The level of bile salt-stimulated lipase in the milk of Chinese women and its association with maternal BMI

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Abstract

This study aimed to investigate the bile salt-stimulated lipase (BSSL) concentration in the milk of Chinese women and its correlation with maternal body mass index (BMI), gestational diabetes mellitus (GDM) and gestational hypertensive disorder (GHD). The BSSL levels in the milk samples were measured by enzyme-linked immunosorbent assay (ELISA). BSSL level in colostrum milk of mothers with full-term infants was positively correlated with pregnancy week and negatively correlated with maternal pre-pregnancy BMI and BMI late in pregnancy. Moreover, the BSSL concentration in mature milk was positively correlated with BMI gain during pregnancy. The BSSL concentration in colostrum milk was lower in GDM mothers than in normal mothers. The BSSL helps infants digest fat in early life and its level was associated with lactation. The changes in BSSL characteristics with maternal BMI and GDM in this study may have clinical implications regarding the effects of pregnancy weight and metabolism on the nutrition and health of the offspring.

Keywords: bile salt-stimulated lipase, breast milk, BMI, gestational hypertensive disorder, gestational diabetes mellitus

Introduction

Bile salt-stimulated lipase (BSSL), also known as carboxylic ester lipase (CEL) and cholesterol esterase, is a lipolytic enzyme that acts on a wide range of substrates, including triglyceride, diglyceride, monoglyceride, cholesterol ester, and fat-soluble vitamin esters, phospholipids, galactolipids, and ceramides[1]. Originally, BSSL was thought to be a digestive enzyme expressed exclusively in the acinar cells of the exocrine pancreas and secreted into the intestinal lumen[1]. However, in some species, including humans and mice but not cows or rats, the BSSL gene is also expressed in the mammary gland during lactation and is secreted into the milk[2]. Recently, BSSL expression has been found in a wide variety of additional cells and tissues, including endothelial cells[3], polymorphonuclear cells[4], macrophages[5], eosinophils[6], platelets[7], and the liver, and it is now considered to be an intriguing multifunctional enzyme with a much broader tissue distribution.

Infancy is a vulnerable period from a nutritional point of view. The rapid growth of newborns requires

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Received 21 October 2018, Revised 26 February 2019, Accepted 09 May 2019, Epub 30 July 2019

© 2020 by the Journal of Biomedical Research. https://doi.org/10.7555/JBR.33.20180107
an energy-dense diet that is rich in fat and essential nutrients. However, a commonly held view is that the exocrine pancreas is immature at birth and that BSSL production is insufficient to support proper fat absorption. Notably, such fat malabsorption is almost exclusively seen in formula-fed infants. Therefore, a more reasonable interpretation is that the digestive functions may be sufficiently developed in breast-fed, but not in formula-fed infants. In 1977, Olle first reported that BSSL is abundantly present in human milk, constituting approximately 1% of the total milk protein in humans. Moreover, active BSSL restores fat malabsorption in BSSL-deficient suckling pups to normal, and the pups do not exhibit intestinal lesions. The survival and growth status of premature mice have been found to be improved in transgenic mice expressing human BSSL. In preterm and low-birth-weight infants, milk-derived BSSL ensures efficient milk lipid digestion and may protect the immature intestinal epithelium from deleterious effects of otherwise incompletely digested milk fat. Moreover, a recent study has shown that recombinant human BSSL improves growth and long chain polyunsaturated fatty acid absorption in preterm infants fed with formula or pasteurized breast milk. Therefore, BSSL is important for the healthy growth and development of infants, particularly preterm infants.

The BSSL level in milk has been reported in Ethiopia, Sweden, Bukavu, Marseilles (France), Nigeria, and Nepal, and BSSL in human milk has been found to change with gestational age, lactation period, and prolactin level. However, the BSSL concentration in the milk of Chinese women is unclear. Moreover, obesity-related biofactors in breast milk, such as adiponectin, leptin, and microRNAs, are influenced by maternal weight and metabolic status or endocrine hormones. Thus, the aim of the present study was to investigate the milk BSSL concentration during lactation in Chinese women and to determine its correlation with maternal weight characteristics. We also sought to determine whether metabolic diseases, such as gestational diabetes mellitus (GDM) and gestational hypertensive disorder (GHD), influence BSSL secretion.

