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$\delta^{15}N$ and $\delta^{13}C$ in hair from newborn infants and their mothers: a cohort study

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INTRODUCTION: Protein intake in fetal life or infancy may play a key role in determining early growth rate, a determinant of later health and disease. Previous work has indicated that hair isotopic composition is influenced by diet and protein intake.

METHODS: This study analyzes the isotopic composition of hair obtained from 239 mother/newborn pairs randomly selected within a larger cohort enrolled in a study of pre- and postnatal determinants of the child's development and health. The isotopic compositions in nitrogen ($\delta^{15}N$) and in carbon ($\delta^{13}C$) were determined by isotope ratio mass spectrometry.

RESULTS: Mother and newborn hair $\delta^{15}N$ were tightly correlated (Pearson $r = 0.88$). The mean $\delta^{15}N$ and $\delta^{13}C$ values of hair from newborn infants were significantly higher than those for the mothers: 9.7 ± 0.7 vs. 8.8 ± 0.6‰ ($P < 0.0001$) for $\delta^{15}N$ and -20.0 ± 0.4 vs. -20.4 ± 0.4‰ ($P < 0.0001$) for $\delta^{13}C$. Maternal hair $\delta^{15}N$ at parturition was slightly and positively correlated with estimates of protein intake ($r = 0.14$, $P = 0.04$).

DISCUSSION: Hair $\delta^{15}N$ of the fetus is both highly dependent on and systematically higher than that of the mother. Whether quantitative and qualitative protein intake, disease, or hormonal status alter hair $\delta^{15}N$ at birth remains to be determined.

Early life programming plays a major role in determining health and disease in later life (1). Unsatisfactory size at birth and/or weight gain velocity in childhood are linked to increased risk of onset of coronary events in adulthood (2). Birth weight for gestational age (GA) is the main variable for fetal growth and adequacy of fetal nutrient intake (i.e., used to measure intrauterine growth retardation of placental origin, for instance). However, although several studies have reported an association between protein intake in infancy, growth velocity, and fat mass development in children (3–5), there are currently no reliable simple indexes for fetal protein intake. We know from animal studies that decreasing protein intake during gestation decreases birth weight and shortens life span in mice (6) and that increasing protein intake during gestation results in lower birth weight and higher fat mass at 3 mo in rats (7). Furthermore, in intrauterine growth retardation caused by an experimentally low protein intake, amino-acid transporter expression is affected before growth restriction, suggesting a causative effect of maternal nutrition (8). In humans, intrauterine growth retardation led to increased plasma amino acid concentrations in the mother coupled with decreased levels in the fetus (9), apparently due to impaired amino-acid transfer from mother to fetus (10,11). Protein intake might modulate epigenetic modification of gene expression in the offspring (12–14).

What we seek therefore is a rapid and robust indirect method by which the protein transfer between mother and infant during gestation can be easily assessed. Isotopic values in hair may fulfill this need. It has been shown that the isotopic content of hair is quantitatively correlated with protein source (animal or vegetable) and intake (15,16) in animals and humans including pregnant women (17) and that the $\delta^{13}C$ and $\delta^{15}N$ values of hair and fingernail tissue taken from newborn babies are related to those of their mothers (18). However, not only was there a considerable degree of variability in the mother/infant pairs but also the small population size makes it difficult to establish whether this tendency would be valid over a wide population, as would be required were the relationship to be exploitable as a potential marker of nutritional status. Furthermore, $\delta^{13}C$ and $\delta^{15}N$ of hair were followed in only one subject.

Considering that hair is the easier tissue to sample from newborn infants, we have, within the context of the EDEN mother–child cohort (prenatal and early postnatal determinants of child development and health), analyzed $\delta^{13}C$ and $\delta^{15}N$ for hair samples randomly taken from 239 newborn babies and their mothers in order to assess correlations between isotopic values of mothers and children in a larger population than in previous publications and to study whether there is a relationship between maternal diet and the isotopic values.

