THE USE OF NUCLEAR MAGNETIC RESONANCE FOR ASSESSMENT OF TIME VARIATION IN COMPOSITION OF HUMAN MILK AND COLOSTRUM: A CASE STUDY DURING PREGNANCY AND POST-PARTUM*

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Abstract: Nuclear Magnetic Resonance (NMR) Spectra were collected on milk and colostrum obtained from the author during the pregnancy and post-partum periods for two births. Peak features in the downfield region, containing antibody and aromatic resonances (6.0 to 9.0 ppm), varied over time. This time variation pattern, while nearly identical for milk following the author's two births, differed dramatically from the colostrom of another woman in labor. A possible pattern unique to each woman should be further investigated by monitoring NMR spectra on a larger sample.

Downfield peak pattern variations were less intense between five women in later stages of lactation. Other spectral regions showed only minor variations with time and patterns did not differ significantly. These five women excreted proteins and/or small aromatic compounds in a time variant pattern assessable with NMR spectroscopy.

Key words: milk colostrum protein carbohydrates post-partum nuclear magnetic resonance (NMR) antibody aromatic fatty acids pregnancy lactation birth midwife

INTRODUCTION

Epidemiological evidence associates breast feeding with many long and short term health benefits (1). Human milk possesses a variety of anti-infective properties (2) and immune factors (3). Human colostrum IgA and IgM autoantibodies may be crucial to proper development of the immune system (4). Many papers have reported that human milk composition varies with the state of lactation (5, 6, 7, 8, 9, 10, 11, 12). Concentration of fatty acids (13) and various macronutrients (14) have

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been shown to vary with lactation time. Various immunological properties, including the concentration of secretary IgA, have been shown to decline over the first three months of lactation (15, 16).

Nuclear Magnetic Resonance (NMR) has been performed on a range of body fluids including human milk (17). Thus, NMR appears as a possible tool for monitoring milk composition variations over time. I have tested the feasibility of this method by collecting a series of NMR spectra on samples of human milk and colostrum. Subjects included myself and four women other than myself. The samples were collected at defined times pre- and post-delivery, and the resulting spectra suggest potential longitudinal patterns in human milk composition.

METHODS

The majority of samples were collected from the author before the births of her second and third children. Both deliveries took place at home with a midwife. The author was lactating from a previous birth at the onset of her second and third pregnancies. The author’s milk dried up between three and five months into both of these pregnancies. The author was able to express colostrum at five months into her second pregnancy and at approximately seven months into her third pregnancy. This colostrum made a visible transition into milk at approximately 48 hours post-partum for both births. The author began her second pregnancy when her oldest child was four years. There was a 29 month time lapse between the second and third births.

Milk and/or colostrum samples were obtained from four women other than the author. One of these samples was collected from a woman in labor. Two women gave samples at seven months post-partum, and one woman gave a sample at 14 months post-partum. All milk and colostrum samples were collected in 1–10 ml quantities and frozen immediately. Samples were thawed, shaken and D2O was added to approximately 10% of sample volume. Any sample not frozen was prepared immediately for NMR data collection.

Proton spectra were collected at 25°C on a Varian Unity spectrometer operating at a proton frequency of 500 MHz. Each experiment was run for approximately one hour using a 90 degree pulse and a recycle delay of 7.8 seconds. The water resonance was presaturated for reduction of intensity. Samples were referenced comparatively against a sample of tsp in water.

RESULTS AND DISCUSSION

Fig. 1 shows the variation in the spectral region between 6.0 and 9.0 ppm for milk and colostrum samples taken from the author. This region contains aromatic and amide resonances. Since amide resonances are found throughout the backbone of antibody proteins, variations in this region could reflect variability in antibody characteristics and concentration. It is evident from Fig. 1 that time dependent post-partum changes in the author’s milk follow a similar pattern for both births.