Subjects

Parturients (including those with term and preterm deliveries) who delivered in Nanjing Drum Tower Hospital (the affiliated hospital of Nanjing University) between 1 Dec, 2013 and 31 Dec, 2014 were selected as research subjects in the colostrum group. Parturients underwent an assessment of metabolic status in the second trimester of pregnancy. Pregnant women with a systolic pressure $\geq 140$ mmHg and/or diastolic pressure $\geq 90$ mmHg after 20 weeks of gestation were diagnosed with GHD. Pregnant women who had abnormal glucose values obtained from a 75 g glucose challenge test at 24–26 weeks of gestation and without pre-gravid diabetes were diagnosed with GDM. The exclusion criteria were mothers who had a history of rheumatologic, autoimmune, respiratory, gastrointestinal diseases, or chronic kidney infections, and babies who suffered from birth injury, asphyxia or a history of rescue, congenital diseases, genetic diseases, metabolic diseases or severe neonatal infections. Additionally, pregnant women without complications, such as GDM, GHD, pulmonary edema, chronic nephritis, eclampsia, and intrahepatic cholestasis, during pregnancy and who experienced a term delivery were assigned to the normal group.

Mature breast milk was collected in Children's Hospital of Nanjing Medical University between 1 Dec, 2013 and 31 Dec, 2014. The inclusion criteria were mothers who were healthy and solely breastfeeding. The gestational weeks of delivery were 37 to 42 weeks, and the birth weights of the infants were between 2500 g and 4000 g. The exclusion criteria were mothers who had a history of chronic diseases, GDM, preeclampsia and other immune diseases and metabolic disease, or infectious diseases during lactation.

Data collection

All mothers underwent a medical examination during hospitalization and were interviewed by trained clinicians. A standardized questionnaire form was used to collect laboratory and anthropometric data from each mother's medical record during pregnancy, which included maternal health status, blood biochemical items, delivery characteristics, and anthropometric measurements (maternal height, weight, and blood pressure). All measurements were performed using a standardized protocol with calibrated equipment.

Human milk collection and preparation

The colostrum samples were collected 2–3 days...
after birth, and mature milk samples were collected on the 14th day after birth. Milk samples (2 to 5 mL) were collected by using a hand breast pump before infant feeding between 9:00 a.m. and 11:00 a.m., and were immediately stored at −80 °C. The interval from the last infant feeding was at least 2 hours.

**BSSL quantification by double antibody sandwich ELISA**

The milk samples were centrifuged at 10 000 g for 40 minutes to remove fat, cells, and large debris, and further liquid was collected for BSSL measurement (1.5 mL). A double antibody sandwich ELISA was designed to quantify the BSSL. The 96-well immunoplates (Beyotime, China) were coated overnight with polyclonal anti-human BSSL (donated by Professor Olle Hernell) diluted in carbonate coating buffer (0.1 mol/L Na-carbonate, pH 8.2) to 1 μg/mL at 4 °C. Plates were blocked for 1 hour with 200 μL/well of blocking buffer (1% skim milk) at 37 °C. Standards (n-BSSL, donated by Professor Olle Hernell) and samples were diluted in dilution buffer (0.05% Tween in PBS) 1 : 5 000 and combined (200 μL/well). A standard curve included n-BSSL concentrations of 64, 32, 16, 8, 4, 2, 1, and 0 ng/mL. Plates were incubated overnight at room temperature. Monoclonal anti-human BSSL (donated by Professor Olle Hernell, diluted to 0.1 μg/mL) in dilution buffer was added (100 μL) and incubated at 37 °C. Two hours later, goat anti-mouse IgG-HRP (Beyotime) (1 : 5 000 dilution) was treated with dilution buffer (100 μL) and incubated for 1 hour at 37 °C. Then, samples were incubated in the dark with TMB (Horseradish Peroxidase Color Development Solution for ELISA, Beyotime) (200 μL/well) at room temperature for 10 minutes. Plates were incubated with 2 mol/L H₂SO₄ (50 μL/well) to end the reaction. Immediately, the absorbance was measured at 450 nm using an ELISA microplate reader (ThermoFisher, USA). The standard curves were obtained using Curve Expert software.

