Vol. 196, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1993 Pages 267-273

INSULIN-LIKE GROWTH FACTORS (IGF) I AND II AND IGF BINDING PROTEINS (IGFBPs) IN HUMAN COLOSTRUM/TRANSITORY MILK DURING THE FIRST WEEK POSTPARTUM: COMPARISON WITH NEONATAL AND MATERNAL SERUM

U. Eriksson, G. Duc*, E.R. Froesch and J. Zapf

Div. of Endocrinology and Metabolism, Dept. of Medicine, University Hospital, CH-8091 Zürich, Switzerland

*Neonatology, Dept. of Gynaecology, University Hospital, CH-8091 Zürich, Switzerland

Received September 2, 1993

Day 1 human colostrum contains 5 times more IGF I than IGF II. By day 3 postpartum, IGF I drops by 80 % to constant levels whereas IGF II increases 3-fold up to day 7. Colostrum contains mainly IGFBP-2, little IGFBP-3 and no detectable IGFBP-1 or IGFBP-4. IGFBP-2 rises 20-fold up to day 6 of lactation. The major IGFBPs of newborn serum are IGFBP-2, -3 and -4. Early maternal serum contains only small amounts of IGFBP-2 and -3 and no detectable IGFBP-4. The pronounced differences between the IGFBP patterns of colostrum and early maternal serum suggest that IGFBP-2 does not pass from maternal blood into colostrum but is produced and secreted by mammary tissue itself. On the other hand, most of the IGF I, but not IGF II, in day 1 colostrum appears to stem from the maternal circulation.

Insulin-like growth factors (IGFs) I and II are polypeptides with a molecular mass of 7.5 kD which are homologous to proinsulin and exert both metabolic and growth effects in vitro and in vivo (1, 2). They are present in serum and other biological fluids and always tightly associated with specific binding proteins (BPs) (2, 3). Beside endocrine, IGFs exert auto/paracrine effects which are modulated by IGFBPs.

Explants of bovine mammary tissue from lactating nonpregnant animals synthesize and secrete both IGF I and IGFBPs, whereas mammary acini cultures synthesize only IGFBPs (4). In human (5), bovine (6,7,8) and porcine (9) colostrum/ transitory milk, IGF I drops during the first days postpartum. Several hormones and growth factors present in milk behave similarly (10,11). This suggests that these growth factors including IGF I may be important in meeting specific requirements of the newborn during the first days of breast feeding.

Abbreviations: IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; SDS-PAGE, sodium dodecyl sulfate polyacrylamide electrophoresis.

0006-291X/93 $4.00

IGF II levels in human colostrum/transitory milk have not been reported. Furthermore, little is known about the species of IGFBPs in human colostrum/transitory milk. Therefore, we addressed the following questions: 1) Do IGF II levels in colostrum change in parallel with IGF I levels? 2) Do IGFBP levels in colostrum change during the first week of breast feeding? 3) Do the BP species of colostrum reflect the IGFBP pattern of maternal serum suggesting passage from blood into milk?

MATERIALS AND METHODS

Milk and serum samples

Milk samples were obtained from healthy women on various days postpartum. They were centrifuged immediately for 30 min at 14'000 g and 4°C. The fat layer was removed with a spatula and the clear infranatant was kept frozen at -20°C until used for analysis. Venous blood was drawn after an overnight fast from the same women from whom milk was obtained. Samples of umbilical cord blood where collected at birth. Blood samples from newborns were taken on the occasion of the routinely performed Guthrie test. After clotting at 4°C, blood samples were centrifuged and the sera were kept frozen at -20°C.

Determination of IGF I and II in milk and serum

250 µl of centrifuged milk or serum was passed through Sep-Pak C_{18} cartridges (Waters Ass., Milford, MA) according to the protocol supplied by Immunonuclear (Stillwater, MN). The eluate was dried under a stream of air, lyophilized and dissolved in 1 ml of phosphate-buffered saline (PBS), pH 7.4, containing 0.2 % of human serum albumin (HSA). IGF I and II were assayed at 3 different dilutions as described earlier (12) using rhIGF I or rhIGF II as standards (kindly provided by Drs. K. Müller and W. Märki, Ciba-Geigy AG, Basel, Switzerland).

