Carbohydrate content of human milk is affected by seasonal variations: a retrospective observational study

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Research

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Abstract

Background

Previous studies have suggested seasonal variation in macronutrient content of milk produced from animals. The influence of seasonal variation upon human milk macronutrient content has not been elucidated. This study aimed to compare the macronutrient content of HM produced by lactating mothers during the winter and the summer seasons.

Methods

We compiled previously generated data on macronutrient content of colostrum milk samples collected from lactating mothers of healthy term infants. The mothers were recruited during their postpartum stay at the Lis Maternity Hospital of the Tel Aviv Medical Center. Macronutrient content was measured by mid-infrared spectroscopy.

Results

The carbohydrate content of the colostrum was significantly higher in the summer season than in the winter season (5.9 ± 1.3 vs 5.4 ± 1.4 g/100 ml, p value <0.001). Protein, fat, and energy contents were similar in both groups.

Conclusion

The carbohydrate content in colostrum obtained from mothers of term infants was affected by seasonal variations.

Background

Breastmilk is a unique and optimal source of nutrition for the health and development of infants (1, 2). The composition of human milk (HM) shows great variability depending upon many factors. There are known mother-to-mother (3), day-to-day (4), and day-to-night (5) variations in HM macronutrients. Moreover, the composition of HM varies as lactation progresses over time (6), as maternal age increases (7, 8), and as gestational age at delivery varies (9, 10). We have recently demonstrated the effect of maternal pre-pregnancy body mass index (BMI) (11) and the effect of infant’s sex on HM macronutrient content (12).

Seasonal variation may have an effect on human milk macronutrient through complex interplay of psychological, emotional, physiological, neurochemical and hormonal mechanisms (13). Indeed, several studies have documented effect of seasonal variation on the composition and physical properties of
cow’s milk (14–17). Furthermore, Davis et al. demonstrated that human milk oligosaccharides profiles were significantly affected by the seasonal changes that were attributed to energy intake and overall output (17). To the best of our knowledge, the effect of seasonal variations on HM macronutrient (fat, protein, and carbohydrate) content has not been investigated to date. Therefore, we reviewed HM macronutrient data from relevant published studies conducted at our department with the aim of testing the null hypothesis that macronutrient content of HM is not affected by the season in which the infant was born and breastfed.

**Materials And Methods**

**Participants**

This is a retrospective analysis of data on the macronutrient content of HM generated in previously published studies from our group (8, 11, 18). The study was approved by our local institutional review board, and informed consent was waived for this anonymous data analysis. The data were handled in accordance with the principles of GCP.

Healthy lactating mothers of healthy singleton term infants (37 to 41 completed weeks of gestation by last menstrual period confirmed by early first trimester ultrasonographic dating) were included. We excluded all births that involved major obstetrical and neonatal complications, such as pregnancy-induced hypertension, maternal diabetes, plastic surgery of the breast, known dyslipidemia, and neonatal asphyxia and/or major neonatal complications. The collected demographic data included maternal age and BMI, mode of delivery, infant gestational age, gender, birth weight and birth date.

The entire investigation took place in central Israel, an advanced subtropical country situated at the eastern end of the Mediterranean Sea in Western Asia. Israel has a Mediterranean climate with hot, rainless summers and cool, rainy winters.

**Sample collection**

In order to maintain uniformity between the subjects and to represent a period that also reflects the season towards the end of the pregnancy, only colostrum samples, taken up to 72 h post-delivery were used for analysis. The samples had been obtained by hand or pump expression any time in the morning at the mother’s convenience. They were then classified according to season of delivery. Seasons in Israel were defined according to the Encyclopedia Britannica as having a cool, rainy winter between October and April, and a dry, hot summer between May and September (https://www.britannica.com/place/Israel/Climate).

**Laboratory methods**

Macronutrient (fat, total protein, and carbohydrate) and energy content were measured by means of the Human Milk Analyzer (HMA, from Miris, Uppsala, Sweden), an instrument based on mid-infrared transmission spectroscopy, as reported in detail in our previous publications (5, 8, 9, 11, 12). Just prior to
analysis, each frozen sample was initially heated to 40°C in a thermostatic bath, and then homogenized with an ultrasonic technique (MIRIS milk sonicator, Miris), as recommended by the manufacturer. The analyses were performed following the manufacturer’s protocol.