**Statistical analysis**

All statistical analyses were performed using SPSS 17.0 (SPSS Inc., USA). Statistical significance was set at P<0.05. The data distribution was assessed by using the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean±standard deviation (SD), and non-normally distributed data were expressed as medians (P₂₅, P₇₅). The difference in the BSSL level between colostrum and mature milk was determined by paired t-tests with data from women who donated milk at both the first-week and 3-month postpartum visits. Additionally, the difference in the BSSL level between the preterm and term milk was analyzed by the independent samples t-tests. The associations between the BSSL level and perinatal maternal clinical characteristics were assessed with Pearson's (normally distributed data) or Spearman's (skewed data) simple correlation tests. The associations of gestational metabolic diseases with the BSSL level or maternal BMI were examined with one-way analysis of variance (ANOVA), and adjustment was made for confounding factors by using covariance analysis (ANCOVA). P values <0.05 were considered to be significant.

**Results**

**Clinical characteristics**

Eighty-one term delivery mothers were selected as research subjects in the colostrum group; 52 were healthy, 16 were diagnosed with GDM, and 13 were diagnosed with GHD in the second trimester of pregnancy. A total of 67 lactating women were included in this study, including 31 normal mothers who came for a follow-up assessment during lactation, and the mothers breastfed their infants for at least 3 months. The participants' clinical characteristics are shown in **Table 1**.

**Effect of lactation on the BSSL level in human milk**

The average BSSL level was lower in colostrum [(71.49±40.61) μg/mL] than mature milk [(236.90±52.75) μg/mL] in 31 paired samples (**Fig. 1A**). Additionally, in the non-paired samples, the BSSL level was lower in colostrum [(96.35±55.66) μg/mL, n=52] than in mature milk [(208.03±66.99) μg/mL, n=67] in healthy mothers (**Fig. 1B**). However, the BSSL level in mature milk decreased after 6 months (**Fig. 2**).

**Effect of maternal BMI on BSSL level in human milk**

As shown in **Table 2**, the BSSL level in colostrum was positively correlated with pregnancy weeks and negatively correlated with maternal BMI pre-pregnancy and in late pregnancy in 52 mothers. Moreover, the BSSL level in mature milk was negatively correlated with the lactation period and positively correlated with BMI increase during pregnancy.

**Comparisons of the BSSL level in colostrum between term and preterm milk and among normal, GHD, and GDM mothers**

There was no difference in the BSSL concentration in colostrum milk between preterm and term milk (**Table 3**).
There was a significant difference in the BSSL concentration in colostrum milk among normal mothers and mothers with GHD or GDM ($P<0.01$, Table 4). The BSSL concentration in colostrum milk was lower in GDM mothers compared with normal mothers, even after adjusting for maternal pre-pregnancy BMI and pregnancy week ($P<0.05$).

**Discussion**

Many clinical trials and animal models have indicated that milk-borne BSSL can compensate for the low expression of pancreatic lipases during infancy and is believed to be important for the healthy growth and development of infants, particularly preterm infants. To the best of our knowledge, this is the first report to indicate that BSSL is abundantly present in both colostrum and mature milk of Chinese women. The BSSL concentration in mature milk was higher than that in colostrum milk, and the high BSSL level in human milk persisted until 6 months after pregnancy.

*Table 1  Participant characteristics*

| Clinical characteristics                     | Colostrum | Mature milk |
|----------------------------------------------|-----------|-------------|
| Number of case                               | 81        | 67          |
| Maternal age (year)                          | 29.51 (27.00, 31.00) | 28.06±3.43  |
| Pregnancy duration (week)                    | 39.35±1.09 | 39.26±1.08  |
| Lactation period                             | 2 days    | 3.24 (2.93, 5.75) months |
| Infant sex (male/female)                     | 37/44     | 37/30       |
| Pre-pregnancy weight (kg)                    | 57.04±10.19 | 56.88±10.22 |
| Pre-pregnancy BMI (kg/m$^2$)                 | 22.08±3.75 | 21.80±3.86  |
| Weight late in pregnancy (kg)                | 72.38±10.89 | 71.88±10.69 |
| BMI late in pregnancy (kg/m$^2$)             | 27.96±3.64 | 27.47±3.76  |
| BMI during lactation (kg/m$^2$)              | –         | 23.44±3.28  |
| Pregnancy-associated complication mother with GDM (case) | 16       | 0          |
| Mothers with GHD (case)                      | 13        | 0           |