RESULTS

Population

Mother and child characteristics did not differ between the Poitiers and Nancy populations (**Table 1**). Diet before

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Table 1. Characteristics of the sample population

Characteristic	Variable[a]	Population			
		Poitiers	Nancy	All	P[d]
	Sample size (n)	166 (69%)	73 (31%)	239	
Mother					
	Age (y)	29.6 (4.6)	29.3 (4.8)	29.5 (4.7)	NS
	BMI (kg/m^2)	23.3 (4.2)	23.1 (4.8)	23.2 (4.4)	NS
	Overweight (25–30) (%)[b]	19 (31)	19 (14)	19 (45)	NS
	Obese (≥30) (%)[b]	7 (12)	7 (5)	7 (17)	NS
	Weight gain (kg)	9.6 (5.6)	7.4 (4.0)	8.9 (5.3)	NS
	$\delta^{13}C$ (‰)	−20.2 (0.4)	−20.7 (0.3)	−20.4 (0.4)	**
	$\delta^{15}N$ (‰)	8.7 (0.7)	9.0 (0.5)	8.8 (0.6)	**
Newborn					
	Sex	64% (107)	52% (38)	61% (145)	NS
	Gestational age (WA)	39.3 (1.7)	39.3 (1.2)	39.3 (1.6)	NS
	Prematurity (<37 WA)[b]	4.8 (8)	1.4 (1)	3.8 (9)	NS
	SGA[b]	7.8 (13)	9.6 (7)	8.4 (20)	NS
	LGA[b]	9.6 (16)	12.3 (9)	10.5 (25)	NS
	Weight (g)	3,369 (513)	3,281 (494)	3,342 (508)	NS
	Length (cm)	50.2 (2.3)	48.6 (2.1)	49.7 (2.3)	NS
	Ponderal index (kg/m^3)	26.5 (2.4)	28.3 (3.0)	27.0 (2.7)	NS
	Head circumference (cm)	34.7 (1.4)	34.5 (1.5)	34.6 (1.4)	NS
	$\delta^{13}C$ (‰)	−19.9 (0.4)	−20.2 (0.3)	−20.0 (0.4)	**
	$\delta^{15}N$ (‰)	9.5 (0.7)	10.0 (0.5)	9.7 (0.7)	**
Last 3 mo of pregnancy					P[e]
	Sample size (n)	146	69	215	
	Energy (kcal/d)	2,469.3 (787.7)	2,345.4 (658.3)	2,429.5 (749.3)	NS
	Proteins—total (g/d)	103.8 (37.2)	103.9 (37.8)	103.9 (37.4)	NS
	Proteins—vegetable (g/d)	26.3 (8.9)	25.0 (8.5)	25.9 (8.7)	NS
	Proteins—animal (g/d)	77.5 (31.6)	79.0 (32.9)	78.0 (31.9)	NS
	Carbohydrates (g/d)	276.1 (87.3)	240.1 (66.4)	264.5 (82.7)	NS
	Lipids (g/d)	104.8 (40.4)	106.7 (37.9)	105.4 (39.6)	NS
	Proteins—total (%DEI)[c]	16.9 (3.1)	17.6 (3.5)	17.1 (3.2)	NS
	Proteins—vegetable (%DEI)[c]	4.3 (0.8)	4.3 (0.9)	4.3 (0.8)	**
	Proteins—animal (%DEI)[c]	12.6 (3.2)	13.3 (3.6)	12.8 (3.4)	NS
	Carbohydrates (%DEI)[c]	45.1 (5.9)	41.6 (6.8)	44.0 (6.4)	NS
	Lipids (%DEI)[c]	37.8 (5.0)	40.4 (5.8)	38.6 (5.4)	*

DEI, daily energy intake; LGA, large for gestational age; NS, nonsignificant; SGA, small for gestational age; WA, weeks of amenorrhea.

[a]Unless otherwise indicated, values are means with SDs in parentheses. [b]Values are percentage of population with n in parentheses. [c]Values are mean percentages of DEI with SDs in parentheses. [d]Comparison between centers: NS $P \geq 0.05$; *$P < 0.01$; **$P < 0.001$. [e]Comparison of intakes before pregnancy and during the last 3 mo of pregnancy: NS $P \geq 0.05$; *$P < 0.01$; **$P < 0.001$.

pregnancy did not differ between these two centers. During the last 3 mo of pregnancy, (i) carbohydrate intake expressed in g/d or % of daily energy intake (%DEI) was slightly higher in Poitiers than in Nancy; (ii) lipid intake (%DEI) was lower in Poitiers than in Nancy; (iii) vegetable protein intake (%DEI) decreased and lipid intake (%DEI) increased in both centers during the last 3 mo of pregnancy. No significant difference was observed for any other parameter of the dietary record (Table 1).

