

# Adiponectin, Adipocyte Fatty Acid Binding Protein, and Epidermal Fatty Acid Binding Protein: Proteins Newly Identified in Human Breast Milk

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**Background:** Breastfeeding may protect children from developing metabolic syndrome and other diseases later in life. We investigated novel proteins in human breast milk that might play a role in this process.

**Methods:** We used ELISA to measure adiponectin, adipocyte and epidermal fatty acid binding proteins (AFABP, EFABP), and leptin concentrations in human breast milk obtained from 59 mothers 48 h after initiation of lactation. Using a questionnaire and medical records, we collected information about the mothers and newborns.

**Results:** Mean (SE) adiponectin concentrations in breast milk were 13.7 (0.8), range 3.9–30.4  $\mu\text{g/L}$ ; AFABP concentrations 26.7 (4.4), range 1.2–137.0  $\mu\text{g/L}$ ; EFABP concentrations 18.1 (1.4), range 0.8–47.0  $\mu\text{g/L}$ ; and leptin concentrations 0.50 (0.05), range 0–1.37  $\mu\text{g/L}$ . We found a significant correlation between AFABP and EFABP concentrations ( $r = 0.593$ ,  $P < 0.0001$ ). Maternal EFABP concentrations were significantly higher in mothers who delivered boys than in those who delivered girls

[21.7 (2.3) vs 15.4 (1.7)  $\mu\text{g/L}$ ,  $P = 0.028$ ] and correlated with newborn birth weight ( $r = 0.266$ ,  $P = 0.045$ ). Maternal leptin correlated with body weight before pregnancy ( $r = 0.272$ ,  $P = 0.043$ ) and at delivery ( $r = 0.370$ ,  $P = 0.005$ ), body mass index before pregnancy ( $r = 0.397$ ,  $P = 0.003$ ) and at delivery ( $r = 0.498$ ,  $P < 0.0001$ ), body weight gain during pregnancy ( $r = 0.267$ ,  $P = 0.047$ ), and newborn gestational age ( $r = 0.266$ ,  $P = 0.048$ ). Leptin was significantly lower in mothers who delivered preterm vs term babies [0.30 (0.09) vs 0.60 (0.05)  $\mu\text{g/L}$ ,  $P = 0.026$ ].

**Conclusions:** Concentrations of adiponectin, AFABP, and EFABP in human breast milk are related to nutritional variables of mothers and newborns and thus may play a role in the protective effects of breastfeeding.

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Nutritional status of newborns might play a role in metabolic syndrome (including insulin resistance, dyslipidemia, and hypertension) and risk of later cardiovascular disease, hypertension, and diabetes (1). Breastfeeding may protect children from developing metabolic syndrome symptoms and other diseases later in life, including insulin-dependent diabetes mellitus and obesity (2–4). Exposure to maternal gestational diabetes mellitus (GDM)<sup>7</sup> predisposes the offspring to develop obesity in childhood and later life, and ingestion of breast milk from diabetic mothers might be a contributing factor (5). In neonatal rats, the source of calories during critical phases of early development affected metabolic programming of islet functions, leading to chronic hyperinsulinemia and adult-onset obesity (6).

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<sup>7</sup> Nonstandard abbreviations: GDM, gestational diabetes mellitus; AFABP, adipocyte fatty acid binding protein; EFABP, epidermal fatty acid binding protein; BMI, body mass index; SDS, SD score.

Although the substances responsible for the effects of breast milk have not been identified, leptin, an adipose tissue-derived cytokine that reflects the total amount of body fat and is a key player in the regulation of the nutritional status of the organism, is present in human colostrum and full breast milk (7). We investigated proteins present in human breast milk that are produced by adipose tissue and are related to lipid metabolism, adiponectin, adipocyte fatty acid binding protein (AFABP), and epidermal fatty acid binding protein (EFABP) (8–23).

## Materials and Methods

### PARTICIPANTS

The study included breastfeeding mothers who gave birth to a single healthy newborn at the Department of Neonatology, University Hospital Motol, Prague. Exclusion criteria were complications during delivery and/or the perinatal period requiring intensive care for the mother or newborn.

We collected breast milk samples 48 h after the beginning of lactation from 59 mothers by manual expression of 5 mL breast milk into tubes containing EDTA and protease inhibitor (aprotinin). The samples were collected after the end of the first morning breastfeeding (after 7:00 am) from the same breast used for breastfeeding the newborn and immediately frozen at  $-20^{\circ}\text{C}$ . All participants gave written informed consent. The study was performed according to conditions of the Helsinki declaration and approved by the hospital ethics committee.

We obtained additional data from questionnaires and medical records. Maternal data included age, height, weight before pregnancy and at the time of delivery, weight gain during pregnancy, the number of previous pregnancies and deliveries, and complications of pregnancy. We calculated the body mass index (BMI) as ( $\text{kg}/\text{m}^2$ ). Data on newborns included sex, gestational age, body weight, and length at delivery. We counted the percentiles and SD score (SDS) of body weight according to gestational age and the ponderal index of newborns, defined as birth weight divided by cubic birth length ( $\text{kg}/\text{m}^3$ ).

