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Decreased Protein and Increased Triglycerides and EPA Concentrations in Human Colostrum Produced Three to Five Days Postpartum by Japanese Mothers

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Abstract

Human colostrum（HC）is generally considered to provide optimal nutrition for newborns. While all breast milk produced up to around the 5th day postpartum is collectively called HC, it is assumed that the composition of HC may differ from day to day. The purpose of this study was to investigate time-dependent changes in the composition of HC, in terms of protein, triglycerides（TG）and fatty acids（FA）. The study participants were 48 lactating mothers（aged 16−38 years）who gave birth from November 2010 through March 2011 in Kagawa, Japan. On days 3, 4 and 5 after giving birth, HC was collected from each mother. The protein level was quantified using a modified Lowry's method. The TG level was quantified using a commercially available kit. FAs were extracted using a modified Folch method and quantified with gas chromatography-mass spectrograph. Compared to day 4 HC or day 5 HC, the protein levels in day 3 HC were significantly higher and the TG levels were significantly lower. Compared to day 4 HC or day 5 HC, the eicosapentanoic acid（EPA）levels day 3 HC were also significantly lower. The EPA/arachidonic acid ratio increased significantly from day 3 to day 4 and from day 4 to day 5. It is suggested that the composition of HC may change from day to day. These findings may be specific to Japanese mothers due to the typical Japanese diet.

要旨

日本人母親の産後３日目から５日目の初乳中では，タンパク質濃度は減少し，トリグリセリドとEPA濃度は増加した．一般的に，初乳は新生児にとって最適な栄養であると考えられている．産後５日目頃までの母乳は全て一括して初乳と呼ばれているが，初乳成分は日ごとに変化している事が想像される．本研究では時間依存的な初乳成分（特に，タンパク質，トリグリセリド，及び脂肪酸）の変化を調べる事を目的とした．研究対象者は，2010年11月から2011年３月に香川県で出産した乳婦48名（16－38歳）であった．産後３，４，及び５日目に，各母親から初乳を採取した．タンパク質濃度は改変ローリー法にて測定を行った．トリグリセリド濃度はキットを用いて測定を行った．脂肪酸は改変ホルチ法にて抽出し，ガスクロマトグラフィー質量分析計にて濃度を測定した．産後４日または５日目に比較して３日目初乳中では，タンパク質濃度は有意に高く，トリグリセリド濃度は有意に低かった．また産後４日または５日目に比較して３日目初乳中では，EPA濃度も有意に低かった．産後３日目から４日目，及び産後４日目から５日目へと，初乳中EPA/アラキドン酸比は有意に高くなった．本研究から，初乳成分は日ごとに変化している事が推測される．こういった変化は，典型的な日本食を摂取しているために起きる，日本人母親に特異的なものなのかも知れない．

Key Words：colostrum, breast milk, EPA, Japanese diet

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Introduction

Human colostrum (HC) is the thick, yellowish, and small quantity of breast milk produced by the mother's mammary glands at the end of gestation [1] that is recommended by World Health Organization as the optimal nutrition for newborns [2]. HC is rich in immunologic components such as secretory immunoglobulin (Ig) A, lactoferrin and leukocytes, and provides immunity to a newborn's throat and digestive organs, in addition to providing nutrition [3]. HC is also rich in growth factors such as insulin-like growth factor and epidermal growth factor [4]. From around the 5th day postpartum, when tight junction closure occurs in the mammary epithelium and secretion of milk is activated, the appearance and components of breast milk change such that it is then termed transitional milk [3]. In transitional milk, the sodium to potassium ratio declines while the concentration of lactose increases [3]. At around 2 weeks postpartum, composition of the breast milk becomes fixed, at which time it is termed mature milk [3]. In mature milk, the levels of fat and sugar are increased compared to the levels found in HC, but the levels of Igs, proteins and minerals are decreased [1].