Resonances falling between 6.8 and 8.0 ppm are present before both births, and...
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The region of 7.8 ppm at two weeks and at 22 hours before the author's second birth. (See arrow in column 1 of Fig. 1A and 1B.) The relative intensity of this second birth peak pattern is diminished at 3 hours following delivery (arrow in first column of Fig. 1C), and is almost gone at 21 hours post-partum (arrow in first column of Fig. 1D). The peak at 7.8 ppm is also present one month before the author's third birth (arrow in second column of Fig. 1A), is diminished in intensity by six hours before delivery (arrow, in second column of Fig. 1B), and changes its features by four hours post-partum (arrow in second column of Fig. 1C). Unlike the second birth this peak pattern persists at 21 hours post-partum (arrow in second column of Fig. 1D). At 50 hours following the third birth this pattern is no longer present (second column of Fig. 1E). It is noteworthy that, in both births, this pattern drops intensity before delivery and disappears within a few days of delivery as colostrum transitions into milk. This 7.8 ppm peak may reflect aromatic resonances located on antibody or hormonal pregnancy-associated proteins. Such proteins may have a protecting function for the infant at birth, and the infants would not have been exposed to these proteins had they not been nursed shortly following delivery.

Fig. 2 contains spectra of samples taken from women all other than the author. Fig. 2A shows a sample taken from a woman in labor. The peak pattern for this woman shows some considerable differences from the sample taken before the author’s two birth (Fig. 1B). For example, Fig. 2A shows a sharp peak at about 6.8 ppm. This peak is broader in the author’s spectra. Fig. 2A
Fig. 2: Downfield region of milk and colostrum samples from subjects other than the author. A: Colostrum sample from subject 1 collected in labor within 10 hours of delivery. B: Milk sample collected from subject 2 approximately 7 months post-partum. C: Milk sample collected from subject 3 approximately 7 months post-partum. D: Milk sample collected from subject 4 approximately 14 months post-partum.

shows another sharp resonance near 7.15 ppm whereas Fig. 1 colostrum spectra show a possible triplet in this region. The peak pattern in the region of 7.0 to 7.9 ppm is considerably different between Fig. 1B and Fig. 2A. The peak pattern present around 7.8 ppm in the author's colostrum is not present in Fig. 2A. The most similarity with the author's milk is seen between 8.0 and 8.6 ppm. Aromatic resonances typically
occur between 6.0 and 8.0 ppm. Thus, these results raise the possibility that aromatic compounds of human colostrum could be unique to each woman. It is noteworthy that the time-dependent variations in aromatic resonances of the author's milk were identical between her births. This result suggests that each woman may have a specific repertoire of antibodies or other aromatic containing compounds that are expressed in her colostrum and change following delivery. This hypothesis could be further investigated with a study on a larger group of women.

Figs. 2B, 2C and 2D show spectra taken from women at seven and fourteen months post-partum. Note that, by these later times, the signal intensity from this region has dropped considerably and patterns are more difficult to discern. However, the basic peak patterns are similar among these spectra and between these spectra and those of the author at six months post-partum (Fig. 1I). The only notable differences occur at around 8.0 ppm. According to these results, aromatic and amide-containing compounds secreted at later stages of lactation are more consistent than those secreted in colostrum.

Fig. 3 shows the peak patterns for resonances between 0 and 6.0 ppm for milk and colostrum samples taken from the author. Peaks below 2.5 ppm correspond to resonances typical of fatty acid side chains. Peaks between 3.0 and 4.0 ppm fall in the region of carbohydrate ring resonances. It is evident from Fig. 3A that the ratio of carbohydrate to fatty acid-like resonances is very low in early pregnancy. The peak pattern for the 3.0–4.0 ppm region showed a pattern that was identical between both

of the author's early pregnancies (Fig. 5A and 5B). However, this pattern differed from that observed consistently for all other stages of lactation and for all women whose milk or colostrum was sampled in this study. (Fig. 5C through 5H). The author was lactating at the time she became pregnant, and a spectrum of milk immediately before pregnancy is not available. Thus, it is
unclear whether the lowered intensity and altered peak patterns in the 3.0–4.0 range result from the onset of pregnancy or from extended lactation following a previous pregnancy.

