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Fatty acids profile in preterm Colostrum of Tunisian women. Association with selected maternal characteristics

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ABSTRACT

Fatty acids (FA), especially arachidonic (AA, 20:4ω6) and docosahexaenoic (DHA, 22:6ω3) acids are critical for the health and development of infants. Colostrum FA composition has been examined in 101 lactating Tunisian women delivering prematurely using gas chromatography. Among polyunsaturated FA, linoleic acid predominated whereas each of the other polyunsaturated FA accounted for 1% or less of total FA. Colostrum AA and DHA contents were lower in women aged above 34 years compared to those less than 34 years. Preeclampsia was associated with lower DHA (0.40 ± 0.22 vs. 0.53 ± 0.27; $p=0.018$), but higher AA (1.14 ± 0.44 vs. 0.93 ± 0.30; $p<0.006$) and AA:DHA ratio (4.31 ± 4.04 vs. 2.29 ± 2.79; $p<0.001$). In multivariate analysis, colostrum DHA correlated with plasma DHA (β, 0.417; $p=0.002$), maternal age (β, −0.290; $p=0.028$) and preeclampsia (β, −0.270; $p=0.042$). Preterm colostrum FA profile in Tunisian women is comparable to those of other populations. Colostrum AA and DHA levels are altered in aged and pre-eclamptic women.

1. Introduction

Fatty acids (FA), mainly long-chain polyunsaturated fatty acids (LCP) are vital for normal growth and development of infants [1]. Arachidonic (AA, 20:4ω6) and docosahexaenoic (DHA, 22:6ω3) acids, the main LCP, are critical for neural and visual development [2]. LCP are precursors for highly bioactive compounds such as prostaglandins, leukotrienes, lipoxins, resolvins and protectins. These compounds are involved in the lunching and resolution of inflammation, which may affect the outcome of acute and chronic diseases [2,3]. Not only the amounts of different polyunsaturated FA are important, but evidence suggests that the balance between ω6 and ω3 FA is also essential for maintaining health [4,5]. Preterm infants are born with a marginal status in LCP due to shortened gestation [6] and low activity of enzymes responsible of their endogen synthesis [7]. These infants have to sustain growth with immature organs, small body stores and increased demand. They must be provided with sufficient amounts of LCP to fulfill their increased needs.

Breast milk is the first natural food taken by the nursing infant; its content in nutrients is critical for his health and development. Fat content of Human milk serves as source of energy, fat-soluble vitamins and essential FA for breast-fed infants [8]. FA content in breast milk is influenced by dietary intake and endogen lipid metabolism [9–12]. Many other factors may affect the milk FA composition, including maternal age, term of delivery, stage of lactation and gestational diseases [13–16]. In Tunisia, scarce data are available on the biochemical composition of breast milk. A previous study revealed low colostrum vitamins A, E and D in Tunisians women [17]. This study aimed to examine preterm colostrum FA profile and its main determinants in Tunisian women.

2. Materials and methods

2.1. Subjects and samples collection

The study included 101 Tunisian lactating women who delivered prematurely (28 weeks to 37 weeks of gestation) at the Center of Maternity and Neonatology of Tunis. Mothers with chronic and acute diseases and those using supplements containing vitamins or FA during pregnancy were excluded. Fresh colostrum was obtained from lactating mothers between the 2^{d} and the 7th day postpartum. Each mother presses one breast totally with a manual breast pump and the colostrum is collected in a sterile glass vial. One milliliter of mixed colostrum was recuperated for the needs of the study and the remaining portion was fed to the infant or discarded. Fasting blood was collected into EDTA-containing tubes in 46 women. Blood was centrifuged at

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2000*g and plasma and colostrum samples were stored at −80 °C until analysis (within 6 months). Required data were collected from the mothers and their medical records. Preeclampsia (PE) and gestational diabetes are defined according to the American College of Obstetricians and Gynecologists criteria [18,19]. All mothers included had been taking habitual Tunisian foods during pregnancy and no one adopted special diet or received FA-containing supplements. The study protocol was approved by the Ethics Committee of Maternity Center and informed consent was obtained from each women.

2.2. Analytical methods

Colostrum and plasma FA were analyzed by capillary gas chromatography according to the method of Moser and Moser [20]. Plasma lipids were extracted by methylene chloride/methanol mixture in presence of heptadecanoic acid as internal standard and hydrolyzed by potassium carbonate, while FA were methylated in presence of acetyl chloride. The fatty acid methyl esters were extracted by hexane and analyzed with a gas chromatograph model 6890N (Agilent Technologies, Santa Clara, CA), equipped with a flame ionization detector. Separation was achieved on capillary column (Innowax; 30 m × 0.25 mm; ID, 0.25 mm; Agilent Technologies) using nitrogen as carrier gas. Oven temperature was programmed from 150 °C to 250 °C. The injector and detector temperatures were 230 °C and 280 °C, respectively. Individual FA were identified by comparison of retention time with standards and results expressed as percent of total fatty acids weight (mol%).

