinary creatinine; adjusted for gestational age and country.

b The optimized model shown has p < 0.001 with F=57.1 (df=3 and n-4; for n, see Table II).
c P-Pb provides a better fit than MB-Pb, or CB-Pb.
d Variables considered: maternal age, maternal smoking frequency, maternal BMI, maternal height, number of deliveries, P-Pb, P-Ni, P-Cu, MB-Pb, maternal urinary creatinine; adjusted for gestational age.
e The optimized model shown has p < 0.001 with F = 36.8 (df=2 and n-3).
f MB-Pb provides a better fit than P-Pb, or CB-Pb.
g Variables considered: maternal age, maternal smoking frequency, maternal BMI, maternal height, number of deliveries, P-Ni, P-Cu, MB-Pb (alternatively P-Pb, or CB-Pb), maternal urinary creatinine; adjusted for gestational age.
h The optimized model shown has p < 0.001 with F = 21.0 (df=2 and n-3).
i Variables considered: maternal age, maternal smoking frequency, maternal BMI, maternal height, number of deliveries, P-Ni, P-Cu, MB-Pb, maternal urinary creatinine; adjusted for gestational age.
j The optimized model shown has p < 0.001 with F = 43.6 (df=3 and n-4; for n, see Table II)
k MB-Pb provides a better fit than P-Pb, or CB-Pb. Maternal smoking is also a close to significant negative predictor of birth weight in the multivariate model for the combined group.
l Variables considered: maternal age, maternal smoking frequency, maternal BMI, maternal height, number of deliveries, P-Ni, P-Cu, MB-Pb, maternal urinary creatinine; adjusted for gestational age.
m The optimized model shown has p < 0.001 with F = 15.3 (df=2 and n-3)
n MB-Pb provides a better fit than P-Pb, or CB-Pb.
o Variables considered: maternal age, maternal smoking frequency, maternal BMI, maternal height, number of deliveries, P-Ni, P-Cu, MB-Pb (alternatively P-Pb, or CB-Pb), maternal urinary creatinine; adjusted for gestational age.
p The optimized model shown has p < 0.001 with F = 14.4 (df=2 and n-3)
The corresponding models for BMIC are also presented in Table VI. An obvious difference for the combined group (Model 4, Table VI) compared to the association with birth weight is the highly significant country factor remaining in the final model. The maternal BMI constitutes the only significant positive predictor (p < 0.025), but the MB-Pb has replaced P-Pb as the negative predictor close to significance (95% CI of -4.21, 0.33; p ≥ 0.05). In the Russian group (Model 5, Table VI), maternal age is close to achieving significance as a positive predictor in the final model (95% CI of -0.0001, 0.09; p ≥ 0.05), while MB-Pb reaches significance as a negative contributor (95% CI of -5.0, -0.19, p < 0.05). In the Norwegian group (Model 6, Table VI), the maternal BMI is the strongest positive predictor, close to significance (95% CI of -0.00004, 0.095; p ≥ 0.05), while maternal smoking again is a significant negative contributor (95% CI of -0.7, -0.1, p < 0.005).

DISCUSSION

Sampling considerations
Guidelines regarding collection and storage of the placenta, tissue sampling and sample preparation are suggested by Iyengar and Rap (4). A sample of "placenta tissue" can be regarded as a heterogeneous mixture of placental, maternal and fetal cells. The reason for this is that the placenta, consisting of chorionic villi, amniotic membranes
and cord tissue at the point of entry to the placenta, is highly vascularized by maternal and fetal blood vessels. Since elements are not uniformly distributed in the tissues of the maternal-foeto-placental unit, our sampling protocol involved a single peri-insertional site and the samples primarily represent villous (embryonic) tissue, because the decidua and chorionic plates were removed. The senior author carried out the first 5 sample collections in the presence of the local midwives and gynecologists to harmonize the protocol. This approach was adapted from Manci and Blackburn (7) and closely corresponds to the Iyengar and Rap recommendations (4).