Mean (SE) age of mothers was 28.9 (0.6) years, mean BMI before pregnancy was 21.4 (0.4)  $\text{kg}/\text{m}^2$  and at the time of delivery 26.8 (0.4)  $\text{kg}/\text{m}^2$ . Mean gestational age of newborns was 39.2 (0.2) weeks, mean SDS of birth weight was 0.08 (0.12) and mean body length 50.1 (0.3) cm. Thirty-two mothers delivered girls and 27 mothers delivered boys. Two mothers had a history of GDM, 1 with hypertension. Eleven newborns were delivered by caesarian section. Five children developed benign neonatal icterus, treated by phototherapy. Two boys were hypotrophic (below the 5th percentile of body weight for gestational age) and 1 boy was hypertrophic (above the 95th percentile). Eight newborns (2 boys and 6 girls) were delivered preterm (at 265 days or less), but all of them had adequate body weight (above the 5th percentile).

### LABORATORY METHODS

*Samples.* Whole breast milk samples were stored frozen until analysis. We assayed adiponectin in whole milk and leptin, AFABP, and EFABP in skim milk. Samples were thawed at  $4-6^{\circ}\text{C}$  overnight and centrifuged at  $2500g$  at  $4^{\circ}\text{C}$  for 20 min to separate the fat milk. We removed the fat layer with a spatula and used the liquid for assays. The centrifugation step was repeated for turbid samples. We used the BCA (BCA-1, Sigma) method to measure total protein concentrations in skim breast milk samples.

*Assays.* We performed all determinations of adiponectin, leptin, and AFABP with commercially available ELISA kits (Biovendor-Laboratory Medicine).

*Adiponectin assay.* The adiponectin ELISA uses specific goat polyclonal antihuman adiponectin antibody coated in microtiter wells and native adiponectin prepared from human serum as a calibrator and biotin-labeled specific goat polyclonal antibody with streptavidin-HRP for detection. The antibody was raised against whole adiponectin (aa 15–244) and reacts with the whole molecule, including the globular domain (aa 104–244). Whole breast milk samples were diluted 3 times with a dilution buffer and assayed according to the manufacturer's instructions. The detection range of the assay was  $1.0-50.0\ \mu\text{g}/\text{L}$ . Intraassay CVs were 3.8% for samples with low concentrations of protein ( $11.8\ \mu\text{g}/\text{L}$ ) and 5.4% for high concentrations ( $22.2\ \mu\text{g}/\text{L}$ ), and interassay CVs were 5.1% and 7.6%, respectively. The limit of detection reported by the manufacturer is  $0.5\ \mu\text{g}/\text{L}$ .

To validate the assay, we used 2 whole breast milk samples with baseline adiponectin concentrations of 8.1 and  $9.4\ \mu\text{g}/\text{L}$  enriched with various amounts of adiponectin calibrator to increase the original adiponectin concentration by +2.0 and  $+5.0\ \mu\text{g}/\text{L}$ . We obtained a mean recovery of 91.6%. Moreover, by diluting the samples 3-, 6-, 9-, and 12-fold, we tested whole breast milk samples from another 2 women with baseline adiponectin concentrations of 22.6 and  $21.4\ \mu\text{g}/\text{L}$ . The mean recovery after dilution was 111.2%. To perform the correlation analysis, we ran the assay on 6 samples each of whole and skim breast milk.

*Leptin assay.* We measured leptin concentrations by ELISA as previously described (24). To improve the lower limit of detection, we modified the manufacturer's protocol. Skim milk samples were diluted 1:1 with dilution buffer; the range of calibrators was  $0.2-10.0\ \mu\text{g}/\text{L}$ , and the incubation time of calibrators and samples in plate was 2 h. Intraassay CVs were 7.6% for samples with low protein concentrations ( $0.43\ \mu\text{g}/\text{L}$ ) and 6.4% for high concentrations ( $1.03\ \mu\text{g}/\text{L}$ ), and interassay CVs were 9.1% and 8.4%, respectively. The limit of detection reported by the manufacturer is  $0.05\ \mu\text{g}/\text{L}$ .

**AFABP assay.** The AFABP ELISA (25) uses specific goat polyclonal antihuman AFABP antibody coated in microtiter wells, recombinant AFABP as a calibrator, and biotin-labeled specific rabbit polyclonal antibody used with streptavidin-HRP for detection.

Skim milk samples were diluted 5 times with dilution buffer and assayed according to the procedure recommended by the manufacturer. Assay results were 0.5–20.0  $\mu\text{g/L}$ , with an intraassay CV of 4.6% for a sample with low concentration of protein (4.5  $\mu\text{g/L}$ ) and 3.9% for a sample with high concentration of protein (36.6  $\mu\text{g/L}$ ). The interassay CVs were 6.6% and 5.1%, respectively. The limit of detection reported by the manufacturer is 0.1  $\mu\text{g/L}$ . The assay has no detectable cross-reactivity to human EFABP, HFABP, IFABP, LFABP, leptin, leptin receptor, adiponectin, resistin, RELM- $\beta$  at 100.0  $\mu\text{g/L}$ , or IL-6 at 2.0  $\mu\text{g/L}$ .

