The regulation of IGFs and IGFBPs by prolactin in primary culture of fetal rat hepatocytes is influenced by maternal malnutrition

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El Khattabi, Ilham, Claude Remacle, and Brigitte Reusens. The regulation of IGFs and IGFBPs by prolactin in primary culture of fetal rat hepatocytes is influenced by maternal malnutrition. Am J Physiol Endocrinol Metab 291: E835–E842, 2006.—During perinatal development, the regulation of IGF system appears to be growth hormone (GH) independent. By using highly purified primary fetal hepatocytes, we investigated the role of prolactin (PRL) in the regulation of IGF system and hepatocyte proliferation. We also analyzed the consequence of a maternal low-protein (LP) diet on the regulation of IGF, IGFBP, and hepatocyte proliferation. Pregnant Wistar rats were fed a control (C) diet (20% protein) or isocaloric LP (8% protein) diet throughout gestation. On day 21.5, fetal hepatocytes were cultured for 4 days and incubated with rat prolactin. In the C hepatocytes, PRL at 100 ng/ml decreased the abundance of IGFBP-1 and IGFBP-2 by 50 (P < 0.05) and 60% (P < 0.01), respectively. It also reduced by 70% the level of IGF-II mRNA (P < 0.01). By contrast, PRL had no effect on either the proliferation or the IGF-I production by C and LP hepatocytes, although it reduced the expression of IGF-II. These results suggest that prolactin influences hepatocyte proliferation in vitro by inhibiting IGFBP-1, IGFBP-2, and IGF-II levels, which may coincide with the decline of IGF-II observed in rodents during late gestation in vivo. On the other hand, maternal LP diet induces a resistance of fetal hepatocytes to PRL.

Prolactin (PRL) and placental lactogens (PLs), also known as “lactogenic hormones,” may also regulate the IGF system during the perinatal period, when such regulation seems to be GH independent (26, 29, 57). PRL and PLs form, along with growth hormone (GH), a group of proteins with structural similarities (20). Depending on the species, PRL or PL circulates at high levels in the fetuses of the same species at late gestation did not influence the IGF production, whereas it altered the IGFBP-3, either by

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reducing the protein level in the circulation (44) or by enhancing its expression in the liver (10). In vitro, hPL stimulated IGFs and IGFBPs in fetal human fibroblasts (25). The interpretation of these data was rendered difficult due to the use of heterospecific system and ligands. Moreover, it has been demonstrated that oPL and bovine PL, as well as hPL, bind to the GH receptor (GHR) in addition to the PRLR (19).

Therefore, we were interested in studying the lactogenic regulation of IGFs, IGFBPs, and prolactin by using a homologous system of primary cultured fetal rat hepatocytes described previously (13). For that purpose, we have used rat PRL instead of rat PL-II, since the latter is not available commercially. In rodents, PRL and PL show closer similarity in their primary sequence than in human and bind with strong and similar affinity to the PRLR but do not show any cross-reactivity to the GHR (19). We also sought to determine in vitro whether PRL could restore some of the alterations previously observed in LP-derived hepatocytes.

**MATERIALS AND METHODS**

**Animals and diets.** Wistar rats bred in our laboratory were placed in a controlled environment (25°C, 12:12-h dark-light cycle) and allowed free access to food and water. Virgin females were housed with males overnight, and copulation was confirmed the next morning by the presence of vaginal plug or spermatozoa after a vaginal smear. Midnight was considered as the time of mating and day 0 of gestation. Pregnant females were then caged individually and randomly assigned to one of two diets throughout gestation: 20% protein diet representing the control (C) group, 8% isocaloric protein diet representing the LP group. As a general characteristic, the body weight gain during pregnancy of the control group, 8% isocaloric protein diet representing the LP group. On the last day of gestation, dams were killed and fetuses exposed by abdominal incision. Both diets were identical in fat content and differed by the addition of carbohydrates in the LP group. Diets were purchased from Hope Farms (Waukon, The Netherlands), for which composition has been previously described by Snoeck et al. (54). Ethical approval for the animal study was obtained from the animal ethics committee of the Catholic University of Louvain, Louvain-la-Neuve, Belgium.