Maternal/Placental/Fetal Associations

Iyengar and Rap (4) suggest that the placenta may serve as a dual biomarker "to monitor both the internal environment of the foetus and that of the external environment of the mother". One might add to that certain intrinsic characteristics of the mother, such as morphometric parameters and health status. This is confirmed by the linear regression analyses conducted in our studies. Associations were observed between: maternal blood (serum) and cord blood concentrations (of Pb, Se); maternal blood (serum) and placental tissue levels (Cd, Pb, Se, Cu); cord blood and placental concentrations (Pb). Of these, placental Ni and Pb appear to repress birth weight (adjusted for gestational age; near significance) in the univariate regression analysis. Lead also acts similarly toward BMIC. This effect is retained only for lead (in placental tissue and whole blood) in the multivariate analysis for both birth weight and BMIC ($p \leq 0.05$ or $\geq 0.05$). Maternal smoking has the same effect. In addition, maternal BMI and age are positively associated with birth weight and BMIC ($p \leq 0.05$ or $\geq 0.05$).

Interpretation of the linear regression analysis

Because placental lead and nickel belonged to the primary group in the principal component analysis (PCA), and thus, like Ca, exhibit a dependance on gestational age, only adjusted birth weight can be considered in any regresional analysis.

The observed impact of maternal blood lead concentrations on birth weight is now well established (for review see 10,12,13). Comparable relationships exist for cord blood lead. There are very few reports of a link between placental lead content and birth weight (14,15). Our observation of this association is not surprising, as it is expected from
the strong relationships found between placental lead and mater- nal, or cord, blood concentrations of this toxic metal (see Table III). While a relationship between placental and cord blood lead was noted by Osman et al. (15), it was absent for the maternal blood and placental lead pair.

Nickel exposure, as measured, by urinary nickel did not show any relationship to birth weight when we focused on the restricted cohort of pregnant women (and the neonate) for whom urinary nickel concentrations were available (8). Since placental nickel lost its statistical significance as a negative factor when examining gestational age-adjusted birth weight, the apparent association is likely related to the membership of this metal to the Factor 1 elements in the PCA (1,2).

The relationship between urine-Ni and -creatinine has been observed previously in male nickel workers and reflects a mutual dependence of these variables on urine flow rate. The latter constitutes the basis for creatinine normalization of urinary nickel (16).

Interpretation of the multivariate linear regression analysis models
Significant, or close to significant, predictors of birth weight in the multivariate models presented in Table VI are: BMI and P-Pb (combined group), maternal age and MB-Pb (Russian group), and maternal smoking and BMI (Norwegian group). Predictors of BMIC include exactly the same variables, except that a non-specific country factor contributes significantly (Table VI). Maternal BMI was very similar in the Norwegian and Russian groups, with no significant difference (Table II).

The positive association between birth weight and maternal BMI is well established (17). These authors demonstrate that pre-pregnancy BMI is an indicator of women’s energy stores before pregnancy, as well as their genetic propensity toward obesity. Unfortunately, in our study, we did not have access to the information needed to calculate pre-pregnancy maternal BMI, only the BMI immediately before delivery. That could be one explanation for the lack of consistency between the multivariate models for the two groups in our study. Maternal height has been shown to be independently associated with birth weight (17,18). However, this could not be demonstrated consistently in our study; it reached significance
only in the univariate analysis for the Norwegian group (weight change of 21 g/cm with 95% CI 6, 36; p < 0.005).

Maternal age is a significant positive contributor in the Russian multivariate model for both birth weight and BMIC (Table VI). This variable is considered to reflect pre-pregnancy weight, weight gain, parity and socio-economic factors known to differ between younger and older women, such as education, smoking and early prenatal care (17,19). In our study, the Russian women were significantly younger than the Norwegian (mean 25.0 versus 28.2) and probably differed considerably in socio-economic status and resources. The retention of the age factor for the Russian group in the multivariate model might therefore have been expected. Cogswell and Yip (17) conclude that much of the difference in pregnancy outcome related to these factors is preventable through education, pregnancy planning and the development of pregnancy care programs. Such programs are now being developed in North-West Russia by the authors.

Maternal smoking as a negative predictor of birth weight is well established in the literature (20,21). In our study the difference in smoking habits, with the Norwegian women smoking substantially more than the Russians, is consistent with the retention of this factor in the Norwegian model. Cadmium, an important toxic substance introduced in the body primarily through smoking (including secondary cigarette smoke) (10), could not be demonstrated as a separate negative predictor of birth weight, or BMIC in our study. Another lifestyle risk factor for low birth weight is alcohol consumption during pregnancy. Unfortunately, this information was not available for many of the respondents, because of a reluctance on their part to report it.