**Statistical analyses**

The data were analyzed with the Statistical Package for the Social Sciences software version 27 (SPSS Inc., Chicago, IL). All statistical tests were two-sided. The Kolmogorov-Smirnov test and the Shapiro-Wilk test were applied to assess the normality of continuous data. The data were expressed as mean ± standard deviation (SD) and range for normally distributed variables and median ± SD and range for skewed distributions. Pearson's Chi-square test was performed to compare the distribution of categorical variables. An independent sample t-test or an independent sample Mann-Whitney test was performed to compare between groups for continuous variables with normal or skewed distribution, as appropriate. A $p$ value $\leq .05$ was considered significant.

**Results**

A total of 189 colostrum samples were used for analysis. Demographic characteristics of the participating mothers and newborns are presented in Table 1. There were 96 infants born during the winter season and 93 during the summer season. An independent t-test revealed that maternal age and infant birth weight were similar in both groups, as were, gestational age, and maternal BMI by Mann-Whitney U tests. There were no significant differences between the groups in the mode of delivery, male-to-female infant ratio, and percentage of small-for-gestational age infants (by Chi-squared tests). Table 2 depicts the total protein (based upon total nitrogen content), carbohydrate, fat, and energy content of the colostrum samples by season of delivery. The Mann-Whitney U tests demonstrated that the carbohydrate content of the colostrum was significantly higher in the summer season than in the winter season (6.2 ± 1.3 vs 5.5 ± 1.4 g/100 ml, $p = < .001$). A trend for higher fat content in the summer was also noted (2.35 ± 1.9 g/ml vs 2.6 ± 1.9 g/ml, $p = 0.07$). Protein and energy content in both groups remained similar; specifically, the protein content was 2.6 ± 2.2 g/ml vs. 2.7 ± 2.1 g/ml, and the energy content was 60.5 ± 21 Kcal/ml vs 62 ± 19.1 Kcal/ml in the winter and summer seasons, respectively, $p = > .05$ for all, Table 2).

**Discussion**

This study is a part of an ongoing research that aims at determining the effects of selected environmental, clinical, and demographic variables upon HM constituents. We found that the carbohydrate content was significantly higher in the summer season (May to September) than in the winter season (October to April) in the colostrum of the HM of lactating Israeli mothers of healthy term infants. Our findings are contrary to our initial hypothesis.

The effects of seasonal variation on composition and physio-chemical properties of raw milk had been described decades ago in animal studies (14–18). Saadi et al. compared the milk composition in cattle
fed either on green grass or on concentrates during winter and summer periods. Those authors showed that the highest percentage of fat was found in the milk of cattle fed on green grass in the winter (16). Heck et al. reported lower fat and protein contents in bovine milk in summer than in winter (17). Few studies, however, have examined the effect of seasonal variation on human breast milk output and macronutrient contents (20, 21). A recent study by Davis et al. assessed the HM oligosaccharides (HMO) composition of breast milk in 33 rural Gambian mothers. They reported that mothers nursing in the wet season (July to October) produced significantly less HMO compared to those nursing in the dry season (November to June) (22). Similarly, Bates et al. documented seasonal variations for ascorbic acid serum levels (23). However, all of these studies had been conducted in countries with a poorly developed economy, unlike the current work.

Seasonal variation in macronutrients in the milk of animals seems obvious. Animals are more vulnerable to changes in weather conditions that can affect workload, food availability, and disease burden (14–17). Moreover, seasonal variability affects the light-to-dark ratio that, in turn, can affect bovine milk production and chemical ingredients through its effect on prolactin concentration (19). However, seasonal variation also affects human physiology and health. In a study by Prentice et al that was conducted four decades ago among rural African mothers, breast milk fat concentrations were affected by season in a manner that correlated with seasonal changes in maternal subcutaneous fat stores, and were unrelated to seasonal variations in maternal energy intake and breast milk output (20). Seasonality in nutrient intakes and meal patterns have also been documented in several studies (24–27). Although daily total caloric intake did not vary significantly by season in some of them (24, 25), others showed differences related to season in HM macronutrient composition (27) that was accompanied by change in specific food ingredients consumption, such as milk products, oils and fats, fruits, and vegetables (25).