**Materials.** Type II collagenase (sp act 387 U/mg), dexamethasone, HEPES buffer, EDTA, and bovine serum albumin (fraction V) were purchased from Sigma (St. Louis, MO). Insulin was from NovoNordisk (Bagsvaerd, Denmark). William’s E medium, 10× HBSS, antibiotics, and fetal bovine serum (FBS) were from Gibco BRL (Merelbeke, Belgium). Desoxyribonuclease I was from Boehringer Mannheim (Mannheim, Germany) and Vitrogen 100 from Collagen (Palo Alto, CA). Percoll was purchased from Pharmacia (Uppsala, Sweden). Goat anti-rat IGFBP-1 and anti-rat IGFBP-2 polyclonal antibodies were obtained from Sanvertech (Santa Cruz, CA). Mouse anti-rat PRLR U5 mAb was from Affinity Bioreagents (Golden, CO). Rabbit anti-mouse and rabbit anti-goat immunoglobulin were from Dako (Merelbeke, Denmark). 4,6-Diamidino-2-phenilindole (DAPI) was from Serva (Heidelberg, Germany) and DNA from calf thymus, used as standard, from Boehringer Mannheim. Protein G-Sepharose beads were from Zymed (San Francisco, CA). The rat PRLR (NIDDK rPRL) was obtained from the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD).

**Fetal hepatocytes isolation and primary culture.** The detailed protocol of isolated fetal hepatocytes and cell culture has been described previously (13). Briefly, minced livers from rat fetuses 1 day before birth were incubated in a balanced salt solution (BSS: 142 mM NaCl, 6.7 mM KCl, 10 mM HEPES, pH 7.4) containing 1 mM EDTA at 37°C under constant agitation. The suspension was centrifuged at 716 rpm for 5 min, and the tissue pellet was then digested in BSS containing 6.7 mM CaCl2, 0.5 mg/ml collagenase, and 0.1 mg/ml deoxyribonuclease I at 37°C under constant agitation. After dissociation, the cell suspension was collected and diluted in William’s E medium supplemented with 0.2% BSA and centrifuged for 5 min at 424 rpm. Subsequently, the supernatants were filtered through nylon meshes (100 and 30 μm, respectively) and washed with WE-BSA medium (424 rpm, 5 min). Fetal hepatocytes were further purified by adding 15 ml of cell suspension to 14.4 ml of diluted Percoll solution (9 volumes Percoll and 1 volume 10× HBSS). The mixture was centrifuged at 1,433 rpm for 8 min. To eliminate the residual hematopoietic cells, the pellet (hepatocyte fraction) was resuspended in WE-BSA medium at low speed (277 rpm, 5 min). Purified fetal hepatocytes were resuspended in William’s E medium supplemented with 10% FBS and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin), and cell number was counted in a hemocytometer. Routinely, 10 × 106 hepatocytes/g liver were obtained with viability above 90–95% as assessed by Trypan blue exclusion.

Fetal hepatocytes were cultured on dishes previously coated with a type I collagen gel at a density of 105 cells/cm2 in William’s E medium supplemented with 10% FBS and antibiotics. Four hours after plating, the cells were washed three times and transferred into hormono-defined medium (10 mM insulin, 10–7 M dexamethasone, 50 mg/ml EGF, 0.1 mM ascorbic acid, 10 μg/ml linoleic acid) without or with rPRL: 10 or 100 ng/ml at final concentrations.

**Preparation of conditioned media and cellular extracts.** For IGF-I and IGFBPs measurements, media were conditioned over 24-h periods of time from day 1 to day 4. Conditioned media were recovered at 4°C, centrifuged to eliminate cells and debris, and kept at −20°C. They were then precipitated with trichloroacetic acid (10% wt/vol final concentration) and concentrated 15 times upon reconstitution in Laemmli sample buffer under nonreducing conditions without β-mercaptoethanol.