To validate the assay, 2 skim breast milk samples with the baseline AFABP concentrations of 8.4 and 6.5  $\mu\text{g/L}$  were enriched with various amounts of AFABP calibrator to increase the AFABP concentrations by +2.0 and +5.0  $\mu\text{g/L}$ . The mean recovery was 92.7%. In addition, we tested skim breast milk samples from another 2 mothers with baseline AFABP concentrations of 11.1 and 15.1  $\mu\text{g/L}$  after the samples were diluted 5-, 10-, 15-, and 20-fold. The mean recovery for the diluted samples was 115.0%.

**EFABP assay.** We established the first immunoassay intended for quantitative measurement of EFABP and evaluated it for skim breast milk samples. We used specific sheep polyclonal antihuman EFABP antibody (Biovendor) coated in microtiter wells (Corning Costar, High Binding type): 100  $\mu\text{L}$ /well, 4  $\mu\text{g/L}$  in 0.1 mol/L carbonate buffer (pH 9.0) overnight at 4 °C. The plate was washed once with TBS-Tw [0.05 mol/L Tris-HCl; 0.15 mol/L NaCl; pH = 7.2; 0.05% (w/v) Tween 20] on the washer Columbus (Tecan). Nonspecific binding sites were blocked with 250  $\mu\text{L}$ /well 1% BSA (w/v) in TBS-Tw for 30-min at 25 °C. After aspiration, diluted samples [50  $\mu\text{L}$  of skim breast milk sample diluted with 200  $\mu\text{L}$  of 5% BSA (w/v) in TBS-Tw] were pipetted in duplicates at 100  $\mu\text{L}$ /well. The plate was incubated for 1 h at 25 °C. After 3 washes with TBS-Tw, 100  $\mu\text{L}$ /well of biotin-labeled specific rabbit polyclonal antibody (Biovendor), 0.13  $\mu\text{g/mL}$  in 1% BSA (w/v) in TBS-Tw was added and the plate was incubated for 1 h at 25 °C. After 3 washes, we added 100  $\mu\text{L}$ /well of streptavidin-HRP conjugate (Research Diagnostics) 0.05  $\mu\text{g/mL}$  in 1% BSA (w/v) in TBS-Tw, incubated the plate for 30-min at 25 °C, and then washed it. We then added 100  $\mu\text{L}$ /well of TMB substrate (KPL) and incubated the plate for another 10-min at 25 °C. The reaction was stopped with 100  $\mu\text{L}$ /well sulfuric acid (0.2 mol/L). The developed color was determined by reading the plate on the microplate reader MRX II (Dynex) at a wavelength of 450 nm.

The protein content was determined by the Bradford method (Sigma-Aldrich) with recombinant EFABP (Biovendor) with >95% purity confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown) as a calibrator, prepared at concentrations of 40, 20, 10, 5, 2, and 1  $\mu\text{g/L}$  in 5% BSA (w/v) in TBS-Tw and 100  $\mu\text{L}$  directly pipetted into the wells.

The specificity of the immunoassay was confirmed by reactivity with recombinant EFABP (Abnova). No cross-reactivity was found with recombinant human AFABP (Biovendor), IFABP (R&D Systems), LFABP (Abnova), or other related proteins: leptin, leptin receptor, adiponectin, resistin, and RELM- $\beta$  at 100  $\mu\text{g/L}$  and IL-6 at 2  $\mu\text{g/L}$  (all provided by Biovendor). We observed 0.15% cross-reactivity with HAFBP (Prospec). No signal was found to the following animal sera: rabbit, horse, pig, chicken, sheep, and bovine.

To validate the assay we tested the accuracy and the precision. Skim breast milk samples from 2 participants with baseline EFABP concentrations of 5.8 and 12.4  $\mu\text{g/L}$  were enriched with increasing amounts of recombinant EFABP (+2.0, +4.0 and +10.0  $\mu\text{g/L}$ ) and assayed. The mean recovery was 98%. We also tested by dilution skim breast milk samples from another 2 participants with baseline EFABP concentrations of 18.3 and 10.5  $\mu\text{g/L}$  (Fig. 1). The mean recovery was 112.4%.