Moreover, the effect of seasonal variation upon HM macronutrients may be mediated by a third factor, such as serum vitamin D levels, which has pronounced seasonal variations and a known systemic effect on glucose homeostasis and insulin sensitivity (28). Cross-sectional studies, including large-scale population studies (29, 30), have shown a significant positive relationship between serum 25-hydroxyvitamin D (25(OH)D) and measures of insulin sensitivity. This relationship has been corroborated in a variety of populations, including pregnant women (31). Since the vitamin D receptor is expressed in normal mammary glands (32), it is possible that variations in the levels of sun exposure affects the serum levels of 25 OH and 1,25-dihydroxycholecalciferol (1,25OHD) which modulates multiple cellular pathways, including those related to energy metabolism and glucose utilization and production.

The mechanism and biological significance of the increase in carbohydrates in colostrum during the summer period remain to be determined. We are aware that this effect seen in our study cannot be attributed to seasonal food shortage or morbidity, as described in developing countries (20–22). We consider that changes in the mothers’ diet or vitamin D serum levels in the summer season might have been instrumental in leading to increased carbohydrate content in their milk's colostrum.
The present study has several limitations. First, HM was expressed either manually or by pump and the sampling time varied throughout the day. While we and others have shown that there are circadian variations in HM composition (9), and that fat content is affected by mode of expression (33), there remains the possibility that this inconsistency in sampling had masked small macronutrient content differences despite the large number of milk samples included in the study. Furthermore, three different investigators performed the milk analysis with the HMA, which might have led to significant scattering of the data, as discussed in a recently published study by Kwan et al (34). Lastly, we only measured macronutrients, and it would be valuable to determine whether micronutrients (vitamins and minerals) and other biologically active components (enzymes, hormones, antioxidants, and so on) of HM exhibit a seasonal variation in their distribution. The major strength of this study lies in its contribution to the sparse knowledge on the effect of seasonal variations of macronutrient content in the colostrum obtained from mothers of healthy term infants in a developed country. If confirmed in larger, prospective studies, these findings may encourage different dietary and supplements recommendations for lactating women in the winter months.

**Conclusion**

In this study, protein, fat, and energy content of colostrum were similar in HM obtained in both the winter and summer seasons in our subtropical country. The carbohydrate content of colostrum, however, was higher during the summer. The mechanism behind the seasonal variation of carbohydrate levels in colostrum is unclear and warrants additional studies.

**Abbreviations**

HM  
human milk  
BMI  
body mass index  
SGA  
small for gestational age  
HMO  
human oligosaccharides  
25(OH)D  
25-hydroxyvitamin D  
1,25OHD  
1,25-dihydroxycholecalciferol

**Declarations**

**Ethics approval and consent to participate:** The study protocol was approved by the “Helsinki” institutional review board of the medical center. Informed consent was waived because of the
The retrospective nature of the study and the analysis used anonymous data. The data were handled in accordance with the principles of the Good Clinical Practice (GCP).

Consent for publication - not applicable

Competing interests - The authors declare that they have no competing interests.

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Authors' contributions - HML, LM and DM conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript.
RL, LM, SV and RM designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.
RL and DM conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.
All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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References