Validation of the Protocol for the Measurement of $\delta^{15}N$ and $\delta^{13}C$ in Hair by Elemental Analyzer-Isotope Ratio Mass Spectrometer

The working sample mass range was determined using one maternal sample and varying the mass of prewashed hair

encapsulated (duplicate samples) from 0.1 to 0.8 mg in 0.1 mg increments. Loss of precision was seen below 0.4 mg (data not shown). Therefore, the sample cutoff was set at 0.4 mg and the mass target at 0.6 ± 0.08 mg.

Feasibility was tested with three mother/infant hair pairs, with and without prewashing. Each pair was analyzed twice and the %C and %N determined. The overall means were %C=45.6 ± 0.3, %N = 14.5 ± 0.9 and C/N = 3.14 ± 0.16. No significant difference was seen for samples with or without prewashing (data not shown). However, for consistency, all samples were prewashed as described earlier.

Repeatability and precision were assessed with 10 samples of prewashed hair from one mother. Values of $\delta^{13}C = -20.03 \pm 0.07‰$ (max, 20.56‰; min, 19.77‰) and $\delta^{15}N = 9.08 \pm 0.13‰$ (max, 9.21‰; min, 8.95‰) were obtained. The precision for the working standard was ± 0.08‰ for both $\delta^{15}N$ and $\delta^{13}C$. Therefore, an acceptable difference between two measurements of hair samples was set to 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$ (95% confidence limit).

Values of $\delta^{15}N$ and $\delta^{13}C$ in Mother/Newborn Hair Pairs by Elemental Analyzer-Isotope Ratio Mass Spectrometer

From the 250 pairs abstracted from the 1,770 available samples in the EDEN cohort, 10 pairs with a technical problem and 1 with insufficient mass were not replaced, meaning that 239 pairs of hair samples were analyzed. These samples all gave measurements in the linear range of the spectrometer with homogeneous values. Samples falling outside the defined acceptable difference between two measurements were reanalyzed or replaced with another sample from the same protein-origin group.

Mean $\delta^{15}N$ for infant hair was systematically significantly higher than the mean $\delta^{15}N$ for the mother's hair ($P < 0.0001$): $\delta^{15}N_{infant} = 9.65 \pm 0.70‰$ and $\delta^{15}N_{mother} = 8.76 \pm 0.62‰$. No case was recorded in which the $\delta^{15}N_{infant}$ was inferior to that of the $\delta^{15}N_{mother}$. Mean $\delta^{13}C$ for infant hair was systematically significantly higher than the mean $\delta^{13}C$ for the mother's hair ($P < 0.0001$): $\delta^{13}C_{infant} = -19.99 \pm 0.40‰$ and $\delta^{13}C_{mother} = -20.36 \pm 0.42‰$. Only one $\Delta\delta^{13}C$ was recorded in which the $\delta^{13}C_{infant}$ was significantly lower than the $\delta^{13}C_{mother}$. As a way of verifying that the samples were not contaminated, the C/N ratios were evaluated. The mean values for mothers and infants were 3.09 ± 0.16 and 2.95 ± 0.12, respectively ($P < 0.0001$). In contrast to the isotope values, no significant correlation was seen for the C/N ratios of mother/infant pairs.

In both mother and newborn hair, mean $\delta^{15}N$ values were lower in the Poitiers population than in the Nancy population, whereas the opposite occurred for $\delta^{13}C$ (Table 1). We did not observe any effect of gender on the mean $\delta^{15}N$ and $\delta^{13}C$ in hair from newborns in either the Poitiers or Nancy groups (data not shown).