The limit of detection was 0.5  $\mu\text{g/L}$ ; intraassay CVs were 5.6% for low protein concentration samples (4.2  $\mu\text{g/L}$ ) and 7.4% for a high protein concentrations

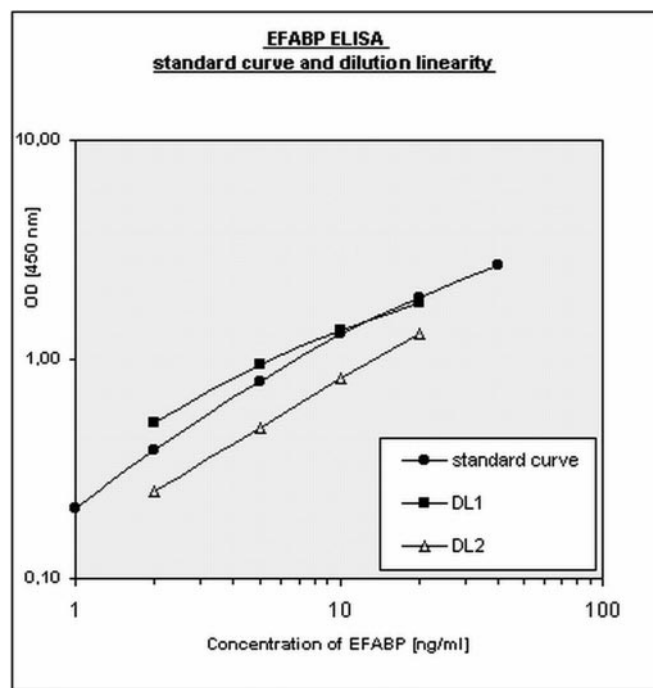


Fig. 1. Evaluation of the human EFABP ELISA.

Two breast milk samples were serially diluted (DL1 and DL2) to determine linearity; the mean recovery was 112.4%.

(21.4  $\mu\text{g/L}$ ); and interassay CVs were 6.2% and 8.1%, respectively.

#### STATISTICS

We performed statistical analysis with Prism 4.0 statistical software (Graph Pad). Results are reported as mean (SE). We used tested correlations with Pearson correlation coefficient and determined differences between groups with the unpaired *t*-test. When biochemical values did not show gaussian distributions, we used Spearman correlation coefficient and Mann–Whitney test for comparison between groups. We used Welch's correction for significantly different variances, 1-sample *t*-test to compare single values in the hypertrophic newborn with the rest of the group, and 1-way ANOVA to compare differences in relation to the number of previous pregnancies or deliveries and for comparison between groups of children of various percentiles of birth weight. A *P* value of  $<0.05$  was considered statistically significant.

### Results

#### SEX DIFFERENCES

Newborn boys had considerably higher absolute body weight at birth than girls ( $P = 0.025$ ), but there was no difference in SDS of body weight, body length, ponderal index, or gestational age with respect to sex. Mothers of boys vs girls showed no statistically significant differences in age, body height, body weight, BMI before pregnancy or at the time of delivery, or body weight gain.

#### ADIPONECTIN CONCENTRATIONS

Mean (SE) adiponectin breast milk concentrations ( $n = 59$ ) were 13.7 (0.8)  $\mu\text{g/L}$ , range 3.9–30.4  $\mu\text{g/L}$ . Concentrations did not differ significantly in mothers who delivered boys ( $n = 27$ ) vs girls ( $n = 32$ ) [12.8 (1.0) vs 14.5 (1.1)  $\mu\text{g/L}$ ,  $P = 0.276$ ], (Fig. 2). We found a positive correlation between adiponectin concentrations and the body weight

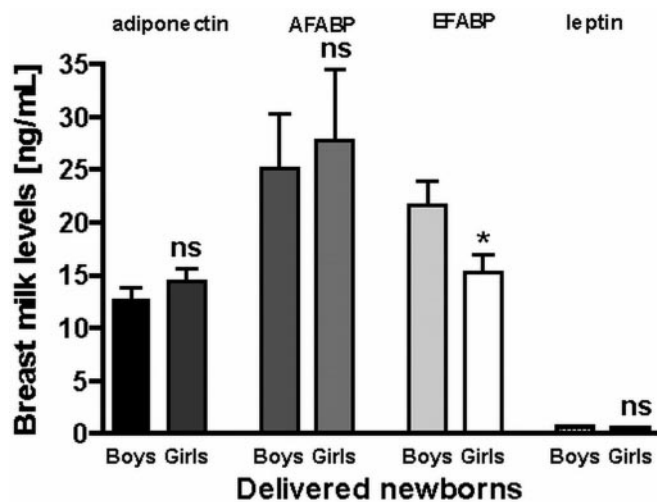


Fig. 2. The relationship between concentrations of different proteins in breast milk and the sex of delivered newborns.

of mothers before pregnancy ( $r = 0.288$ ,  $P = 0.027$ ), but no correlation was found with body weight at the time of delivery nor with the birth weight of newborns, either in absolute values ( $r = -0.119$ ,  $P = 0.371$ , see Fig. 3A), or in SDS. There was no relationship with the number of previous pregnancies or deliveries or any other variable in mothers or newborns, nor with AFABP, EFABP, or