1. Ballard O, Morrow AL. Human milk composition. Pediatr Clin North Am 2013;60:49–74.
2. Eidelman E, Schanler R. Breastfeeding and the use of human milk, section on breastfeeding. Pediatrics 2012;129;e827.
3. Harzer G, Haug M, Dieterich I, Gentner PR. Changing patterns of human milk lipids in the course of the lactation and during the day. Am J Clin Nutr. 1983;37:612-21.
4. Mitoulas LR, Kent JC, Cox DB, Owens RA, Sherriff JL, Hartmann PE. Variation in fat, lactose and protein in human milk over 24h and throughout the first year of lactation. Br J Nutr. 2002;88:29-37.
5. Lubetzky R, Mimouni FB, Dollberg S, Salomon M, Mandel D. Consistent circadian variations in creamatocrit over the first 7 weeks of lactation: a longitudinal study. Breastfeed Med. 2007;2:15-8.
6. Mandel D, Lubetzky R, Dollberg S, Barak S, Mimouni FB. Fat and energy contents of expressed human breast milk in prolonged lactation. Pediatrics 2005;116:e432-5.
7. Hausman Kedem M, Mandel D, Domani KA, Mimouni FB, Shay V, Marom R, Dollberg S, Herman L, Lubetzky R. The effect of advanced maternal age upon human milk fat content. Breastfeed Med. 2013;8:116-9.
8. Lubetzky R, Sever O, Mimouni FB, Mandel D. Human Milk Macronutrients Content: Effect of Advanced Maternal Age. Breastfeed Med. 2015 Jul 14. -Not available-, ahead of print. doi:10.1089/bfm.2015.0072.
9. Lubetzky R, Littner Y, Mimouni FB, Dollberg S, Mandel D. Circadian variations in fat content of expressed breast milk from mothers of preterm infants. J Am Coll Nutr. 2006;25:151-4.

10. Gidrewicz, D.A., Fenton, T.R. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. BMC Pediatr 14, 216 (2014).

11. Mangel L, Mimouni FB, Feinstein-Goren N, Lubetzky R, Mandel D, Marom R. The effect of maternal habitus on macronutrient content of human milk colostrum. J Perinatol. 2017;37:818–21.

12. Mangel L, Morag S, Mandel D, Marom R, Moran-Lev H, Lubetzky R. The Effect of Infant's Sex on Human Milk Macronutrients Content: An Observational Study. Breastfeed Med. 2020 Sep;15(9):568-571.

13. Krauchi K, Wirz-Justice A. The four seasons: food intake frequency in seasonal affective disorders in the course of year. Psych Res, 198, 25(3): 323–338.

14. Chen B, Lewis MJ, Grandison AS. Effect of seasonal variation on the composition and properties of raw milk destined for processing in the UK. Food Chem. 2014 Sep 1;158:216-23.

15. D'Alessandro AG, Martemucci G, Jirillo E, De Leo V. Major whey proteins in donkey's milk: effect of season and lactation stage. Implications for potential dietary interventions in human diseases. Immunopharmacol Immunotoxicol. 2011 Jun;33(2):259-65.

16. Saadi AM, Hasan GM. The Effect of Nutrition and the Seasons of the Year on the Composition of Cow's Milk in Two Different Areas of the Province of Mosul. Annals of Agri-Bio Research 24 (1) : 148-152, 2019.

17. Heck JM, van Valenberg HJ, Dijkstra J, van Hooijdonk AC. Seasonal variation in the Dutch bovine raw milk composition. J Dairy Sci. 2009 Oct;92(10):4745-55.

18. Shapira D, Mandel D, Mimouni FB, Moran-Lev, Marom R, Mangel L, Lubetzky R. The effect of gestational diabetes mellitus on human milk macronutrients content. J Perinatol. 2019; 39(6):820-823.

19. Ozrenk, E. and Inci, S. S. (2008). The effect of seasonal variation on the composition of cow milk in Van Province. Pak. J. Nutr. 7 : 161-164.

20. Prentice A, Prentice AM, Whitehead RG. Breast-milk fat concentrations of rural African women. 1. Short-term variations within individuals. Br J Nutr. 1981 May;45(3):483-94.

21. Whitehead RG, Rowland MG, Hutton M, Prentice AM, Müller E, Paul A. Factors influencing lactation performance in rural Gambian mothers. Lancet. 1978 Jul 22;2(8082):178-81.

22. Davis JC, Lewis ZT, Krishnan S, Bernstein RM, Moore SE, Prentice AM, Mills DA, Lebrilla CB, Zivkovic AM. Growth and Morbidity of Gambian Infants are Influenced by Maternal Milk Oligosaccharides and Infant Gut Microbiota. Sci Rep. 2017 Jan 12;7:40466.