For immunoprecipitation and DNA experiments, cellular extracts were collected as follows: cells were washed three times with phosphate-buffered saline (PBS) and were incubated in presence of collagenase (1 mg/ml, 1 ml/dish) for 5 min at 37°C to digest the collagen gel. The cell suspension was washed twice with PBS at 4°C (300 g, 5 min), and pellets were then stored at −20°C until utilized.

For IGF-I, IGFBPs, and PRLR measurements in vitro we used the same starting volumes of conditioned media, and therefore, we expressed our values relative to total protein content of the cell layer having produced the conditioning of the medium.

**Quantification of cell proliferation.** Cell proliferation was evaluated by measurement of DNA concentration on day 4 of culture by DAPI method. For this purpose, pellets of cellular extracts were resuspended in a lysis buffer (Tris-HCl 0.1 M, pH 7.4, containing 0.5% Triton X-100). After 30 min of incubation on ice, the extracts were centrifuged at 10,139 rpm for 15 min, and the supernatants were collected. Five hundred microliters of cellular extracts were transferred into tubes, and 2 ml of a buffer containing 12 mM NaCl, 5 mM HEPES, 5 mM EDTA, pH 7, was added to each tube. Thereafter, 500 μl of DAPI solution (120 ng/ml) were added. The fluorescence was measured in a Kontron fluorimeter (Zurich, Switzerland), using calf thymus DNA as standard. The excitation and emission wavelengths were 372 and 454 nm, respectively.

Western immunoblot analysis. Aliquots of conditioned medium from culture were subjected to Western blot analysis according to the method described by Hossenlop et al. (28). Briefly, samples were boiled in Laemmli buffer (Tris-HCl, 0.625 mol/l, pH 6.8, 10% glycerol, 2% SDS, and 0.0125% bromophenol blue) and loaded onto 12% SDS polyacrylamide gels under nonreducing conditions. Standards were run under similar conditions in adjacent lanes. After separation, proteins were transferred electrophoretically to nitrocellulose membranes. The latter were blocked with 5% nonfat dry milk for 1 h in Tris-buffered saline (TBS; 0.05 mol/l Tris, 0.2 mol/l NaCl, pH 7.4)
Membranes were then incubated with antiserum against rat IGFBP-1 and IGFBP-2 (1:500 to 1:1000 dilutions). Antigen-antibody complexes were identified with rabbit anti-goat immunoglobulin G labeled with horseradish peroxidase. Specific binding was visualized by chemiluminescence [enhanced chemiluminescence (ECL) detection system; Amersham]. Autoradiograms were analyzed using the NIH Image 1.55 software. The relative amount of each major band was assessed on the basis of its surface area and mean density.

Liver collection. On the last day of gestation, dams were anesthetized with pentobarbital sodium (55 mg/kg body wt), and fetuses were exposed after abdominal incision along the midline. Fetal livers were removed rapidly, frozen, and stored at −80°C until utilized. Frozen tissues were homogenized with a Polytron in lysis buffer (10 mM Tris, pH 7.5, 5 mM EDTA, 150 mM NaCl, 50 mM Na-fluoride, 30 mM Na pyrophosphate, 1 mM orthovanadate, 10% glycerol, 0.5% Triton-X 100) containing protease inhibitors (1 mM PMSF, 1 mg/ml pepstatin, 2 mg/ml leupeptin, 5 mg/ml aprotinin). The homogenate was then centrifuged at 3,000 rpm for 5 min at 4°C, and the supernatant was centrifuged again at 12,000 rpm for 30 min at 4°C. The clear supernatant was carefully removed and its protein content determined, and then the aliquots were stored at −20°C.