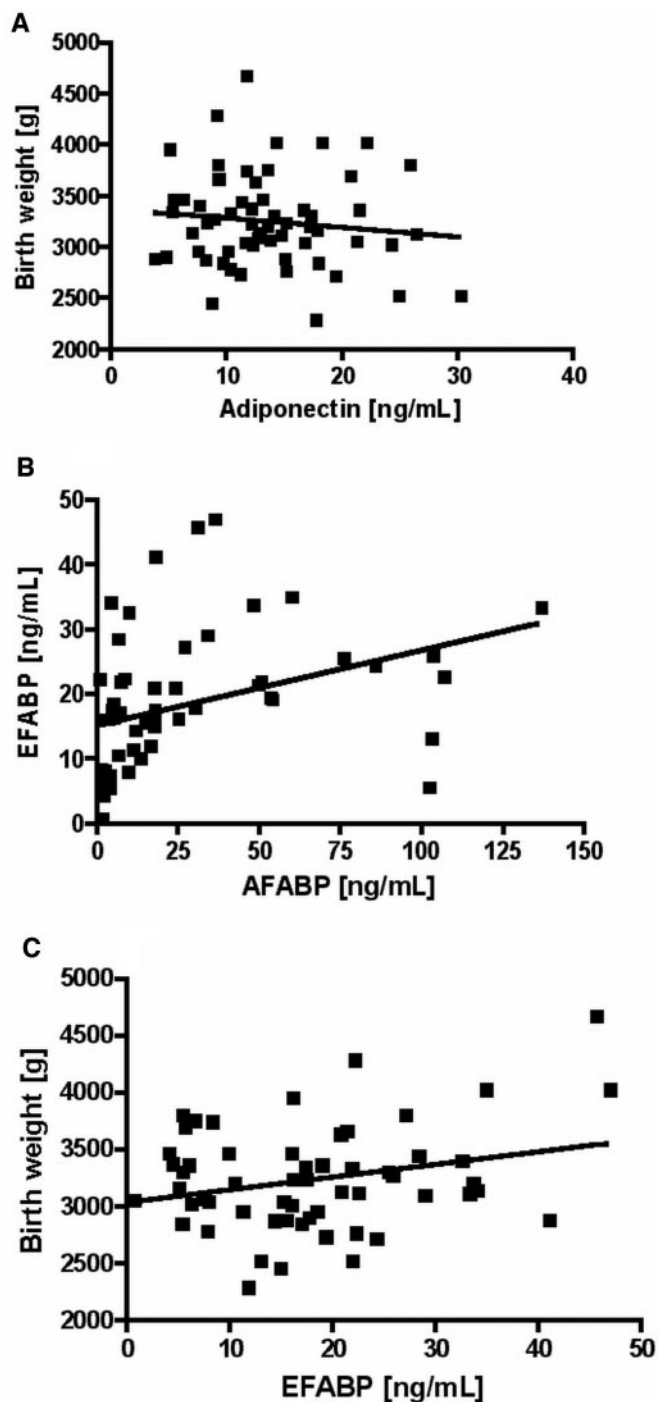


Fig. 3. Correlations of (A) adiponectin with birth weight ( $r = -0.119$ ,  $P = 0.371$ ); (B) AFABP with EFABP ( $r = 0.593$ ,  $P < 0.0001$ ); and (C) EFABP with birth weight of newborns ( $r = 0.266$ ,  $P = 0.045$ ).

leptin milk concentrations. We found a strong correlation between adiponectin concentrations before and after the second freezing/thawing cycle ( $n = 25$ ,  $r = 0.894$ ,  $P < 0.0001$ ) and a correlation between adiponectin concentrations in full breast milk and skim breast milk ( $n = 6$ ,  $r = 0.958$ ,  $P = 0.003$ ).

#### AFABP CONCENTRATIONS

Mean (SE) AFABP breast milk concentrations ( $n = 57$ ) were 26.7 (4.4)  $\mu\text{g/L}$ , range 1.2 to 137.0  $\mu\text{g/L}$ . AFABP concentrations were not gaussian in distribution ( $P = 0.002$ ). Concentrations were not significantly different in mothers who delivered boys ( $n = 25$ ) or girls ( $n = 32$ ) [25.1 (5.2) vs 27.9 (6.8)  $\mu\text{g/L}$ ,  $P = 0.469$ ]. We found a strong positive correlation between AFABP and EFABP ( $r = 0.593$ ,  $P < 0.0001$ ) (Fig. 3B) but not between AFABP and leptin. We found a significant decrease in AFABP concentrations in mothers with a higher number of previous deliveries (1-way ANOVA,  $P = 0.040$ ) (Fig. 4) but not pregnancies. No correlation was found between AFABP and birth weight of newborns either in absolute values or in SDS.