$^a$Kolmogorov-Smirnov test: $P=0.024$. Skewed data are indicated with medians and quartiles. $^b$Kolmogorov-Smirnov test: $P=0.015$. Skewed data are indicated with medians and quartiles. BMI: body mass index; GDM: gestational diabetes mellitus; GHD: gestational hypertensive disorder.

![Fig. 1](image1.png)  

**Fig. 1** BSSL level in human breast milk samples. The difference in the BSSL level between colostrum and mature milk in 31 paired samples was calculated using paired $t$-tests (A). Non-paired samples were assessed using independent samples $t$-tests (B). $^{**}P<0.01$.

![Fig. 2](image2.png)  

**Fig. 2** Correlation between post-gestational time and BSSL level. The difference in the BSSL level between the groups on the basis of 67 mature milk samples was calculated using one-way ANOVA. The BSSL levels in the groups were (218.52±56.34) μg/mL (1–3 months, $n=31$), (219.30±71.04) μg/mL (3–6 months, $n=23$), (165.21±71.77) μg/mL (6–9 months, $n=12$), and 137.70 μg/mL (9–12 months, $n=1$).
reach 100–300 μg/mL and that BSSL levels increase significantly from the 3rd to the 60th postpartum day[21]. The BSSL secretion pattern was similar to the pattern that has been reported for the fat content of human milk; that is, it was higher in mature milk and lower in colostrum. It has been reported that mouse pancreatic BSSL expression increases after the first 4 days of life and peaks on day 7[22], whereas pancreatic lipase secretion in humans remains mature for two years after parturition. Therefore, milk-borne BSSL enhancement may improve fat absorption and compensate for the poor exocrine pancreatic function in infancy, whereas BSSL declines when infants develop a mature digestive capability.

In this study, the BSSL level in preterm milk was higher than that in term milk, although this difference was not significant. Previous studies have shown that the BSSL in preterm milk is higher than[23] or similar to[24] that in term milk and that the lipolytic enzyme activity (i.e., hydrolysis of long-chain triglycerides) does not change with lactation length. However, Pamblanco et al.[25] have posited that gestational age may influence the BSSL activity. Together, these observations suggest that breastfed preterm infants may obtain the same or greater fat digestion capability compared with term neonates, thus improving energy acquisition and allowing infants to catch up in early life.

Obesity-related cytokines in breast milk, such as leptin[18], microRNAs[19], and adiponectin[17], dynamically change over the course of lactation and are influenced by maternal BMI. In the present study, the BSSL level in colostrum was negatively correlated with maternal pre-pregnancy BMI rather than BMI late in pregnancy. The milk BSSL level may be influenced by changes in maternal endocrine hormones (such as prolactin) during pregnancy. Prolactin regulates the nuclear factor 1-C2 protein level, which can activate and regulate the BSSL milk gene in mammary epithelial nuclei[26], and obese mothers have lower prolactin levels during lactation[27] thus resulting in decreased BSSL secretion. Interestingly, the BSSL level in mature milk was positively associated with BMI gain throughout pregnancy. Being overweight and obese during pregnancy increases the risk of fetal macrosomia[28], and the composition of breast milk can influence the metabolism of the offspring through breastfeeding[29]. Therefore, increased BSSL secretion in the presence of a high gestational BMI gain suggests the influence of milk-borne BSSL on the health of the offspring by promoting the efficient digestion of milk lipids, which may in turn increase infant weight. Many researchers have confirmed that prenatal nutritional exposure not