Immunoprecipitation and Western blot analysis of PRLR. Liver homogenates were diluted 1:1 in lysis buffer containing protease inhibitors, and total cellular extracts were lysed in 1 ml of the same buffer for 30 min at 4°C. Cell lysates were then centrifuged at 14,000 rpm at 4°C for 10 min and resuspended in 1 ml of lysis buffer. Five microliters of milliliter anti-receptor mAb U5 were added, and all samples were then rotated at 4°C for 1 h. Sixty microliters of protein G-Sepharose were added, and rotation at 4°C was continued overnight. The suspension was then centrifuged at 14,000 rpm, and the pellet was washed three times with cold lysis buffer. For Western blots, washed protein G pellets were boiled in 60 μl of Laemmli lysis buffer for 30 min at 4°C. The clear supernatant was carefully removed, and its protein content determined, and then the aliquots were stored at −20°C.

Measurement of IGF-I. In plasma and in culture medium IGF-I was measured by enzyme immunoassay (EIA), using rat IGF-I EIA kit from Diagnostic System Laboratories (Webster, TX). The IGF-I EIA is a homologous assay that uses a pretreatment step (Diagnostic System Laboratories) to eliminate the interference of IGFBPs. After the pretreatment step, neither IGFBP-1 nor IGFBP-2 was detected by EIA in culture medium of C and LP hepatocytes isolated from fetuses isolated from fetuses 1 day before birth. The addition of PRL at 10 or 100 ng/ml for 48 h from day 1 to day 3 of culture had no effect on the production of IGF-I by C and LP hepatocytes. Because prolactin was reported to stimulate the expression of its own receptors (1), we then prolonged the time of incubation during an additional 24-h period of time, and again no effect was observed on the IGF-I levels in both C and LP groups (data not shown).

The level of IGF-II mRNA was evaluated by real-time PCR on day 4 of culture in cellular extracts of C and LP hepatocytes isolated from fetuses isolated from fetuses at 21.5 days of gestation (Fig. 1). The addition of PRL at 10 or 100 ng/ml for 72 h from day 1 to day 4 of culture decreased significantly the IGF-II mRNA levels of C and LP hepatocytes relative to untreated hepatocytes (P < 0.01, C of prolactin (10 or 100 ng/ml) vs. untreated C;bb

RESULTS

C and LP hepatocytes were cultured for 3 days with PRL (10 or 100 ng/ml), and hepatocyte-conditioned culture medium was collected at the end of days 3 and 4. The level of IGF-I was evaluated by EIA in culture medium of C and LP hepatocytes isolated from fetuses 1 day before birth. The addition of PRL at 10 or 100 ng/ml for 48 h from day 1 to day 3 of culture had no effect on the production of IGF-I by C and LP hepatocytes. Because prolactin was reported to stimulate the expression of its own receptors (1), we then prolonged the time of incubation during an additional 24-h period of time, and again no effect was observed on the IGF-I levels in both C and LP groups (data not shown).

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Statistical methods. The significance of mean differences was calculated by using Scheffé’s test after analysis of variance (ANOVA 1 or 2 as specified). Significance was assumed when P < 0.05. Results were expressed as means ± SE.

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Fig. 1. Effect of prolactin on the relative content of IGF-II mRNA as measured by real-time PCR on day 4 of culture in cellular extracts of control (C) and low-protein (LP) hepatocytes isolated from fetuses isolated from fetuses at 21.5 days of gestation. Hepatocytes were cultured for 3 days in the absence (0 ng/ml) or presence of 2 concentrations of prolactin: 10 or 100 ng/ml. Cellular extracts were collected on day 4 of culture. Values reported are means ± SE for 3–4 different cultures for each group (C and LP), expressed in relative content after normalization with endogenous housekeeping gene (18S) and relative to the untreated C group. Mean value of IGF-II mRNA level in untreated C hepatocytes is 100%.