Analysis of IGFBPs

IGFBPs were identified by Western ligand blotting (13,14).7 µl of defatted mild or 2 µl of serum was subjected to SDS-PAGE under nonreducing conditions. ^{14}C-labeled molecular weight marker (Rainbow Marker, Amersham International, UK) was run under reducing conditions. Gels were electroblotted on nitrocellulose, the washed membranes incubated with ^{125}I-labeled IGF II (4 x 10^6 cpm, specific activity 300-400 µCi/µg, ANAWA Trading SA, Wangen, Switzerland), washed again, air-dried and exposed to an X-ray film at -70°C.

Densitometric analysis of IGFBPs

Ligand blots of colostrum were evaluated by scanning densitometry (Video Densitometer, Model 620, BioRad, Richmond, CA). Colostrum IGFBP-2 and -3 were expressed as OD units relative to IGFBP-2 and -3, respectively, present in a normal adult serum pool.
Protein was determined by the method of Lowry et al. (15) using bovine serum albumin (BSA; Fluka) as a standard.

RESULTS

IGF I and IGF II concentrations in colostrum and maternal sera at various days postpartum

The protein concentration in pooled colostrum decreased from 4.1 g/dl on day 1 to 1.8 g/dl on day 6. As shown in figure 1A, IGF I in colostrum decreased during the first 3 days postpartum from 52±2.3 ng/ml to 8.6±3.0 ng/ml (mean ± SD, n=8) and re-

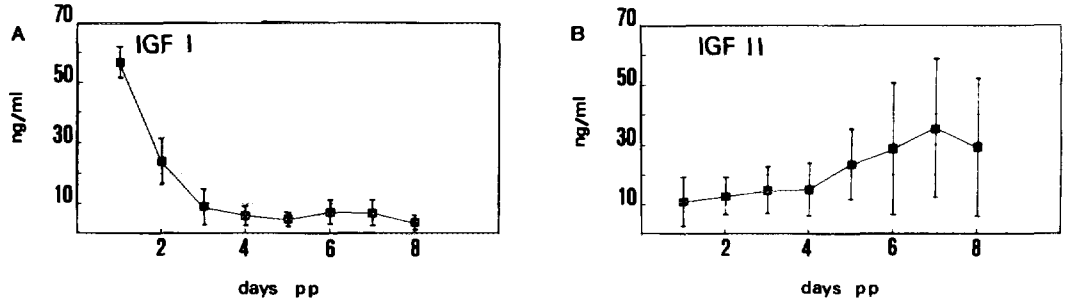

Figure 1.
IGF I (A) and IGF II (B) levels in human colostrum/transitory milk between day 1 and 8 postpartum. Milk samples were processed and analysed by RIA as described in Materials and Methods and in ref. 12. IGF levels are expressed in ng/ml. Squares correspond to mean values ± SD of 8 different subjects.

mained more or less constant at 3-6 ng/ml between days 4-8. In contrast to IGF I, IGF II was low in day 1 colostrum (10.5±8.5 ng/ml) and increased 3-fold (35±21 ng/ml) up to 6 days postpartum (fig. 1B). IGF I levels in maternal serum were 142±45 and 105±35 ng/ml (mean ± SD, n=3) on day 2 and 6 postpartum, respectively, and IGF II levels 717±205 and 487±81 (mean ± SD, n=3).