Strong correlations were observed between mother/newborn pairs of data. The stronger correlation was for $\delta^{15}N$ (Figure1a: Pearson correlation coefficient $r = 0.88$ (0.84–0.90)), but a clear correlation was also observed for $\delta^{13}C$ (Figure1b: Pearson correlation coefficient $r = 0.74$ (0.68–0.79)). It was particularly noticeable that the one pair seen as an outlier in $\delta^{15}N$ still fits the mother/newborn correlation (Figure1a,b). The mean $\delta^{15}N$ for newborn hair was systematically higher than the mean $\delta^{15}N$ for mother's hair (Table 1). Similarly, the mean $\delta^{13}C$ for newborn hair was systematically higher than the mean $\delta^{13}C$ for the mother's hair (Table 1). In only one case was the $\delta^{13}C_{newborn}$ significantly lower than the $\delta^{13}C_{mother}$.

Isotope values were also compared for mother/infant pairs from small-for-GA (SGA), appropriate-for-GA (AGA), and large-for-GA neonate populations. The birth weight of the SGA population ($n = 20$) was significantly lower than that for the AGA population ($n = 219$): SGA birth weight 2,582 ± 301 g vs. AGA birth weight 3,412 ± 465 g ($P < 10^{-4}$), but this had no impact on the $\delta^{15}N$ value for hair (SGA 9.82 ± 0.68‰ vs. AGA 9.63 ± 0.71‰, not significant). Similarly, mothers of SGA children did not have a lower $\delta^{15}N$ than mothers of AGA children (8.90 ± 0.75 vs. 8.78 ± 0.61‰, not significant). GA and age at birth of the mothers with SGA babies were not different from those of mothers of babies with an AGA weight. The BMI of mothers with an SGA child was lower than that of mothers of AGA children (20 ± 2 vs. 24 ± 4 kg/m^2, $P < 0.001$), and their total energy intake during pregnancy was higher (2,851 ± 1,125

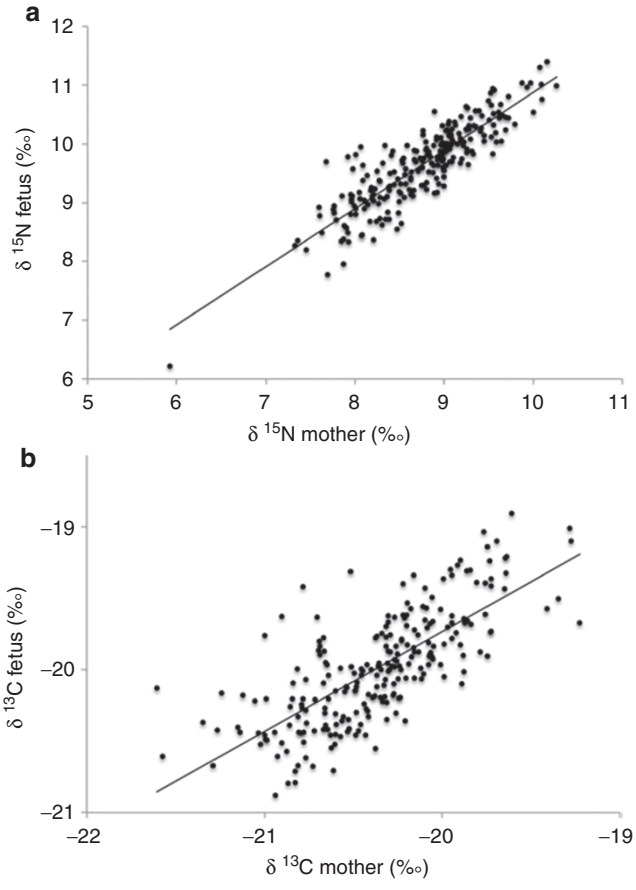

Figure 1. Correlation between (**a**) $\delta^{15}N$ (‰) values and (**b**) $\delta^{13}C$ (‰) values for hair samples from mother/newborn pairs. For $\delta^{15}N$ (‰), linear correlation is $y = 0.99x + 0.99$, $R^2 = 0.76969$, Pearson correlation coefficient $r = 0.88$ (0.84–0.90). For $\delta^{13}C$ (‰), linear correlation is $y = 0.70x - 5.77$, $R^2 = 0.55018$, Pearson correlation coefficient $r = 0.74$ (0.68–0.79). Confidence limits (95%) of the measurements are 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

kcal/d vs. 2,396 ± 704 kcal/d, $P < 0.05$). No significant differences were observed between isotope values ($\delta^{13}C$ or $\delta^{15}N$) of hair in mother/infant pairs for large-for-gestational age infants vs. the AGA population.