| Table 2 | Bivariate associations between BSSL level in breast milk and maternal characteristics |
|---------|---------------------------------------------------------------------------------|
| Characteristics | Colostrum (n=52) | r/F | P |
| Maternal age | −0.084 | 0.563 |
| Gestational week | 0.379 | 0.006 |
| Pre-pregnancy weight | −0.267 | 0.056 |
| Pre-pregnancy BMI | −0.397 | 0.004 |
| Weight late in pregnancy | −0.146 | 0.301 |
| BMI late in pregnancy | −0.292 | 0.036 |
| Weight gain during pregnancy | 0.239 | 0.088 |
| BMI gain during pregnancy | 0.172 | 0.224 |

| Table 3 | Comparison of BSSL levels between preterm and term colostrum milk |
|---------|-------------------------------------------------------------------|
| Group | n | BSSL (μg/mL) |
| Full-term | 52 | 96.35±55.66 |
| Preterm | 24 | 103.73±43.22 |

P=0.574, P=0.568; Data on the BSSL concentration in full-term and preterm colostrum milk are shown as mean±SD; The differences were calculated using independent samples t-tests.

| Table 4 | Comparison of BSSL levels in colostrum milk between normal, GHD, and GDM mothers |
|---------|---------------------------------------------------------------------------------|
| Group | n | BSSL (μg/mL) |
| Normal | 52 | 96.35±55.66 |
| GHD | 13 | 69.59±42.86 |
| GDM | 16 | 41.63±27.58 |

P=0.001; One-way ANOVA was used to compare the normal, GHD, and GDM mothers with full-term infants; P=0.05 vs. normal. GDM: gestational diabetes mellitus; GHD: gestational hypertensive disorder.

birth. These results were in agreement with those of previous reports from Oklahoma[20], which have revealed that BSSL concentrations in human milk reach 100–300 μg/mL and that BSSL levels increase
only influences children’s growth and development, but also affects adulthood health. Notably, early overnutrition is a risk factor for obesity and metabolic syndrome[30–33]. However, further studies are needed to determine whether the higher BSSL level in breast milk merely represents the nutritional status of overweight and obese mothers or influences the health of their children.

Moreover, GDM women had a lower BSSL concentration in colostrum than that of normal women. Increased glucose and insulin levels have been reported in human breast milk from diabetic mothers, owing to diffusion of glucose and insulin from the maternal circulation into breast milk[32–33]. Insulin is secreted by pancreatic endocrine cells and is not only involved in glycometabolism but also regulates pancreatic lipase through the islet-acinar shaft and reduces pancreatic lipase activity[34]. However, the small sample size in this study limited our ability to discover any additional correlations between milk-borne BSSL and maternal lipid and glucose metabolic indexes. Further study is required to determine whether BSSL secretion and synthesis in the mammary gland are regulated by insulin.

In this first reported investigation on the BSSL concentration in the milk of Chinese women, we confirmed that the milk-borne BSSL level is correlated with the maternal lactation length and the BMI at pre-pregnancy, late pregnancy, and the BMI gain during pregnancy. However, we measured the BSSL level, but not activity, in the present study. Published reports have demonstrated that the activity of BSSL, instead of its level, reflects its function[9,14,35,23–24]. Moreover, the small sample size and small number of mature milk samples from mothers with GDM/GHD and preterm groups limited our ability to discover any additional effect of the BSSL level in mature breast milk on the maternal metabolic status or gestational age. Furthermore, additional research is needed to determine whether the BSSL level in offspring is regulated by the milk-borne BSSL level and facilitates weight control in breastfed infants.

In conclusion, our findings demonstrated that pancreatic BSSL, which is involved in lipid absorption, was abundantly present in human milk, particularly in mature milk. The BSSL level in human milk was mainly correlated with maternal BMI. These findings suggest that preterm milk has the same fat digesting potential as term milk. GDM may affect BSSL secretion in colostrum milk. The changes in BSSL features in this study may have clinical implications associated with the weight and metabolic changes in pregnant women with respect to the nutrition and health of the offspring.

Acknowledgments

We would like to thank the mothers and their babies who participated in this study, as well as the doctors and nurses at Nanjing Drum Tower Hospital. We would also like to acknowledge Professor Olle Hernell at Umeå University, who consulted us on the quantitative methods and provided an antibody used in this study. This work was supported by grants from the National Basic Research Program of China (973 Program, Grant No. 2013CB530604) and the key program of the Nanjing Health Bureau (ZKX15043).

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