ANOVA 2 followed by Scheffé’s test tested differences between means. $P < 0.01, C of prolactin (10 or 100 ng/ml) vs. untreated C; $P < 0.01, LP of prolactin (10 or 100 ng/ml) vs. untreated LP; $P < 0.05, LP of prolactin (100 ng/ml) vs. C of prolactin (100 ng/ml).
0.01; Fig. 1). This reduction was more pronounced in LP groups at the highest concentration of the hormone (P < 0.05; Fig. 1).

As mentioned elsewhere (13), only one band situated at 29–32 kDa was detected in purified hepatocyte cultures, as performed by Western ligand blot, using 125I-IGF-II to detect all the IGFBP species. Also, a major band in the similar range of 29–32 kDa was detected in vivo. This band had been demonstrated to correspond to IGFBP-1 and IGFBP-2 as revealed by immunoblot.

We therefore investigated whether PRL had any regulatory role on IGFBPs, in particular IGFBP-1 and IGFBP-2, since they represent the predominant binding proteins in the fetal period (21, 55). In our previous work, we had reported that growth retardation caused by maternal protein restriction was associated with increased IGFBP-1 and IGFBP-2 (13). To observe such differences, the C and LP samples had to be analyzed in the same gel. However, in the present study, having focused on the regulation by PRL on IGFBPs in both C and LP groups, these differences are not apparent since we used independent gels for the two groups. The immunoblot analysis was performed using specific polyclonal antibodies to all the IGFBP species used (Fig. 2, A and B) than untreated hepatocytes. By contrast, when LP hepatocytes were exposed to PRL for 48 h, there was no difference in the amount of IGFBP-1 (Fig. 2A) relative to their untreated controls. After 72 h of incubation, LP cells produced less IGFBP-1 (P < 0.05; Fig. 2B) than untreated hepatocytes but only at the higher concentration of the hormone without any effect being observed at 10 ng/ml, whereas this reduction was less pronounced than in C hepatocytes.

A similar pattern was detected for IGFBP-2 (Fig. 3). In C hepatocytes, after 48 h of incubation, PRL at 10 and 100 ng/ml also provoked a marked diminution in the abundance of IGFBP-2 (P < 0.05; Fig. 3A) with 50 and 60% less, respectively, than the untreated cells. After 72 h of treatment (Fig. 3B), IGFBP-2 showed a similar decrease in the presence of PRL. Regarding LP hepatocytes, there was no significant effect of this hormone on IGFBP-2 at both times and concentrations used (Fig. 3, A and B) compared with their respective controls (no PRL).

Because we found that PRL was able to regulate hepatocytes IGF-II and IGFBPs in vitro and that these binding proteins were able to modulate cell growth, we studied the effect of PRL on fetal hepatocytes proliferation. DNA content, as measured by the DAPI method on day 4 of culture, was unchanged in C and LP hepatocytes incubated with PRL (10 or 100 ng/ml) for 3 days compared with untreated cells (data not shown). Because of the presence of dexamethasone in culture medium, which has an antimitotic effect on hepatocytes (13), we reproduced the same experiment with cells incubated first with a hormono-defined medium for 2 days and with PRL alone at the two concentrations cited above for the next 2 days. Nevertheless, PRL was again ineffective in both C and LP hepatocytes even in the absence of dexamethasone (data not shown).

As reported above, PRL inhibits the IGFBP production in C fetal hepatocytes; however, this was not the case in LP cells. Therefore, we tested the hypothesis of a PRL resistance due to lactogenic receptor defect. For that purpose, PRL receptors were immunoprecipitated using an anti-PRLR mAb U5 and analyzed by Western blot with the same monoclonal antibody. This antibody is specific to the extracellular domain of the PRLR and is able to detect both forms of receptor: the LPRLR and the SPRLR. Interestingly, U5 mAb is directed to an epitope outside the binding domain and should be able to recognize the bound receptor (43).