The significant difference in BMIC for our two groups might point to nutritional deficiencies among the Russian group. The Barker theories (22) about associations between malnutrition and cardiovascular diseases and diabetes in adult life fit in well with the recent documentation of increases in cardiovascular disease and diabetes in the adult population of Russia (23). However, Barker’s views are not without controversy (24). Interestingly, Mathews et al. (25) have concluded that maternal intake of macro-nutrients, at least in industrialized populations, seems to have only a small effect on placental and birth weights. By contrast, vitamin C appeared to be the only micro-nutrient predictive of placental weight.
The observed impact of maternal blood lead concentrations on birth weight discussed earlier in the paper and elsewhere (10-12) constitutes an important predictor (Table VI), but not for the Norwegian group, who had considerably lower blood and placenta lead levels. As explained in our previous publications (1,2,10), the prolonged use of leaded petrol in Russia, and perhaps the presence of lead-containing paints in homes, likely explain the higher blood and placental lead levels for the Russian subjects. The strong association of lead levels between compartments explains the link between placental lead and birth weight (13,14). Recent studies (see 26, for example) indicate that the effects of lead on development in young children occurs at lower levels than previously reported, suggesting that no safety margin exists. Elimination of lead exposure therefore remains an important objective.

In a discussion of risk factors for low birth weight, the entire distribution of birth weight must be considered (17). In our study, both the Norwegian and Russian groups exhibited normal distribution patterns, but with significant differences in the mean birth weights and SDs. Only short gestational age seems to affect the low end of the curve (17). In our study maternal age, maternal smoking and BMI seem to affect the entire birth weight distribution, suggesting that these factors have a general effect.

Different studies of pregnancy outcome in Eastern European countries having experienced socio-political changes have been presented recently (27-29). The Estonian study of Koupilova et al. (27) demonstrates that the mean birth weight increased and the pre-term rate decreased in general during the transition. There were significant differences between different social groups. The study also noted, as Nordstrøm and Cnattingius have demonstrated (19), that an increase in variation in birth weight by maternal education was particularly notable. Unfortunately, we did not collect information about maternal education in our study. Nolte et al. (28) conclude that, since the unification of the two parts of Germany, a complex process has led to a convergence of parameters of infant health most likely to be a result of improvements in the quality of pregnancy care and perinatal care. Hesse et al. (29) conclude that the change in socio-economic conditions during the German reunification was associated with a significant alteration in anthropometric measures, even within such a short period. The
mean birth weight increased by 151 g during the period 1984 – 1997, length increased by 0.2 cm, and length-related birth weight increased by 2.3 g/cm. Finally, a potential one obvious two limitation of our study is the lack of information on blood mercury levels and alcohol consumption.

Concluding remarks
A practical conclusion of recent studies of pregnancy outcomes in societies undergoing socio-economic transitions, including our own, is that future work should focus on preventive measures rather than curative work. This has already begun in the Kola Peninsula. Efforts are under way to improve the pregnancy care practices with the help of the World Health Organization and other agencies. Further, a comprehensive birth registry has been set up for the period 2003 back to 1973 through international cooperation, in order to monitor and standardize medical practices and reporting, and to permit retrospective and prospective epidemiological studies (30).

Aknowledgements
This work has been supported by the University of Tromsø, Steering Group of Medical Research in Finnmark and Nordland, and the Royal Norwegian Department of Foreign Affairs, East-European Secretariat. The authors wish to thank the staff at the Obstetric Departments of Bergen, Kirkenes, Hammerfest, Nikel, Monchegorsk and Arkhangelsk for their excellent cooperation in the administration of the questionnaires and collection of specimens. Acknowledgment is also extended to Knut Dalaker, Kåre Augustsen, Babill Stray-Pedersen, Alexander Duriagin, Elvira Khotova, Leonid Zhivakov, Irina Perminova, Jevgenij Bojko, Anatoli Tkatchev, Tone Smith-Sivertsen, Gunhild Sand, Per Einar Fiskebeck and, especially, midwife Marie Hallonen, for their kind support in the different phases of the project.
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