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CORRECTION

Correction to: Human baby hair amino acid natural abundance ^{15}N-isotope values are not related to the ^{15}N-isotope values of amino acids in mother's breast-milk protein

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The Authors wish to correct the data published in the original article. Detailed information is given in the full ERRATUM Article.

Introduction

In the article (Romek et al. 2013) we reported the values of $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) obtained by isotope ratio monitoring by mass spectrometry (irm-MS) in a series of samples taken from mother's milk and the hair of their babies after 1 month of exclusive breast feeding. We examined first the bulk values for the whole milk ($\delta^{15}N_W$ (‰) and $\delta^{13}C_W$ (‰)) and hair ($\delta^{15}N_H$ (‰) and $\delta^{13}C_H$ (‰)) samples by irm-MS coupled with an elemental analyser (irm-EA/MS), then the $\delta^{15}N$ (‰) for the individual amino acids ($\delta^{15}N_{AA}$ (‰)) obtained by acid hydrolysis of the protein fraction. The later data was obtained from the derivatized amino acids by gas chromatography coupled to an irm-MS (irm-GC/MS).

We reported (in Fig. 1 of Romek et al. 2013) a large difference in the bulk values ($\delta^{15}N$ (‰) and $\delta^{13}C$ (‰)), measured by irm-EA/MS for the whole milk relative to the infant hair. We have recently had occasion to revisit these samples and to measure the isotope values for the protein present in the milk ($\delta^{15}N_P$ (‰) and $\delta^{13}C_P$ (‰)). To our concern, this was overall 2–3‰ richer relative to the values obtained for the whole milk (see Supplementary Information, Table SI1). This was surprising as the major source of N in human milk is the proteins (Carratù et al. 2003). In addition, measurement of the $\delta^{15}N$ (‰) values for the non-protein N in the milk indicated a value lower than that of the protein (data not shown) but insufficiently so to explain the difference in $\Delta\delta^{15}N_{(P\text{-}W)}$ (‰) observed. We therefore ran several pairs of samples for whole milk and milk protein on a different mass spectrometer and found that the two measurements gave comparable values (data not shown), indicating a possible error in the initial values as reported for whole milk. Re-examining the initial data we have reached the regrettable conclusion that a large part of the difference previously reported was generated by a technical problem in the irm mass spectrometer used. In this apparatus, the N_2 and CO_2 generated by combustion of the sample in an oxygen-rich atmosphere are separated by passage through a GC column and then passed to the MS. The isotope ratio is obtained from the ratio of the current generated from the different ions at *m/z* 28, 29 and 30 for N_2 and at *m/z* 44, 45 and 46 for CO_2. Due to the high concentration of lactose, the C/N ratio in whole milk is approx. 10-fold that of milk protein (Table SI1). In the first series of measurements on whole milk, the eluting peak for CO_2 swamped the signal for the tail of the eluting peak for the ^{15}N signal, causing a switch in the detector from N_2 to CO_2 before the former peak was fully eluted. This was only apparent on close examination of the

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chromatogram in magnified conditions (Fig. SI1). However, it had a differential impact on the mV values for the signals at *m/z* 28 and *m/z* 29, meaning that the $\delta^{15}N_W$ (‰) values computed from the areas under the peak had a lower value than they should have.

In this corrigendum, we wish to put right this error by presenting the $\delta^{15}N_P$ (‰) values for the bulk protein extracted from the milk samples and to indicate how this impacts on the discussion of the data. Crucially, because the paper focussed largely on the $\delta^{15}N_{AA}$ (‰)) values obtained from the protein, the overall conclusions of the paper are not affected.