Lipids in breast milk supply about 50% of the daily energy requirements of a baby and are also the source of the essential fatty acids (FA) and fat-soluble vitamins necessary for growth [5]. The FA composition of HC is known to be markedly affected by maternal dietary composition during the latter period of gestation [4]. For example, high concentrations of n 3 long chain polyunsaturated FA (n 3 LCPUFA), such as eicosapentaenoic acid (EPA) (20:5 (n 3)) and docosahexaenoic acid (DHA) (22: 6 (n 3)), have been detected in the HC of mothers living in area of high fish intake [6]. Based on these findings, it was suggested that the levels of fish intake might influenced n 3 LCPUFA levels in HC during the latter period of gestation [4]. DHA is necessary to promote the growth and development of the retina and central nervous system [5].

In adults, arachidonic acid (ARA, 20: 4 (n 6)) is metabolized from linoleic acid (LA, 18: 2 (n 6)), and EPA and DHA are metabolized from alpha-linolenic acid (ALA, 18: 3 (n 3)) (reviewed in [7]). In contrast, mothers provide these FA to infants, directly through breast milk because the insufficient metabolism of infants does not allow them to metabolize the FA on their own [8]. Therefore, it is proposed that FA intake and the balance of n 6 /n 3 FA are important for the regular function of infants [8].

While breast milk up to around the 5 th day postpartum is collectively called HC, it is assumed that the composition of HC may differ from day to day. The purpose of the present study was to determine any time-dependent changes in the composition of HC, in terms of protein, triglycerides (TG) and FA.

Materials and Methods

Study population and sample collection

The present study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki. All procedures were reviewed and approved by the Ethics Committees at Kagawa Prefectural University of Health Sciences (No.53) and Kaisei Hospital (No. 2010-11). Forty-eight pregnant women (aged 16–38 years) were voluntarily enrolled in the study. They provided written informed consent for themselves and for their newborns. The mothers were scheduled to deliver at Kaisei Hospital (located in Kagawa, Japan), between November 2010 and March 2011. The ages and physical characteristics of the mothers were obtained from their hospital records (Table 1). The increase in each mother's body weight during pregnancy was obtained by subtracting the mother's pre-pregnancy body weight from her body weight at delivery. On days 3, 4 and 5 postpartum, a midwife manually collected HC from each mother at 10 am before she was going to feed her baby. Each HC sample was kept at -20℃ or -80℃ until analysis.

HC composition analysis

All chemicals were purchased from Wako Pure Chemical Industries Inc. (Osaka, Japan) or Sigma-Aldrich Co. (St. Louis, MO, USA), unless mentioned otherwise. For protein and TG analyses, HC was diluted 5 -10 fold with distilled water. Protein quantification was performed using a modified Lowry's method [9]. TG quantification was performed using a Triglyceride E-test Wako kit (Wako Pure Chemical Industries Inc.). FA were extracted following a modified method originally reported by Folch et al [10]. Briefly, HC was mixed with a 2 : 1 (v/v) chloroform : methanol mixture containing one μg of heptadecanoic acid as an internal standard. Total lipids were extracted from the chloroform layer and dissolved in hexane. Subsequently, transmethylation was performed using an FA methylation kit (Nacalai Tesque, Inc., Kyoto, Japan). The FA analysis was performed using gas chromatography-mass spectrograph (GC-MS) (Shimadzu GCMS-QP50 50A, Shimadzu Corporation, Kyoto, Japan). The methylated FA were separated with

Table 1. Age and physical characteristics of mothers (n=48)

Age (years)	Height (cm)	Body weight (kg)			BMI	
		(1) Pre-pregnancy (kg)	(2) Delivery (kg)	(3) Increase (3)=(2)−(1) (kg)	Pre-pregnancy	Delivery
30.0 ± 5.3	157.0 ± 4.5	51.5 ± 7.3	62.7 ± 8.5	11.2 ± 3.7	21.0 ± 2.9	25.5 ± 3.6

Data are presented as means ± SD
BMI= body mass index

a DB-5 column (J and W Scientific, Folsom, CA, USA) using helium as the carrier gas. The methylated FA were then identified by a comparison of retention times with authentic standards and from fragmentation patterns in the mass spectra. The EPA/ARA ratio in the HC was calculated by dividing the mean EPA level by the mean ARA level.