Peak pattern for the 0–6.0 ppm resonance region shows little variation for up to six months post-partum (Fig. 3B through 3D). However, relative intensity of peaks does vary. The colostrum sample (Fig. 3B) shows a higher intensity of carbohydrate peaks relative to fatty acid peaks. This relative intensity declines as lactation time progresses (Fig. 3C and 3D). The peak present at approximately 2.0 ppm in all spectra shows a remarkably increased intensity in the colostrum spectrum (Fig. 3B). This peak decreases in intensity with increasing time post-partum. The relative intensity of the peak at 1.25 ppm in the author's colostrum varies between the two births (Fig. 3B).

Fig. 4 shows the 0–6.0 ppm range of the spectra shown in Fig. 2. Peak patterns do not vary significantly among these or between these and the author's spectra shown in Fig. 3. However, relative peak intensities do vary. Fig. 4A shows a colostrum sample where the carbohydrate resonances are considerably lower than the resonance at 2.0 ppm. This is not the case for the spectrum of the author's colostrum shown in Fig. 3B. Fig. 4D shows the spectrum of a client at 14 months post-partum where the peaks in the carbohydrate region are significantly higher than all other peaks in the 0–6 ppm range. This is different from the author's spectra where relatively high intensity of carbohydrate peaks is observed only in the colostrum (Fig. 3B).
Thus, concentration of compounds giving rise to peaks in the 0–6 ppm range varies somewhat between the author's two births and varies extensively between the author's spectra and that of two other women (Fig. 4A and 4D). Fig. 4B and 4C, taken from two different clients both at approximately seven months post-partum, show spectra with peak patterns and relative intensities that are comparable to that of the author's at six months post-partum (Fig. 3D). The relative high intensity of the 2.0 resonance was seen only in colostrum samples (Fig. 3B and 4A).

Conclusion

This work is suggestive of some noteworthy trends for molecules expressed in human milk at various lactation stages. The aromatic region of the colostrum spectrum (7.0–7.9 ppm) showed the greatest peak pattern variability. This peak pattern was nearly identical for colostrum samples collected at comparable times relative to the author's second and third births. However, for colostrum samples within 24 hours of delivery, the aromatic region of the author's spectrum differed dramatically from that of another subject.

The time-dependent peak profile of the aromatic region shows protein peak variability over as few as four hours post-partum. (See arrows in Fig. 1). These results indicate that the hours immediately following delivery represent a dynamic period for colostrum composition. It may be especially important for infants to nurse during this time when unique colostrum molecules are expressed. Thus, hospital practices, where babies are removed from

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Fig. 5: Expansion of resonances in the carbohydrate frequency range. A: Milk collected from author two weeks into her second pregnancy. B: Milk collected from author 17 weeks into her third pregnancy. C: Author's colostrum three hours following her second delivery. D: Author's milk six months following her third delivery. E: Colostrum from subject 1 collected in labor within 10 hours of delivery. F: Milk from subject 2 at seven months post-partum. G: Milk from subject 3 at seven months post-partum. H: Milk from subject 4 at approximately 14 months post-partum.
their mothers for the first few hours postpartum, may warrant reconsideration.

The carbohydrate region (3.0-4.0 ppm) showed reduced intensity and modifications in peak patterns for early pregnancy. The aliphatic region (0-6.0 ppm) showed nearly identical peak patterns for all women at all stages of lactation. However, relative aliphatic peak intensities did vary. Further studies, involving NMR spectra on milk and colostrum from a larger sample of women, would determine if the trends of this study reflect general patterns in human lactation.

REFERENCES