23. Bates CJ, Prentice AM, Prentice A, Paul AA, Whitehead RG. Seasonal variations in ascorbic acid status and breast milk ascorbic acid levels in rural Gambian women in relation to dietary intake. Trans R Soc Trop Med Hyg. 1982;76(3):341-7.
24. Hackett AF, Appleton DR, Rugg-Gunn AJ, Eastoe JE. Some influences on the measurement of food intake during a dietary survey of adolescents. Hum Nutr Appl Nutr. 1985 Jun;39(3):167-77

25. Rossato SL, Olinto MT, Henn RL, Moreira LB, Camey SA, Anjos LA, Wahrlich V, Waissmann W, Fuchs FD, Fuchs SC. Seasonal variation in food intake and the interaction effects of sex and age among adults in southern Brazil. Eur J Clin Nutr. 2015 Sep;69(9):1015-22

26. de Castro JM. Seasonal rhythms of human nutrient intake and meal pattern. Physiol Behav. 1991 Jul;50(1):243-8.

27. Prentice, A. M., Whitehead, R. G., Roberts, S. B. & Paul, A. A. Long-term energy balance in child-bearing Gambian women. The American Journal of Clinical Nutrition 34, 2790–2799 (1981).

28. Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol. 2010;2010:351385

29. Scragg R, Sowers M, Bell C; Third National Health and Nutrition Examination Survey. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care. 2004 Dec;27(12):2813-8

30. Chonchol M, Scragg R. 25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutrition Examination Survey. Kidney Int. 2007 Jan;71(2):134-9

31. Clifton-Bligh RJ, McElduff P, McElduff A. Maternal vitamin D deficiency, ethnicity and gestational diabetes. Diabet Med. 2008 Jun;25(6):678-84

32. Welsh J. Function of the vitamin D endocrine system in mammary gland and breast cancer. Mol Cell Endocrinol. 2017 Sep 15;453:88-95.

33. Mangel L, Ovental A, Batscha N, et al. Higher fat content in breastmilk expressed manually: A randomized trial. Breastfeed Med 2015;10:352–354.

34. Kwan C, Fusch G, Rochow N, et al. Milk analysis using milk analyzers in a standardized setting (MAMAS) study: A multicentre quality initiative. Clin Nutr 2020;39:2121-2128.

Tables

Table 1

Demographic and clinical characteristics of mothers and neonates
| Variable                  | Winter season (October - April) | Summer season (May - September) | p value |
|---------------------------|---------------------------------|---------------------------------|---------|
| Maternal age, years       | 33.2 ± 4.4 (20 - 43)            | 32.8 ± 4.2 (22 - 41)            | .556    |
| BMI*                     | 22.7 ± 5.4 (15.6 - 49.5)        | 21.8 ± 5 (16.3 - 37.8)          | .914    |
| Cesarean section         | 16 (16.7)                      | 17 (18.3)                       | .770    |
| Gestational age*, weeks   | 39 ± 1.2 (37 - 41)              | 39 ± 1 (37 - 41)                | .403    |
| Birth weight, g           | 3291.8 ± 460.2 (2335 - 4354)    | 3337.4 ± 413.2 (2510 - 4505)    | .475    |
| Male sex                 | 55 (57.3)                      | 42 (45.2)                       | .095    |
| SGA                      | 8 (8.3)                        | 2 (2.2)                         | .06     |

Data are expressed as mean or *median ± standard deviation (range) or n (%).

Note: BMI = Body Mass Index; SGA = Small for Gestational age.

**Table 2**

Macronutrient and energy content of colostrum milk according to season of infant birth
| Colostrum milk samples | Winter season (October - April) | Summer season (May - September) | p value |
|------------------------|--------------------------------|--------------------------------|---------|
|                        | N = 96                         | N = 93                         |         |
| Fat, g/100 ml          | 2.35 ± 1.9 (0.1 - 9.8)         | 2.6 ± 1.9 (0.5 - 10)           | .075    |
| Protein, g/100 ml      | 2.6 ± 2.2 (0.1 - 8.7)          | 2.7 ± 2.1 (0.8 - 9.2)          | .391    |
| Carbohydrate, g/100 ml | 5.5 ± 1.4 (0.8 - 7.2)          | 6.2 ± 1.3 (1.3 - 8.3)          | < .001  |
| Energy, kcal/100 ml    | 60.5 ± 21 (22 - 124)           | 62 ± 19.1 (26 - 130)           | .165    |

Data are expressed as median ± standard deviation (range)