Statistical Analyses

Statistical analyses were performed using the Origin Software package (Microcal Software Inc., MA, USA) and Excel (Microsoft, WA, USA) software. Temporal changes of protein, TG and FA levels (Table 2) were tested by repeated measures analysis of variance, and when significant effects were present, post-hoc comparisons were carried out using the Tukey test. Pearson correlation coefficients and their significance were calculated for the relationships between the physical characteristics of the mothers (Table 1) and the levels of protein, TG or FA (Table 2) with the Origin Software package. Data are presented as means ± standard deviation (SD) (Table 1) or means ± standard error of the mean (SEM) (Table 2). Differences were considered significant when p was ≤ 0.05.

Results

Forty-five mothers gave birth vaginally, and three delivered by Caesarean section. The mean height and prepregnancy weight of the mothers (Table 1) were consistent with the means (height : 158.4cm and weight : 51.5kg) for Japanese women aged 16−39 years as reported in 2012 [11]. The body sizes of the babies were also consistent with the Japanese mean[11].

Compared to day 4 HC or day 5 HC, the protein levels were significantly greater in day 3 HC ($p \leq 0.05$, Table 2). The TG and EPA levels in day 3 HC were significantly lower than those in day 4 or day 5 HC ($p \leq 0.05$, Table 2). The DHA level in day 4 HC tended to be higher than that in day 3 HC ($p = 0.07$, Table 2). The levels of stearic acid (STE, 18 : 0), linoleic acid (LA, 18 : 2 (n 6)) and ARA did not differ between day 3 HC, day 4 HC and day 5 HC (Table 2). The EPA/ARA ratio was significantly lower in day 3 HC compared to day 4 or day 5 HC ($p \leq 0.05$, Table 2). The EPA/ARA ratio in day 4 HC was significantly lower than that in day 5 HC.

There were no significant correlations between the mother's physical characteristics (Table 1) and the HC composition (Table 2).

Table 2. Levels of protein, TG and FA in days 3 - 5 HC after delivery (n=48)

		Days after delivery 3	4	5
Protein	mg/ml	1. 66±0. 10	1. 35±0. 06 a	1. 30±0. 04 a
TG	mg/ml	1. 91±0. 12	2. 73±0. 12 a	2. 59±0. 13 a
FA				
STE	mg/ml	0. 21±0. 01	0. 22±0. 01	0. 20±0. 01
LA	mg/ml	0. 55±0. 03	0. 59±0. 03	0. 56±0. 02
EPA	mg/ml	0. 13±0. 01	0. 18±0. 03 a	0. 19±0. 01 a
DHA	mg/ml	1. 39±0. 12	1. 67±1. 22	1. 54±0. 08
ARA	mg/ml	0. 69±0. 05	0. 71±0. 04	0. 62±0. 03
EPA/ARA		0. 19	0. 25 a	0. 31 a,b

Data are presented as means ± SEM
a indicates significant difference compared to Day 3 HC ($p \leq 0.05$)
b indicates significant difference compared to Day 4 HC ($p \leq 0.05$)
STE : stearic acid, LA : linoleic acid, EPA : eicosapentaenoic acid, DHA : docosahexaenoic acid, ARA : arachidonic acid, EPA/ARA : the EPA/ARA ratio

Discussion

In the present study, lower protein levels and higher TG levels were observed in day 5 HC compared with day 3 HC. Although such time-dependent changes in protein and fat were not previously identified in HC, it has previously been reported that the levels of protein decreased and the levels of fat increased in mature milk compared with HC [1]. Therefore, based on the present findings and those of previous reports [1], it is suggested that the decrease of protein and the increase of fat may already begin in day 3 HC and then continue until the production of mature milk.