#### EFABP CONCENTRATIONS

Mean (SE) EFABP breast milk concentrations ( $n = 57$ ) were 18.1 (1.4)  $\mu\text{g/L}$ , range 0.8 to 47.0  $\mu\text{g/L}$ . Concentrations were significantly higher in mothers who delivered boys ( $n = 25$ ) than those who delivered girls ( $n = 32$ ) [21.7 (2.3) vs 15.4 (1.7)  $\mu\text{g/L}$ ,  $P = 0.028$ ] (Fig. 2). In the weight-selected group of newborns (2500–3500g, 15 boys and 29 girls), we did not see any difference between sexes in EFABP concentrations. An interesting finding was the positive correlation between EFABP and the birth weight of newborns ( $r = 0.266$ ,  $P = 0.045$ ) (Fig. 3C) and borderline correlation with SDS of birth weight ( $r = 0.237$ ,  $P = 0.076$ ). Correlations between EFABP and leptin, the

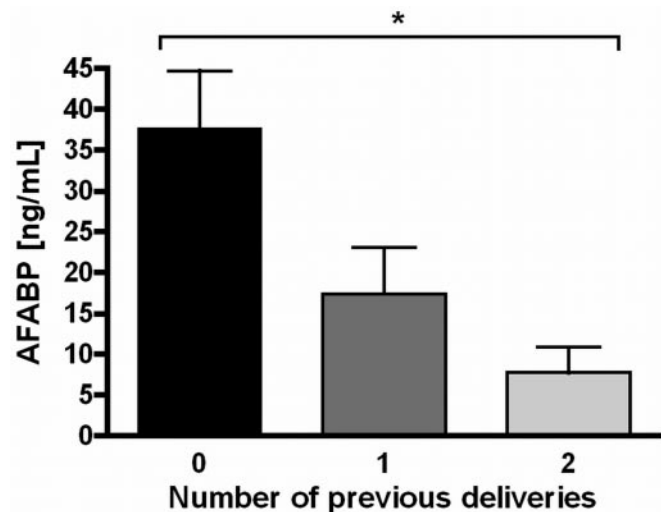


Fig. 4. Decrease of AFABP concentrations in mothers with higher number of previous deliveries.

number of previous pregnancies or deliveries, were not significant.

#### LEPTIN CONCENTRATIONS

Mean (SE) leptin breast milk concentrations ( $n = 56$ ) were 0.50 (0.05)  $\mu\text{g/L}$ , range 0 to 1.37  $\mu\text{g/L}$ , (6 samples were below measurable range). One sample was excluded (4.42  $\mu\text{g/L}$ , more than 10 SD from the mean). Leptin concentrations were slightly, but not considerably, higher in mothers who delivered boys ( $n = 25$ ) than girls ( $n = 31$ ) [0.60 (0.07) vs 0.50 (0.07)  $\mu\text{g/L}$ ,  $P = 0.062$ ] (Fig. 2). We found positive correlation between leptin breast milk concentrations and the body weight of mothers before pregnancy ( $r = 0.272$ ,  $P = 0.043$ ) and at the time of delivery ( $r = 0.370$ ,  $P = 0.005$ ); BMI before pregnancy ( $r = 0.397$ ,  $P = 0.003$ ) and at the time of delivery ( $r = 0.498$ ,  $P < 0.0001$ ), and body weight gain during pregnancy ( $r = 0.267$ ,  $P = 0.047$ ) (Fig. 5A,B). There was no relationship between body weight or BMI before pregnancy and body weight gain during pregnancy. We found a positive correlation between leptin concentrations and the gestational age of the newborns ( $r = 0.266$ ,  $P = 0.048$ ) (Fig. 5C), and we found lower leptin concentrations in breast milk of mothers who delivered preterm babies ( $n = 8$ ) than in those who delivered at term ( $n = 48$ ) [0.30 (0.09) vs 0.60 (0.05)  $\mu\text{g/L}$ ,  $P = 0.026$ ] (Fig. 6). We found a borderline correlation between leptin and birth weight of newborns in absolute values ( $r = 0.242$ ,  $P = 0.072$ ) but not in SDS ( $r = 0.066$ ,  $P = 0.646$ ).

#### TOTAL PROTEIN CONCENTRATION IN SKIM BREAST MILK SAMPLES

The mean (SE) total protein concentration in skim breast milk samples ( $n = 57$ ) was 13.7 (0.5) g/L, ranging from 1 to 34.3 g/L. The values were not gaussian in distribution. There was a strong positive correlation with both AFABP ( $r = 0.482$ ,  $P = 0.0001$ ) and EFABP ( $r = 0.354$ ,  $P = 0.007$ ) but no correlation with adiponectin ( $r = -0.442$ ,  $P = 0.744$ ) or leptin ( $r = 0.206$ ,  $P = 0.152$ ).

After correction to total protein content in breast milk, we found a positive correlation between adiponectin and leptin ( $r = 0.320$ ,  $P = 0.023$ ). Moreover, there were similar appreciable correlations found, as mentioned above (AFABP vs EFABP,  $r = 0.471$ ,  $P = 0.0002$ ; EFABP vs birth weight,  $r = 0.289$ ,  $P = 0.029$ ; and leptin vs body weight before pregnancy,  $r = 0.418$ ,  $P = 0.003$ ; at the time of delivery,  $r = 0.410$ ,  $P = 0.003$ ; BMI before pregnancy,  $r = 0.513$ ,  $P = 0.0001$ ; and at the time of delivery,  $r = 0.487$ ,  $P = 0.0003$ ).