In mothers but not in newborns, $\delta^{15}N$ was positively associated with estimates of intake of protein, specifically animal protein, and lipids during the last 3 mo of the pregnancy (Table 2). The $\delta^{13}C$ in mothers and newborns was negatively associated with mean energy, carbohydrate, and lipid intakes (Table 2). We did not observe any correlation between $\delta^{15}N$ and $\delta^{13}C$ in mothers and newborns and estimates of energy, total and animal proteins, lipids, and carbohydrates before pregnancy (data not shown). Head circumference was significantly smaller in newborns in the upper quartile of hair $\delta^{15}N$ values ($P = 0.004$ and $P = 0.06$ comparing the upper quartiles vs. the three lower quartiles of, respectively, newborn and mother $\delta^{15}N$ values after adjustment for GA). Newborn hair $\delta^{15}N$ showed a negative correlation with GA (−0.14; Table 2) and children within the upper quartile were of lower GA at birth (38.9 ± 1.9 vs. 39.5 ± 1.4 wk, $P < 0.05$). No significant correlation was observed with any other parameter of fetal growth, although a negative trend was found with birth weight (Table 2).

DISCUSSION

The data obtained in this study yielded three main results. First, the $\delta^{15}N$ and $\delta^{13}C$ values for individual mother/newborn pairs were strongly correlated. Second, the $\delta^{15}N$ and $\delta^{13}C$ values for newborn hair were consistently higher than for the mother. Third, a slight positive correlation between the $\delta^{15}N$ of mother's hair at parturition and the total protein intake in the last trimester of gestation was observed.

The $\delta^{15}N$ and $\delta^{13}C$ values of hair from newborns were strongly correlated with those of their mothers in the entire population tested. That the correlation is less strong for $\delta^{13}C$ is to be expected because the carbon pool is influenced by glucose and lipid metabolism as well as protein, whereas the nitrogen pool is specific to protein metabolism. Because the fetus obtains its entire nutrition from the mother, this might at first sight not be surprising. However, the linear aspect of the correlation surprised us. The common belief is that fetal metabolism is the priority. If that were the case, we would expect a stronger relationship between maternal and fetal values for low values of $\delta^{15}N$ than for high values. A linear relationship suggests that fetal protein metabolism might be directly determined by the mother.

Whether the mother's protein intake may affect fetal protein metabolism and growth remains to be tested. It must be emphasized that this study aimed at establishing whether there exists a robust correlation between maternal protein intake and $\delta^{15}N$ from the hair of mothers and newborns within the normal population. The correlation was not found for newborn $\delta^{15}N$. Many factors (level of protein intake, protein percentage derived from vegetal or animal sources, energy intake, nutritional status, whole-body protein turnover in each mother and child pair) were not controlled in this cross-sectional observational study and could have their own effect on fetal protein metabolism and ^{15}N transfer to hair protein. In addition, we were not able to detect an association between fetal growth restriction and $\delta^{15}N$. This warrants further studies in pregnant animals to determine how maternal nutrition

Table 2. Partial correlations for hair $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) in mothers and newborns