Experimental

Protein extraction from whole milk

Milk was thawed, homogenised by vigorous vortex mixing and an aliquot (1 mL) taken to prepare the milk protein fraction. The sample was de-fatted essentially by the procedure of Bligh and Dyer (1959). To 1 mL milk in a glass centrifuge tube was added 1 mL H_2O, 2 mL MeOH and 2 mL CH_2Cl_2. Following vigorous vortexing, the sample was centrifuged (4500*g*, 15 min, 4 °C). Protein precipitated as a white layer at the aqueous/organic interface. The liquid layers were removed and the protein precipitate was vigorously washed with a mixture of 2 mL H_2O, 2 mL MeOH and 2 mL CH_2Cl_2 then again recovered following centrifugation as above. The pellet was suspended in cold (− 20 °C) pure acetone and left overnight at − 20 °C. Following centrifugation (4500*g*, 15 min, 4 °C), the pellet was carefully recovered, washed with acetone (− 20 °C) and, following centrifugation and removal of the acetone, dried at room temperature. The resulting while pellet was ground to a fine homogeneous powder and used for isotope analysis.

Analysis of total $\delta^{13}C$ and $\delta^{15}N$ values of milk and hair samples

For milk or protein ~ 5 or ~ 0.7 mg, respectively, was weighed with 10^{-6} g precision (balance, Ohaus Discovery DV215CD, Pine Brook, New Jersey, USA) into each of two tin capsules (solids "light" 5 × 9 mm, Thermo Fisher Scientific, http://www.thermo.com) for isotope analysis.

The $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) isotope ratios were obtained by isotope ratio measurement by mass spectrometry (irm-MS) using an Integra2 spectrometer (Sercon Instruments, http://www.sercongroup.com) linked to a Sercon elemental analyser in three configurations:

1. With the window for N_2 set from 120 to 205 s (Fig SI1a). These were the conditions used previously (Romek et al. 2013).

   In the re-evaluation two options were used:
2. Samples were passed in 'N-only' mode, without switch to measure the CO_2 peak.
3. The window for N_2 was set from 120 to 250 s (Fig SI1b).

Results

Isotope measurements of $\delta^{15}N$ (‰) values in whole lyophilised milk and in protein obtained from milk

In Fig. 1 is presented the comparable $\delta^{15}N$ (‰) values for the 42 samples taken from the study for which protein ($\delta^{15}N_P$ (‰)) could be obtained. This shows that the initial values obtained for whole milk (($\delta^{15}N_W$ (‰)) analysed by Method 1 were systematically lower than those for the protein ($\delta^{15}N_P$ (‰)) extracted from the same sample and analysed by Method 3. (Methods 2 and 3 gave comparable data on a subset of 6 samples (data not shown)).

In order that both ($\delta^{15}N_P$ (‰) and $\delta^{13}C_P$ (‰) could be measured, method 3 was adopted. This had the advantage that the $\delta^{13}C_g$ (‰) values could also be checked. The $\delta^{13}C_P$ (‰) values were systematically higher than those for the whole milk (Fig. SI2), indicating a strong influence of the lactose present on the $\delta^{13}C_W$ (‰) values. Similarly, the $\delta^{15}N_P$ (‰) values were systematically higher than those for the whole milk (Fig. 1), but in this set of data the difference can be explained by the difficulties with the irm-MS measurements (Table SI1). It is notable that the range of values for $\delta^{15}N_P$ is lower (2.2‰) for the protein samples than for the whole milk analyses (4.4‰).

Figure 2 shows the distribution on a 2D isotope plot for $\delta^{13}C$ and $\delta^{15}N$ (‰) values for total hair and total milk protein for the 42 samples measured [Figure equivalent to Fig. 1