#### OTHER RESULTS

We found no relationship between adiponectin, AFABP, EFABP, or leptin and age, body height of mothers, or ponderal index of newborns. Measurement results did not considerably differ for babies born by caesarian section ( $n = 11$ ) compared with those born vaginally. Breast milk protein concentrations in mothers of newborns who de-

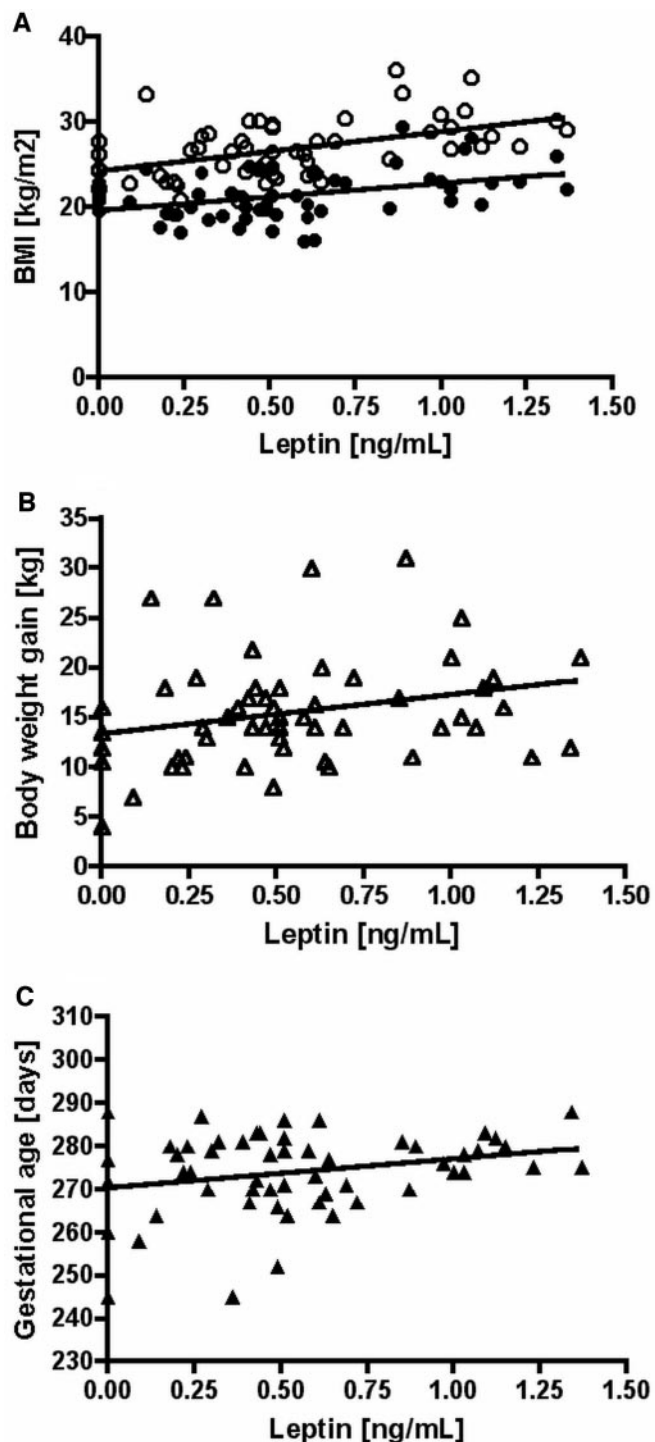


Fig. 5. Correlation of leptin and (A) BMI of mothers before pregnancy (dark spots,  $r = 0.397$ ,  $P = 0.003$ ); BMI at the time of delivery (light spots,  $r = 0.498$ ,  $P < 0.0001$ ); (B) body weight gain during pregnancy ( $r = 0.267$ ,  $P = 0.047$ ); and (C) gestational age of newborns ( $r = 0.266$ ,  $P = 0.048$ ).

veloped neonatal icterus ( $n = 5$ ), mothers with a history of GDM ( $n = 2$ ), and mothers of hypotrophic newborns ( $n = 2$ ) did not differ considerably from those of the rest of the study group, except in 1 mother with GDM who