2.3. Statistical Analysis

Statistical computations were performed using SPSS 15.0 for Windows software (SPSS Inc., Ill.). Continuous variables were compared using independent-samples T test. The relationship between continuous variables was tested using Pearson's correlation. To identify the independent predictors for selected FA and groups of FA in the colostrum, multiple logistic regression models were applied with the FA as response variable. Independent variables were the correspondent plasma FA, maternal age, gestational age, multiple pregnancies, PE and gestational diabetes. Goodness-of-fit of logistic models were satisfactory. A p value <0.05 based on two-sided calculation was considered significant.

3. Results

The main characteristics of lactating women and their infants are shown in Table 1. Saturated and monounsaturated FA accounted for about 40% each, and polyunsaturated FA accounted for about 20% of total FA. Three major FA; palmitic, oleic and linoleic acids accounted for around 80% of total FAs in the colostrum, whereas each one of LCP accounted for 1% or less of total FA (Table 2). Colostrum AA and DHA contents were lower in older women (age >34 years) compared to women under 34. No differences in colostrum LCP profile were observed according to term of delivery, multiple pregnancies, gestational diabetes or cesarean section. Women experiencing PE showed lower DHA levels, but higher AA levels, and AA:DHA and ω6:ω3 ratios in the colostrum (Table 3). Plasma AA and DHA, maternal age, term of delivery and infant birth weight did not differ according to PE (data not shown). Colostrum AA and DHA were positively correlated with the correspondent FA in plasma and gestational age (Fig. 1). In multiple regression analysis, colostrum AA was positively associated with plasma AA [standardized β coefficient (β), 0.318; p=0.022] and negatively associated with maternal age (β, −0.300; p=0.032). Colostrum DHA was positively associated with plasma DHA (β, 0.417; p=0.002) and negatively associated with maternal age (β, −0.290; p=0.028) and PE (β, −0.270; p=0.042).

Table 1
Main characteristics of lactating mothers and their infants (n=101).

Maternal characteristic	
Maternal age, years	31.7 ± 4.92
Multiple pregnancies, %	34.7
Cesarean section, %	73.0
Preeclampsia, %	34.7
Gestational diabetes, %	14.9
Infant characteristic	
Sex-ratio (male/female)	50.05
Term of delivery, weeks of gestation	30.4 ± 2.63
Birth weight, g	1224 ± 191

Values were expressed as mean ± standard deviation or as percent

Table 2
Fatty acid profile (mol%) in preterm colostrum from Tunisian women (n=101).

	mol% of total fatty acids
Saturated fatty acids (SAFA)	39.6 ± 3.21
Myristic acid (14:0)	7.90 ± 2.92
Palmitic acid (16:0)	25.0 ± 1.61
Stearic acid (18:0)	6.67 ± 1.57
Monounsaturated fatty acids (MUFA)	40.9 ± 3.98
Palmitoleic acid (16:1ω7)	3.74 ± 1.01
Oleic acid (18:1ω9)	37.2 ± 3.73
Polyunsaturated fatty acids (PUFA)	19.5 ± 3.40
ω6 PUFA	18.1 ± 3.13
Linoleic acid (LA, 18:2ω6)	15.2 ± 3.02
γ-Linolenic acid (18:3ω6)	0.15 ± 0.15
Eicosadienoic acid (20:2ω6)	0.92 ± 0.35
Dihomo-γ-linolenic acid (20:3ω6)	0.78 ± 0.22
Arachidonic acid (AA, 20:4ω6)	1.01 ± 0.37
ω3 PUFA	1.42 ± 0.54
α-Linolenic acid (ALA, 18:3ω3)	0.63 ± 0.41
Eicosapentaenoic acid (EPA, 20:5ω3)	0.08 ± 0.03
Docosapentaenoic acid (22:5ω3)	0.23 ± 0.10
Docosahexaenoic acid (DHA, 22:6ω3)	0.48 ± 0.26
Essential fatty acids (EFA)	15.8 ± 3.29
LA:ALA ratio	38.8 ± 32.3
AA:DHA ratio	2.99 ± 2.79
ω6:ω3 PUFA ratio	14.7 ± 6.71

Values are expressed as mean ± standard deviation; SAFA, 14:0+16:0+18:0; MUFA, 16:1+18:1; EFA, 18:2ω6+18:3ω3; PUFA, ω6 PUFA (18:2ω6+18:3ω6+20:3ω6+20:4ω6)+ω3 PUFA (18:3ω3+20:5ω3+22:5ω3+22:6ω3)

4. Discussion

The study revealed high levels in saturated and monounsaturated FA and linoleic acid, with low amounts in LCP in preterm colostrum from Tunisian women. While comparable to profiles in other populations, preterm colostrum FA profile in Tunisian women is characterized by contents in oleic, linoleic and arachidonic acids among the highest reported (Table 4).

The milk FA composition results from some combination of FA uptake from circulating lipoproteins and albumin-bound free FA, and de novo lipogenesis (DNL) from glucose in the breast [26]. Circulating lipoproteins originate from diet and liver DNL while free FA mainly derive from adipose tissue lipolysis. It was estimated that 60% of milk FA derive from maternal stores, including hepatic and adipose tissue DNL; 10–12% derive from DNL in the mammary gland, and 29% originate directly from the diet [27]. Since they cannot be synthesized de novo in the breast, milk LCP derive from circulating lipoproteins and tissue stores [28]. Special