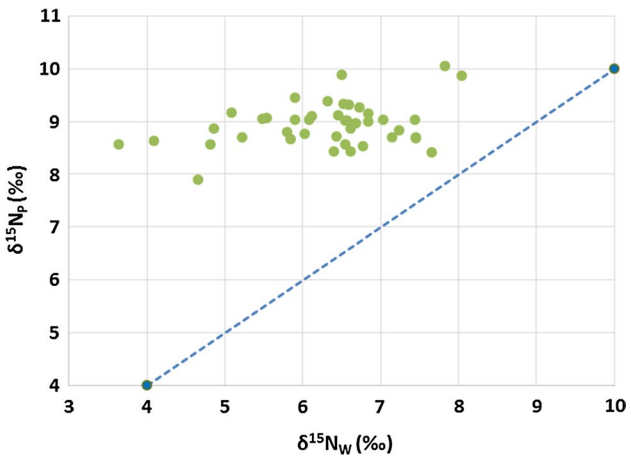

Fig. 1 2D isotope plot for $\delta^{15}N_W$ versus $\delta^{15}N_P$ (‰) values for whole milk and total milk protein, respectively. Each data point represents one sample, analysed by irm-EA/MS, following Method 1 ($\delta^{15}N_W$) or Method 3 ($\delta^{15}N_P$). The broken line indicates the 1:1 correlation fit

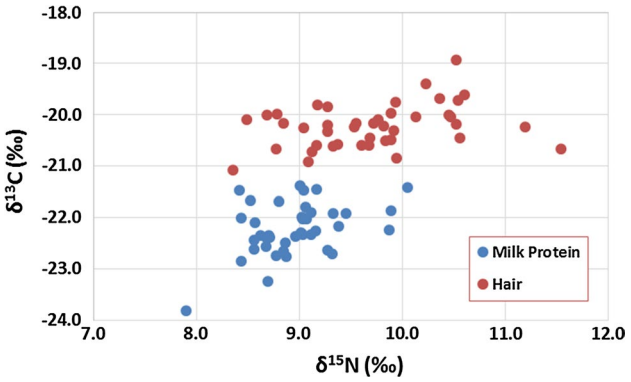

Fig. 2 2D isotope plot for $\delta^{13}C_g$ and $\delta^{15}N_g$ (‰) values for total hair and total milk protein. Each data point represents one mother–infant pair, analysed by irm-EA/MS

of (Romek et al. 2013)]. Despite the smaller size of the separation between the clusters for hair and for milk protein, as previously observed, the milk protein and hair samples cluster as two distinct groups separated from each other. The extent is lower: a mean value of 0.8‰ on the $\delta^{15}N$ axis and 1.9‰ on the $\delta^{13}C$ axis. The infant hair group is still found to be richer in heavy isotope for both elements ($\delta^{15}N$, $P < 0.01$; $\delta^{13}C$, $P < 0.01$).

Correlation of the isotopic composition of individual amino acids in a mother: infant population

No correlation was observed between the infant hair $\delta^{15}N_H$ values and the mother milk protein $\delta^{15}N_P$ values ($R^2 = 0.201$, $N = 46$, $P > 0.05$) as found for the whole milk (Fig. SI3).

On the basis of the amino acid compositions of hair and milk protein, determined by irm-GC/MS (Tea et al. 2013) as reported in (Romek et al. 2013), the $\delta^{15}N$ data for each sample can be used in combination with the percentage composition of each sample to calculate the overall $\delta^{15}N$ for each sample of milk, milk protein and hair. This can then be compared with the data obtained by direct measurement with EA-irm-MS data. In Table 1 are given the values previously reported, with the values for milk protein included. This shows that the calculated value for the milk protein is lower than the measured value, the inverse of that for the whole milk. This does not modify the overall argument made in (Romek et al. 2013).

Discussion

The second and third paragraphs are modified to read

Prior to focussing on the individual amino acids and then the individual mother–infant pairs, it is necessary to discuss to what extent there is variation within the populations. The 42 samples of milk taken from breast-feeding mothers 1 month post-parturition gave a mean value of $\delta^{15}N_P = 8.95 \pm 0.42$‰—Table SI1) with a 75%/25% quartile range of only 0.43‰. This indicates that the mothers were eating a regular diet, introducing a low level of variation into the population. The 42 samples of hair from 1-month-old infants for which the milk protein was measured gave a mean value of $\delta^{15}N_H = 9.7 \pm 0.7$‰ with a 75%/25% quartile range of only 0.8‰, indicating that fluctuation in the $^{15}N/^{14}N$ ratio was of the same order as in the maternal nutrient supply.