As shown in Fig. 4A, a very faint band situated at ~100 kDa corresponding to LPRLR was detected in liver homogenates of rat fetuses 1 day before birth in the C but not in the LP group. In the positive control represented by a liver homogenate of an adult female rat, a band of the same relative mass was detected but expressed more strongly than in fetuses. On the other hand, another band between 32 and 47 kDa, which appeared as a
between means.

Cytos is 100%. ANOVA 1 followed by Scheffe’s test tested differences (optical density/ cellular extracts); mean value of IGFBP-2 content in untreated fetal hepatocytes is 100%. ANOVA 1 followed by Scheffe’s test tested differences between means. aP < 0.05, C of prolactin (100 ng/ml) vs. untreated C.

**DISCUSSION**

Our data revealed that in C hepatocytes PRL had no effect on the hepatic IGF-I production, whereas, unexpectedly, it decreased the mRNA level of IGF-II that may reflect a decrease in the production of IGF-II, since a strong correlation has been made between circulating IGFs and abundance of hepatic IGF mRNAs (22, 50). We also found that PRL reduces the production of both IGFBP-1 and IGFBP-2 in a dose-dependent manner. In the literature, very limited results are available about the IGF and IGFBP response to lactogenic hormones. Hill et al. (25) have already reported that hPL, but not GH, stimulated the in vitro release of IGF-I, IGF-II, and IGFBP by human fetal fibroblasts. In vitro, PRL has been found to stimulate IGF-II in mouse primary mammary epithelial cells (8). Previously, Fielder et al. (14) reported that mouse PL-I, PL-II, or PRL stimulated 29-kDa IGFBP (IGFBP-1 and/or IGFBP-2) secretion by cultured mammary epithelial cells isolated from pregnant mice. Also, Phillips et al. (49) noted that PRL stimulated the secretion of IGFBP-2 into the culture medium of a mouse mammary epithelial cell line. Thus, contrary to our study, most of these findings demonstrated a stimulatory response of IGFs and IGFBPs (IGFBP-1 and -2) to lactogenic hormones. These discrepancies in PRL activities may be due to the type and level of PRLR expression in target tissues, which vary in a tissue-specific manner. For example, in the mammary gland and the liver of adult rats, reverse ratios of long and short PRLR exist. In mammary epithelial cells, there is a predominance of LPRLR, whereas in liver the short form has been described as the major lactogenic receptor (30, 45). In the present study, both LPRLR and SPRLR were clearly doublet, exhibited the expected relative mass (in the range of 34–44 kDa) of the short form of PRLR. Densitometric quantitation of this doublet showed that the amount of SPRLR tended to decrease in liver homogenates of LP fetuses compared with C samples, although it was not significant (Fig. 4A).

To know whether this alteration persists in vitro, we used cellular extracts of cultured hepatocytes originated from C and LP rat fetuses on the last day of gestation. The C and LP cells were maintained for 4 days under similar conditions (see MATERIALS AND METHODS), and collection of cellular extracts was performed at the end of the last day of culture. After quantification of the immunoblot by densitometry, the statistical analysis of the doublet corresponding to SPRLR immunoblots showed clearly that maternal LP diet reduced significantly the abundance of SPRLR in cultured LP hepatocytes compared with C hepatocytes (P < 0.05; Fig. 4B).

**Fig. 4.** A: immunoprecipitation and Western blot analysis of prolactin receptors in liver homogenates; B: total cell lysates derived from C and LP 21.5-day-old rat fetuses. Liver homogenates and whole cell lysates were immunoprecipitated using mAb U5 (1 μg/ml), and immune complexes were separated by 10% SDS-PAGE. Western blot analysis was performed using mAb U5 (1 μg/ml). Bars represent quantification of the 32–47-kDa band after densitometry analysis of 5–7 autoradiograms for SPRLR. Values reported are means ± SE relative to arbitrary units of optical density expressed as follows in % (optical density/g of proteins in liver homogenates or cellular extracts); mean value of SPRLR content in untreated fetal hepatocytes is 100%. ANOVA 1 followed by Scheffe’s test tested differences between means. aP < 0.05, LP vs. 100% of C.
expressed in the liver of adult rat, whereas in both fetal liver homogenates and cultured hepatocytes the short PRLR was mainly detected. Contradictory data have been reported with regard to the SPRLR (see the beginning of this article). In the rat, it has been postulated that SPRLR cannot mediate cell proliferation and inhibits the function of the LPRLR (3, 46, 52). Therefore, the inhibitory response that we observed for IGF-II and the IGFBPs (IGFBP-1 and -2) may be due to the largely dominant expression of SPRLR in fetal liver.