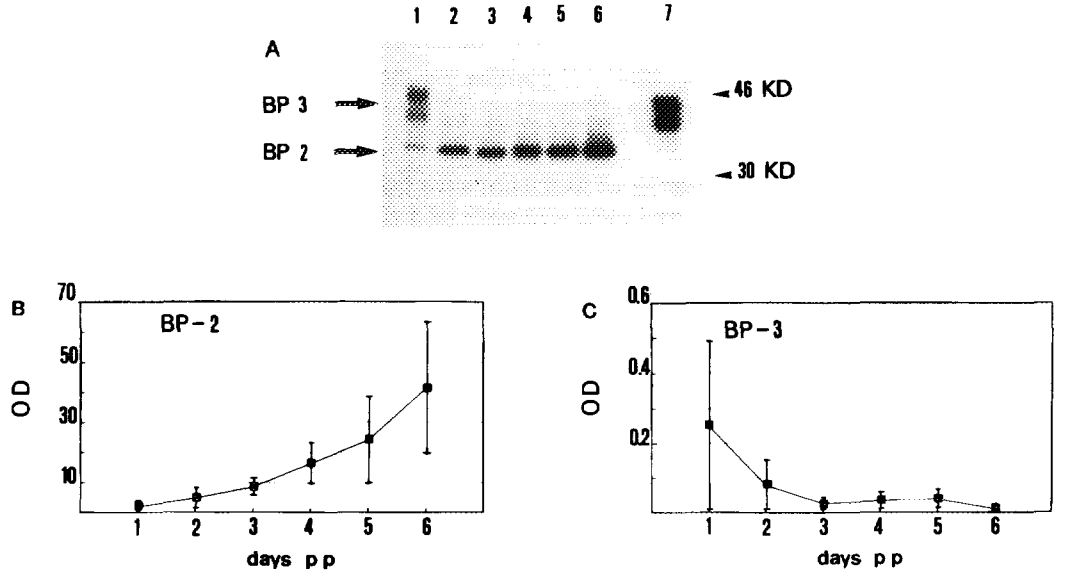

Figure 2.
^{125}I-IGF II ligand blot pattern (A) and densitometric evaluation of IGFBP-2 (B) and -3 (C) of human colostrum/transitory milk from days 1-6 postpartum. Milk (lanes 1-6) or pooled normal adult serum (lane 7) were electrophoresed processed as described in Materials and Methods and in refs. 13 and 14. A typical blot out of 8 different blots with colostrum from different women is shown. Blots were evaluated by scanning densitometry. Mean values ± SD are given.

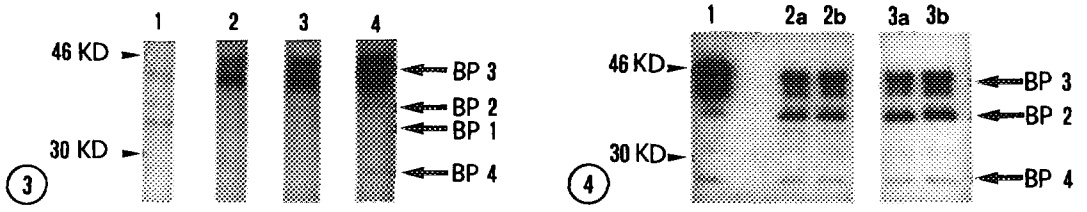

Figure 3.
^{125}I-IGF II ligand blot pattern of maternal sera on various days postpartum. Serum was electrophoresed and processed as described in Materials and Methods and in refs.13 and 14.Lanes 1, 2 and 3: serum from the same woman on days 2, 4 and 6 postpartum. Lane 4: pooled normal adult serum. A typical blot out of 3 different blots is shown.

Figure 4.
^{125}I-IGF II ligand blot pattern of two umbilical cord sera (lanes 2a and b) and sera from the same two breast-fed children on day 4 postpartum (lanes 3a and b). Lane 1 represents pooled normal adult serum. 2 µl of serum was processed as described in Materials and Methods and in refs. 13 and 14. A typical blot out of 3 different blots is shown.

^{125}I-IGF II ligand blot patterns of colostrum/transitory milk between days 1 and 6

The ligand blot pattern of human colostrum/transitory milk differs significantly from that of adult normal (fig. 2) or maternal serum (fig. 3). A 36 kD band, identified as IGFBP-2 by immunoblotting (not shown) with IGFBP-2 antiserum (kindly provided by Dr. M. Rechler, NIH, Bethesda) was the most prominent signal in colostrum between days 2-6 and increased continually during lactation (figs. 2A and 2B). In contrast to "milk" obtained between days 3 and 6, day 1 colostrum contained less IGFBP-2 and largely variable amounts of a 42/45 kD doublet (fig. 2A and 2C) identified earlier as IGFBP-3 (13,16).