Despite this, the $\delta^{15}N_P$ and $\delta^{13}C_P$ values for the milk protein samples are significantly lower ($P < 0.05$) than the equivalent $\delta^{15}N_H$ and $\delta^{13}C_H$ values for infant hair at 1 month old (Fig. 2, Table SI1) as well as mother's hair and infant hair at birth (de Luca et al. 2012). We found an excellent agreement of the natural $\delta^{15}N$ or $\delta^{13}C$ values in hair (9.7 ± 0.7‰ for $\delta^{15}N$, -20.2 ± 0.46‰ for $\delta^{13}C$, $N = 42$, see Table SI1) with the values determined in our earlier study (de Luca et al. 2012), in which these parameters were measured at birth in hair taken from new-born infants from the same vicinity (9.7 ± 0.7‰ for $\delta^{15}N$ and -20.0 ± 0.4‰ for $\delta^{13}C$, $N = 249$). In that study, maternal hair $\delta^{15}N$ (8.8 ± 0.6‰), while always lower than that of the new-born infant for each mother–infant pair, was significantly superior to the milk values presented here (Table SI1). Overall, then these data indicate that there is neither a dramatic shift in N-metabolism from intra- to exo-uterine status nor a wide variation in the breast-fed population. They also indicate that it is the ^{15}N in milk that is relatively impoverished, rather than that the infant protein is enriched. This might reflect fractionation in

Table 1 Comparison of $\delta^{15}N$ (‰) values obtained by irm-EA/MS with those obtained by calculation based on the individual $\delta^{15}N$ and quantities of amino acids obtained by irm-GC/MS

Sample	Milk			Milk protein			Hair		
	Calc $\delta^{15}N$ (‰)	EA $\delta^{15}N$ (‰)	Δ (C-E)	Calc $\delta^{15}N$ (‰)	EA $\delta^{15}N$ (‰)	Δ (C-E)	Calc $\delta^{15}N$ (‰)	EA $\delta^{15}N$ (‰)	Δ (C-E)
N	41	45	40	41	42	41	13	33	8
Mean	7.55	5.91	1.51	7.55	8.95	− 1.40	10.37	9.46	1.41
SD	1.25	1.09	1.16	1.25	0.42	0.83	1.39	1.05	2.18

^{15}N of amino acids during the synthesis of milk proteins in lactatory glands, or be due to the non-protein nitrogenous compounds present in milk. Human milk protein content is principally composed of casein (30%), α-lactalbumin (30%), lactoferrin (20%), immunoglobulin IgA (10%), lysozyme (5%), and albumin (5%) (Davis et al. 1994). Human milk also has a variable non-protein nitrogen content, which may contribute up to 30% of total nitrogen, primarily accounted for by urea, with lesser amounts of uric acid, ammonia, creatinine, and traces of free amino acids and growth factors (Carratù et al. 2003).

The fifth paragraph is modified to read

A key further observation is that the difference between hair and milk $\delta^{15}N$ values reported in Fig. 2 is maintained moderately well when the measured and calculated values of $\delta^{15}N$ (‰) are estimated. The calculated value for the protein is about 1.4‰ lower than for the measured, while the difference is in the opposite sense for the hair values. Nonetheless, the fit is reasonable in both cases, especially taking into account (1) that not all amino acids are measured in the $Calc\delta^{15}N_g$ values and (2) that the composition of hair and milk proteins are considerably different. Consequently, we can eliminate the hypothesis that the difference is purely an effect of the amino acid composition of the two materials analysed: otherwise, the shift ($\Delta Calc\delta^{15}N$ (H-M)) should be negligible.

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