Furthermore, because of the role of IGFs and IGFBPs in cell proliferation (35), and the ability of PRL to induce proliferation in many cell types in vitro (2, 5, 7, 8), we examined the effect of PRL on fetal hepatocytes proliferation. PRL was inefficient to enhance DNA synthesis of fetal hepatocytes. Once again, this may be related to the dominant presence of the short form of PRL receptor. Our results indicate that the decrease in IGFBP production would normally lead to an enhancement of the availability of free IGF, and hence, stimulate proliferation. At the same time, we found that PRL decreases the levels of hepatic IGF-II mRNA, which suggests that PRL could maintain low IGF-II levels to limit hepatocytes proliferation. Indeed, in vivo, the proliferative capacity of fetal hepatocytes declines with advancing gestational age, and it has been suggested that inhibitory factors are involved in the growth arrest observed at term (24). Cortisol has also been reported (36) to suppress IGF-II gene expression in the fetal liver near term, and we have previously reported (13) that glucocorticoids inhibited fetal hepatocytes proliferation. Therefore, it is possible that PRL and related placental lactogens act synergistically with glucocorticoids to maintain a low rate of fetal hepatocytes growth towards term.

In hepatocytes of protein-restricted animals, PRL also reduced significantly the IGF-II mRNA, and this effect appears more pronounced than in hepatocytes of normally fed progeny. As we mentioned above, gestational protein restriction increases IGFBP-1 and IGFBP-2 levels in serum and the production by fetal hepatocytes (13). In the present work, PRL failed to modulate the IGFBP production in the LP hepatocytes as it did in control, which indicates that this hormone is unable to restore the alteration. Because control and LP cells were kept under the same culture conditions for 3 days, this indicates a persistence of programming due to fetal malnutrition already observed in basal conditions (13), and it reveals that maternal malnutrition may provoke a resistance to PRL in the response of IGFBPs due to a receptor or postreceptor defect. At this point, it has to be mentioned that in our model, an increase in carbohydrates was necessary to compensate for hypocaloric status in the protein-restricted animals. This fact may influence our results; however, no significant increase in glycemia occurs in LP animals, in either the mothers or their fetuses.

Our results showed the predominance of short PRLR in the liver at late fetal age, and we found that the abundance of this form is reduced in LP hepatocytes, which may explain in part the absence of PRL activity with regard to IGFBPs. A reduced mRNA for the long form of PRLR in adipose tissue has been shown in growth-retarded sheep fetuses, whereas both types of PRLR mRNA were unchanged in the liver (48, 56). In these studies, lowering placental irrigation has induced growth retardation. Finally, our data indicate that PRL may regulate differentially IGF and IGFBPs in fetal hepatocytes, since in protein-restricted animals IGF-II expression is more sensitive to PRL, whereas IGFBP production is blind to the hormone. These results suggest that the IGF-II PRL action could be mediated either through different receptors or via the same receptor but different intracellular signaling pathways.

In conclusion, our study revealed that maternal LP diet might alter the PRL regulation of IGF-II and IGFBPs and added further weight to the concept of programming in utero. This study also demonstrated in vitro a novel possible role of PRL in the regulation of IGF axis that seems to coincide with the requirement during late gestation in vivo, when a decline of IGF-II levels was observed in rodents and sheep (12, 36) and when there is a need to shift from a phase of dominant cellular proliferation to the differentiation of function required after birth.

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