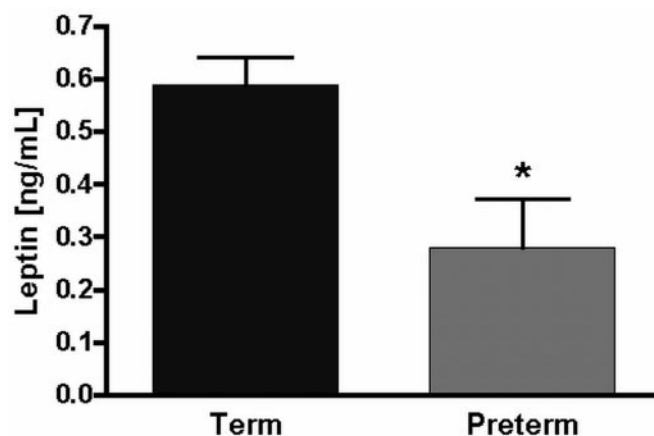


Fig. 6. Difference between leptin breast milk concentrations in mothers who delivered term vs preterm newborns.

had a significantly higher concentration of leptin ( $P = 0.0049$ ). In 1 hypertrophic boy, we found a significantly decreased concentration of adiponectin ( $P = 0.0134$ ) and increased concentrations of leptin ( $P < 0.0001$ ) and EFABP ( $P < 0.0001$ ).

#### Discussion

We demonstrated, for the first time, the presence of adiponectin, AFABP, and EFABP in human breast milk. Adiponectin serum concentrations are higher in women than in men (10, 26, 27), but in breast milk we did not find any considerable difference in adiponectin concentrations related to the sex of the newborn. Mean serum adiponectin concentrations reported by various authors (11–16, 28, 29) vary from 10 to 30 mg/L, 1000-fold higher than the concentrations in breast milk measured in this study, suggesting that adiponectin probably does not pass to breast milk from serum by simple diffusion. The composition of breast milk changes in the course of lactation, particularly during the first week after delivery, within one breastfeeding, and according to the gestational age of the newborn. Thus, adiponectin concentrations should be measured in various kinds of breast milk (preterm milk, colostrum, full milk during a longer period of lactation, foremilk, and hindmilk). Studies of the distribution and kinetics of adiponectin ingested by newborns should be conducted to show whether adiponectin survives the acid environment in the stomach and whether receptors are present in the gastrointestinal tract of newborns.

We found a positive correlation of adiponectin breast milk concentrations and the body weight of mothers before pregnancy. We do not have any explanation for this phenomenon, which we consider unlikely to be clinically important. Previous studies have shown that serum adiponectin concentrations correlate negatively with body weight, BMI, and the amount of body fat in adult humans (10), although Kotani et al. showed a positive correlation of adiponectin plasma concentrations with birth weight in newborns (15).

Although women with a history of GDM have hypoadiponectinaemia (30), a recent study did not show any relationship between adiponectin concentrations and glucose loading response or insulin sensitivity in pregnant women (31), and we did not find any difference in adiponectin breast milk concentrations in 2 mothers with GDM.

Adiponectin might be involved in fetal growth and development (27, 32), but we did not find any remarkable difference in adiponectin breast milk concentrations in mothers who delivered hypotrophic or hypertrophic newborns. Nevertheless, because the nutritional programming hypothesis regarding the protective effects of breastfeeding is generally tested on preterm newborns (33), we suggest that adiponectin breast milk concentrations should be measured in these high-risk patients. Moreover, long-term follow-up studies are needed to assess the development of symptoms of metabolic syndrome in relationship to adiponectin breast milk concentrations.

Birth weight correlated positively with EFABP concentrations in breast milk. Results of an animal model study suggested that FABP deficiency increases the ratio of short (C14) to long (C18) fatty acid in adipose and muscle tissues and in circulating lipids and that absence of both FABPs led to significant protection against obesity, insulin resistance, type 2 diabetes mellitus, and fatty liver diseases (34, 35). Low EFABP in human breast milk might be related to increasing concentrations of long chain fatty acids in breast milk, influencing the risk of insulin resistance.

The stoichiometry of AFABP:EFABP in breast milk was 1.5:1 in our study, which is very different from adipose tissue and surprisingly similar to the stoichiometry found in macrophages (36). We found a positive correlation between AFABP and EFABP rather than a compensatory regulation as reported in adipose tissue (36), indicating an important role for EFABP.

We demonstrated significantly higher EFABP concentrations in breast milk of mothers who delivered boys, but when we corrected for body weight regardless of sex we found that the positive correlation between EFABP concentrations and the body weight of newborns was not related to sex but to higher body weight in boys.

Our study corresponds to previous studies showing that leptin in human breast milk is related to maternal plasma leptin concentration and adiposity (37, 38), but other studies did not confirm this relationship (39). Although previous studies have shown that leptin concentrations did not considerably differ in preterm and full-term breast milk (40), we found significant differences. Our findings may be attributable to the small number of mothers in the preterm group. On the other hand, these data are supported by the positive correlation between leptin and gestational age in our study. Dundar et al. (41) showed decreased breast milk leptin concentrations in mothers of children small for gestational age. We could

not confirm their results because the majority of the children in our study were eutrophic.

In conclusion, it is well known that regulatory proteins may influence human growth and development (42). Identification and characterization of additional regulatory proteins in breast milk may lead to changes in the composition of infant formulas to ensure better nutritional support for newborns to improve their health later in adulthood.

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