	Correlation[a]			
	Mother		Newborn	
Characteristic	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Mother				
BMI (kg/m^2)	0.03 (0.61)	−0.03 (0.66)	0.06 (0.36)	0.01 (0.84)
Weight gain (kg)	−0.12 (0.07)	−0.005 (0.94)	−0.04 (0.56)	−0.08 (0.21)
Gestational age (WA)	−0.03 (0.60)	−0.14 (0.40)	0.06 (0.40)	−0.11 (0.09)
Newborn				
Weight (g)	−0.03 (0.69)	−0.11 (0.10)	−0.07 (0.29)	−0.10 (0.16)
Length (cm)	−0.01 (0.85)	−0.08 (0.22)	−0.02 (0.76)	−0.04 (0.51)
Ponderal index (kg/m^3)	−0.02 (0.74)	−0.04 (0.56)	−0.08 (0.24)	−0.06 (0.33)
Head circumference (cm)	0.036 (0.38)	−0.13 (0.05)	0.02 (0.80)	−0.13 (0.04)
Last 3 mo of pregnancy				
Energy (kcal/d)	−0.18 (0.01)	0.12 (0.08)	−0.15 (0.03)	0.09 (0.21)
Proteins—total (g/d)	−0.02 (0.77)	0.14 (0.04)	−0.03 (0.61)	0.12 (0.08)
Proteins—vegetable (g/d)	−0.06 (0.40)	0.08 (0.27)	−0.13 (0.05)	0.06 (0.36)
Proteins—animal (g/d)	−0.007 (0.92)	0.14 (0.04)	−0.003 (0.96)	0.12 (0.07)
Carbohydrates (g/d)	−0.20 (0.003)	0.05 (0.50)	−0.15 (0.03)	0.01 (0.92)
Lipids (g/d)	−0.19 (0.01)	0.15 (0.03)	−0.16 (0.02)	0.12 (0.07)

WA, weeks of amenorrhea.

[a]Values are Pearson's correlation coefficient adjusted for center and gestational age with *P* value in parentheses.

and intrauterine growth retardation determine ^{15}N transfer from mother to fetus.

Less obvious, however, is why the $\delta^{15}N$ values for hair were consistently higher in newborns than in their own mothers. Because the amino-acid pool of the fetus comes entirely from that of the mother, it might be expected that these two pools would be at equilibrium. However, as pointed out by several authors (16), this observation is compatible with the well-described trophic effect: the $\delta^{15}N$ value for protein increases through the food chain from plants to herbivores to omnivorous species such as humans (15,16).

The factors leading to differential $^{15}N/^{14}N$ retention in omnivorous species need to be further studied, but a number of mechanisms have been put forward. It has been suggested that hepatic transamination favors ^{15}N retention in body proteins(19). Studies using amino acids labeled with stable isotopes showed that leucine (20,21) or glutamine (22–24) turnover was higher in premature babies than in term babies as well as adults. It can be speculated that the high protein turnover of the fetus may augment transamination, thus favoring ^{15}N retention.

In mothers, an increase in nitrogen retention in the later stages of gestation, a decrease in urea excretion and synthesis (25,26), and a decrease of branched-chain amino-acid transamination (27) will all act to lower the $\delta^{15}N$ in hair of mothers and increase the differential between a mother and her offspring. This is consistent with a study showing a decrease in $\delta^{15}N$ of maternal hair throughout pregnancy (17) and with studies of eating disorders (28) and digestive malfunction (29), both of which lead to depletion in ^{15}N.

Head circumference at birth was smaller in babies within the upper quartile of $\delta^{15}N$. This intriguing observation persists when data are adjusted to GA. The effect of maternal protein intake on brain development is poorly documented. A recent article showed that a 30% reduction in total energy intake resulted in structural abnormalities of the brain without any change in brain mass (30). On the other hand, a 10-g/d increase in protein intake led to an 18-g reduction in birth weight without any observed change in head circumference (31). While clearly observational studies such as ours cannot test the effect of protein intake on brain development, they do support a need for further investigations, initially through animal studies. Others nutrients, such as fatty acids, are associated with food proteins and their impact needs to be taken into account.

The slight correlation between stable isotope ratios ($\delta^{15}N$) in mothers' hair and dietary protein intake during the last 3 mo of pregnancy is, in contrast, readily explained. Such correlations exist because the $\delta^{15}N$ values of the food intake are influenced by the nature of the protein source consumed. Thus, vegans (people who do not eat any animal-derived food) have a low $\delta^{15}N$ relative to the average for the population, whereas vegetarians (people who do not eat any animal food) have values midway between vegans and omnivores (15). In accordance, we found a significant correlation mainly with proteins of animal origin. The value of the correlation coefficient (0.14) is not very high because food frequency questionnaires do not allow a precise measurement of nutrient intake and this finding needs further investigation. Our study is observational and establishes that the correlation previously indicated (15–17) is robust within a large population. Thus, thanks to the large cohort available, this study suggests that hair $\delta^{15}N$ values show promise as a nutritional biomarker in European **postpartum** mothers and their newborn babies, for example, in potentially estimating the impact of maternal protein intake on fetal growth in normal and obese women (32). Controlled studies are now necessary to test the direct impact of protein intake on $\delta^{15}N$ of mothers' hair and newborns.