^{125}I-IGF II ligand blot patterns of maternal sera between days 2 and 6 postpartum, and of umbilical cord sera and sera of 4 day old breast-fed newborns

As shown in fig. 3, the BP-3 signal in maternal serum 2 days postpartum (lane 1) was almost lacking (lane 4), but became near normal on day 6 postpartum (lane 3). The intensity of the IGFBP-2 band at 36 kD varied little between days 2-6 and was of similar intensity as the BP-2 band of normal adult serum. Maternal serum on day 2 postpartum shows an additional 33 kD band (most likely IGFBP-1) which disappears later and is lacking in normal adult serum. Furthermore, the 24 kD band of normal serum, identified as IGFBP-4 (17) is lacking in maternal serum between days 2 and 6 postpartum.

No differences in the IGFBP patterns were found between umbilical cord serum at birth and serum of breast-fed 4 day old newborns (fig. 4).However, the IGFBP pattern of these sera differed from that of maternal serum on day 2 postpartum (fig. 3). The former sera contain considerably more IGFBP-2 and -3 than the latter. In contrast to maternal serum up to 6 days postpartum, umbilical cord and newborn serum, like normal adult serum, does contain IGFBP-4.

DISCUSSION

We recently found that the predominant IGFBPs in sera from premature neonates of gestational weeks 28-34 were IGFBP-2 (36 kD band) and IGFBP-1 (33 kD band), whereas only little IGFBP-3 (42/45 kD doublet) was detected (13). Similar results were obtained by Lassarre et al. (18). In this study we show that umbilical cord serum at birth or newborn serum on day 4 postpartum contain similar and significant levels of IGFBP-3 (fig. 4). This IGFBP-3 does not appear to stem from maternal blood via placental transfer, because preterm and term pregnant serum, like early maternal serum (fig. 3), contain little, if any, intact IGFBP-3 due to the presence of an IGFBP-3 protease (19,20). The mature newborn is, therefore, able to produce substantial amounts of IGFBP-3.

The increase of IGFBP-2 in colostrum postpartum is not paralleled by an increase of IGFBP-2 in maternal serum. This finding indicates that colostrum IGFBP-2 is not "extracted" from maternal blood but rather produced by the mammary gland itself. In vivo findings in the rat (21) and in vitro findings in bovine mammary explants (4) are compatible with this conclusion.

A rapid decrease of IGF I in colostrum has been observed earlier in humans (5), cows (6,7,8) and sows (9). It is unlikely that this decrease is due to the loss of IGF I by suckling, because IGF II behaves in an opposite manner to IGF I (fig. 1B). When expressed per mg of protein, the IGF I levels in maternal serum and in day 1 and day 2 colostrum are comparable. However, 4 days postpartum the IGF I concentration per mg of protein is considerably lower in colostrum than in maternal serum. These data suggest that passage of IGF I from maternal serum into milk may occur during the first 2 days postpartum, but not later on. This conclusion is compatible with the following findings: most of the IGFBP-3 present in maternal serum during the last two thirds of pregnancy and during the first 2 days postpartum occurs as a truncated form due to proteolytic cleavage by an IGFBP-3 protease (19,20). The binding capacity of truncated IGFBP-3 for IGF I is significantly reduced, whereas binding of IGF II is less affected (Zapf, unpublished). Thus, the IGF I content of the 150 kD IGFBP complex of term pregnant and early maternal serum is decreased (Eriksson and Zapf, unpublished), whereas the total IGF I level is the same as in nonpregnant serum. Decreased binding of IGF I to the 150 kD IGFBP complex in term pregnant serum may result in higher bioavailability of IGF I and thus in its passage into colostrum. On the other hand, the decrease of IGF I in colostrum coincides with the increase of intact IGFBP-3 (42/45 kD doublet) in maternal serum (fig. 1 and 3). Reappearance of intact IGFBP-3 goes along with formation of an "intact" 150 kD IGFBP complex with increased binding capacity for IGF I, but restricted capillary passage (22,23).

The IGF II concentration per mg of protein is lower in day 1 colostrum than in maternal serum and stays lower, although colostrum IGF II increases between days 2 and 6. This concentration gradient of IGF II between maternal blood and colostrum suggests that transport of IGF II from the circulation into the milk compartment is

restricted already at the beginning of the postpartum period. As pointed out above, the higher binding capacity of truncated IGFBP-3 for IGF II than for IGF I is compatible with this reasoning. Apart from transient passage of IGF I into colostrum, synthesis and secretion by mammary tissue of both IGF I and II are likely, as also shown in vitro for IGF I in bovine mammary explants (4).