In a previous study, comparing HC (collected on days 2 – 4 postpartum), transitional milk (collected on days 5 –15 post-partum) and mature milk (collected during three different time periods, specially, days 16–30, days 31–60 and days > 60 postpartum), no significant changes in the levels of n 3 LCPUFA (EPA, DHA, ALA and clupanodonic acid ((22 : 5) (n 3)) were found between HC and transitional milk, whereas the levels of all n 3 LCPUFA decreased significantly from HC to mature milk (> 60 days postpartum)[12]. In another study comparing HC (collected on days 3 – 5 postpartum) and mature milk (collected on days 40–45 postpartum)[13], the levels of EPA and DHA seemed to have decreased during the transition from HC to mature milk, although the authors did not perform any statistical analysis of their results. From these reports, it maybe suggested that the levels of EPA and DHA decrease concomitantly with the transition from HC to mature milk. However, these past studies did not separate HC by day, but instead combined the HC samples collected on days 3 – 4 [12] or on days 3 – 5 [13] postpartum and then quantified the 3 LCPUFA levels. If the levels of n3LCPUFA in HC samples collected on different days postpartum were compared, the EPA or DHA levels in those HC samples from different days could potentially indicate difference not determined by previous studie[12]. Taken together, the findings of the present study indicate that, contrary to the previous belief that the composition of HC is constant, it may actually change significantly from day to day.

The present study found that the EPA/ARA ratio was increased in day 4 HC compared to day 3 HC and in day 5 HC compared to day 4 HC. The ratio of EPA/ARA is an indicator of inflammation[14]. More specifically, an EPA/ARA ratio indicates an increase in the effects of pro-inflammatory eicosanoids and cytokines[14]. Therefore, it may be considered that the increase in the EPA/ARA ratio seen in this study indicates the resolution of inflammation in the mother's body. Consistent with this, it has previously been reported that the number of leukocytes in the mother's body rises from approximately 40 weeks of gestation until childbirth and then decreases promptly after the birth, returning to a normal level at around the 4th day postpartum[15]. From these changes in the mother's leukocyte numbers, it can be speculated that the mother's body may be in a state of inflammation throughout the delivery, before returning to a normal state after the mother has given birth[15]. Therefore, along with the decrease in a mother's leukocyte numbers, it may also be suggested that the time-dependent higher EPA/ARA ratios in HC reflect the resolution of inflammation in the mother's body. Furthermore, the results of the present study regarding the FA composition of HC could reflect a phenomenon specific to the region where the study was conducted, because FA composition in HC has previously been reported to be markedly effected by maternal dietary composition during the latter period of gestation[4].

In a typical Japanese diet, fats are primarily sourced from seafood, such that the mean n3 FA intake (more than 550 mg/day) of the Japanese diets is the highest in the world[16]. Further research into FA levels in HC should be undertaken, with greater attention paid to their relationship with the details of maternal dietary intake.

In the present study, no correlation was observed between the mother's physical characteristics and the HC composition. A 7–12 kg increase in body weight during pregnancy is recommended for Japanese women with an average BMI (18–21.5)[17], and the increase in body weight among the mothers enrolled in this study (11.2 ± 3.7kg, Table 1) was within the normal range. There have been few past reports regarding any correlation between the composition of HC and the mother's physical or physiological states. One study[12] reported no correlation between the mother's weight at delivery and the n3LCPUFA composition of HC. Accumulation of more data in this field of study is needed for better understanding of breastfeeding. Overall, based on the findings of the present study, it is suggested that the composition of breast milk may start to change in the very early days of HC production. This may be proposed as a new definition for HC and transitional milk. Because milk is only one source of nutrients for the newborn, its composition and any changes therein as well as how they may be influenced by the mother's lifestyle during pregnancy are a matter of great importance. Further research into study of breast milk is needed to inform ongoing efforts to ensure the healthy growth and development of infants.

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