METHODS

Ethics Statement

The study was conducted according to the principles expressed in the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Bicêtre hospital (Kremlin-Bicêtre, France) on 12 December 2002. Written consent was obtained from the mother for herself at inclusion and for her newborn child after delivery.

Subjects

The EDEN mother–child cohort is a study of the prenatal and early postnatal determinants of child development and health from birth to 5 y (**Figure 2**). Pregnant women seen for a prenatal visit between February 2003 and January 2006 at the Departments of Obstetrics and Gynecology of the University Hospitals of Nancy (France) and Poitiers (France) before 24 wk of amenorrhea were invited to participate. Exclusion criteria were twin pregnancies; known diabetes mellitus before pregnancy; French illiteracy, plan to move out of the district.

Dietary Data

A food frequency questionnaire was completed twice by each mother: once at recruitment (on average 15 wk of amenorrhea) and a second time during the first few days postparturition. These allowed an estimation of food intake in the first and third trimester of pregnancy, respectively (33). Energy and nutrient intakes were computed from questionnaires with portion size determined using pictures (34) and the SU.VI.MAX nutrient composition database (35).

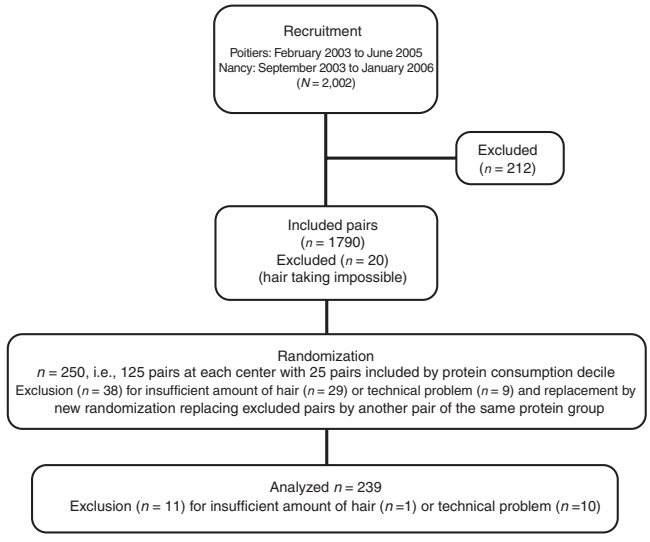

Figure 2. Inclusions flowchart.

Clinical Data

At a first visit performed between 24 and 28 wk of amenorrhea by midwife research assistants, maternal height was measured with a wall stadiometer (Seca 206; Seca, Hamburg, Germany) to the nearest 0.2 cm and maternal weight was measured using electronic scales (TerraillonSL 351; Hanson, Hemel Hempstead, UK) to the nearest 0.1 kg. Weight before pregnancy was obtained by interview. Pre-pregnancy BMI was computed by the standard formula. By reference to the International Obesity Task Force, overweight was defined as a BMI ≥ 25 kg/m^2 and obesity as a BMI ≥ 30 kg/m^2. GA at delivery (determined from the date of the last menstrual period and early ultrasound assessment), newborn admission to an intensive care unit or neonatal unit, birth weight, length, and head circumference were extracted from clinical records, and mother's weight after delivery was obtained with the same protocol as above.

In the two obstetric departments, electronic Seca scales (Seca 737 in Nancy and Seca 335 in Poitiers) were used to measure newborn weight and a wooden somatometer (Testut, Béthune, France) to measure newborn length. Large-for-gestational-age and SGA neonates were defined as babies with a birth weight over the 90th percentile and below the 10th percentile, respectively, of French GA and gender-specific reference curves (36). AGA neonates are defined as between the 90th and 10th percentile.