The presence of high concentrations of IGF I in day 1 and day 2 colostrum may be of physiological importance for the neonate: The combination of high levels of EGF (10,11) and of IGF I, a differentiation factor (24,25), may enhance closure of the intestine-blood barrier postpartum. In this context, Schober et al. have shown that IGF I in small intestine of suckling pigs increases after birth, whereas the IGF I mRNA remains unchanged (26). The increase in IGF I coincides with intestinal villous growth and closure of the intestine-blood barrier (26).

ACKNOWLEDGMENT

This work was supported by grant no. 32-31281.91 from the Swiss National Science Foundation.

REFERENCES

1. Daughaday, W.H. and Rotwein, P. (1989) Endocrine Reviews **10**,68-91
2. Humbel, R.E. (1990) Eur. J. Biochem. **190**,445-462
3. Baxter, C.R. and Martin J.L. (1989) Progress in Growth Factor Research **1**,49-68
4. Campbell, P.G., Skaar, T.C., Vega, J.R., and Baumrucker, C.R. (1991) J. Endocrinol. **128**,219-228
5. Baxter, C.R., Zaltsman, Z., and Turtle, J.R. (1984) Clin. Endocrinol. Metab. **58**,955-959
6. Campbell, P.G. and Baumrucker, C.R. (1989) J. Endocrinol. **120**,21-29
7. Schams, D., Einspanier, R. (1991) Endocr. Regul. **25**,139-143
8. Skaar, T.C., Vega, J.R., Pyke, S.N., and Baumrucker, C.R. (1991) J Endocrinol **131**(1),127-133
9. Simmen, F.A., Whang, K.Y., Simmen, R.C., Peterson, G.A., Bishop, M.D., and Irvin, K.M. (1990) Domest Anim Endocrinol **7**,199-206
10. Carpenter, G. (1989) Science **210**,198
11. Schams, D. and Karg, H. (1986) Ann NY Acad Sci **464**,75
12. Zapf, J., Walter, H., and Froesch, E.R. (1981) J Clin Invest **68**,1321-1330
13. Zapf, J., Schmid, Ch., Guler, H.P., Waldvogel, M., Hauri, Ch., Futo, E., Hossenlopp, P., Binoux, M., and Froesch, E.R. (1990) J Clin Invest **86**,952-961
14. Hossenlopp, P., Seurin, D., Segovia, B., Hardourn S., and Binoux, M. (1986) Analyt Biochem **104**,138-143
15. Lowry, O.H., Rosebrough, NJ., Farr, L.A., and Randall, R.J. (1951) J Biol Chem **192**,265-275
16. Zapf, J., Kiefer, M., Merryweather, J., Masiarz, F., Bauer, D., Born, W., Fischer, J.A., and Froesch, E.R. (1990) J Biol Chem. **265**,14892-14898
17. Kiefer, M.C., Masiarz, F.R., Bauer, D.M., and Zapf, J. (1991) J Biol Chem **266**,9043-9049
18. Lassarre, C., Hardouin, S., Daffos, F., Forestier, F., Frankenne, F., and Binoux, M. (1991) Pediat Res **29**,219-225

19. Hossenlopp, P., Segovia, B., Lassarre, C., Roghani, M., Bredon, M., and Binoux, M. (1990) J Clin Endocrinol Metab **71**,779-805
20. Guidice, L.C., Farell, E.M., Pham, H., Lamson, G., and Rosenfeld, R.G. (1990) J Clin Endocrinol Metab **71**,806-815
21. Donovan, S.M., Hintz, R.L., Wilson, D.M., and Rosenfeld, R.G. (1991) Pediatric Research **29**,50-55
22. Binoux, M. and Hossenlopp, P. (1988) J Clin Endocrinol Metab **67**,509-514
23. Guler, H.P., Zapf, J., Schmid, C., and Froesch, E.R. (1989) Acta Endocrinol. **121**, 753-758
24. Schmid, C., Steiner, T., and Froesch, E.R. (1984) FEBS Lett **173**,48-52
25. Florini, J.R. Ewton, D.Z., Roof, S.L. (1991) Mol Endocrinol **5**,718-724
26. Schober, D.A., Simmen, F.A., Hadsell, D.L., and Baumrucker, C.R. (1990) Endocrinology **126**,1125-1132