Hair Sample Collection and Preparation

Hair samples were collected from 1,770 pairs within the cohort. On the basis of the dietary data, the population was divided into 10 equal groups according to protein intake; 250 pairs were randomly taken (125 from each center), with 25 pairs being included from each protein group. The choice of number of pairs ≈250 was calculated so as to show a correlation coefficient ≈0.2 with a power of 90% and an α risk of 5%. Eleven pairs were not included in the final data set due to technical problems with analysis.

Mother and baby hair samples were taken 3 d after birth. A tuft of hair was cut in the occipital area as close as possible to the scalp. We deliberately chose to analyze a "random" sample of maternal hair so as not to bias the data toward a particular period of pregnancy. The longest hair present was selected and the hair root was not included. Samples were blind-coded with an identification number for each pair and labeled "M" or "E" to indicate mother or newborn baby, respectively. The complete hair sample of infants and a representative hair sample of mothers were transferred to a small glass bottle and cut into small sections (1 mm or less). This sample was washed in cyclohexane (20 min) to remove sebum (lipids) and residue (e.g., shampoo). Residual traces of solvent were removed by evaporation at 45°C under a stream of pure nitrogen gas until completely dry. An aliquot of each sample (~0.6 mg, giving ~0.08 mg N) was weighed with 10^{-5} g precision (balance; Ohaus Discovery DV215CD, Pine Brook, NJ) into two tin capsules (solids "light" 5 ×9 mm, Thermo Fisher Scientific, Bremen, Germany, http://www.thermo.com). Each hair sample was analyzed in duplicate (with a few exceptions where the infant sample mass was insufficient to prepare two capsules). When the total sample mass was <0.4 mg, the pair was excluded and replaced by new pair from the same protein group; 38 mother/child pairs required replacement, the substitute samples being selected by the same randomized procedure.

Isotope Analysis

The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios and the N and C percentage compositions were determined as described previously (14). Briefly, capsules containing hair samples were flash combusted in an oxygen atmosphere using an elemental analyzer Flash EA 1112 HT (Thermo Fisher Scientific) and the resultant gases (CO_2 and N_2) carried in a stream of He to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific) coupled online via a Conflo III interface (Thermo Fisher Scientific). Following sample combustion, water was removed by a $Mg(ClO_4)_2$ water trap and N_2 was completely separated from CO_2 using a Porapak Q gas chromatography column (Thermo Fisher Scientific). Ion currents were measured for *m/z*28, 29, 30 and *m/z* 44, 45, 46, for N_2 and CO_2, respectively, from which the $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values could be calculated. Stable isotope ratios were expressed as the $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) ratios relative to reference pulse peaks of laboratory CO_2 and N_2 respectively, calculated as follows

$$\delta\,(‰) = \left(\frac{R}{R_{std}} - 1\right) \times 1{,}000,$$

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ isotope ratio of the sample and R_{std} is the $^{13}C/^{12}C$ isotope ratio of Vienna Pee Dee Belemnite reference standard (R_{std} = 0.0112372) or of atmospheric N_2 (R_{std} = 0.0036765). Normalization was made using a working standard of glutamic acid defined as having an isotopic composition of $\delta^{15}N = -4.80 \pm 0.08$‰ and $\delta^{13}C = -27.48 \pm 0.05$‰ relative to these standards.

The C/N ratio was calculated in hair samples from the weight percentages determined from the integrated peak areas for CO_2 and N_2 from the mass spectrometer ion currents and sample weights. The same working standard (glutamic acid) was used for the N and C weight percentage determinations.

Statistical Analysis

Data were initially processed using Microsoft Excel 2003. All statistical analyses were carried out using SAS 9.1.3 (SAS Institute, Cary, NC) on the AIX 5.1 platform (IBM, New York, NY). Pearson correlations and partial Pearson correlations taking into account center and GA were used to assess the strength of the association between mother or newborn $\delta^{15}N$ and $\delta^{13}C$ values, and anthropometry or diet, respectively. Confidence interval for correlation coefficients have been computed using the Fisher's z transformation. To study the shape of the relationships, means of anthropometric variables were estimated by quartiles of $\delta^{15}N$ and $\delta^{13}C$ (adjusted for potential confounders: i.e., GA and/or center) using multivariate general linear regression models

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