Acta Medica Okayama

Volume 47, *Issue* 1 1993 *Article* 2

FEBRUARY 1993

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Abstract

We recently reported that epidermal growth factor (EGF) levels in the first urine to be voided by intrauterine growth retardation (IUGR) and heavy-for-dates (HFD) infants were lower than control infants (8). In this study, we analyzed EGF receptors to reveal the mechanisms controlling EGF levels. EGF binding to fetal rat liver increased markedly from day 19-21 of gestation. Fetal rats were divided into IUGR, control and HFD groups. EGF binding to the liver in each group was as follows, IUGR; 380 +/- 57 fmol/mg protein, control; 258 +/- 47, and HFD; 545 +/- 112. The binding to IUGR and HFD rat liver was significantly greater than in the control group ($p < 0.05$). These data suggest that IUGR rats compensate for a lack of EGF by increased receptor expression and that HFD rats consume more EGF and have decreased urinary EGF excretion. These data also suggest that EGF is closely related to fetal growth and may play some important roles in fetal growth.

KEYWORDS: EGF, IUGR, HGD, fetal rat liver, EGF receptor

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*PMID: 8460559 [PubMed - indexed for MEDLINE]

Acta Med Okayama 47 (1) 7-11 (1993)

Rat Liver Epidermal Growth Factor Receptors in Intrauterine Growth Retarded and Heavy-for-Date Fetuses

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We recently reported that epidermal growth factor (EGF) levels in the first urine to be voided by intrauterine growth retardation (IUGR) and heavy-for-dates (HFD) infants were lower than control infants (8). In this study, we analyzed EGF receptors to reveal the machanisms controlling EGF levels. EGF binding to fetal rat liver increased markedly from day 19-21 of gestation. Fetal rats were divided into IUGR, control and HFD groups. EGF binding to the liver in each group was as follows, IUGR; 380 ± 57 fmol/mg protein, control; 258 ± 47, and HFD; 545 ± 112. The binding to IUGR and HFD rat liver was significantly greater than in the control group ($p < 0.05$). These data suggest that IUGR rats compensate for a lack of EGF by increased receptor expression and that HFD rats consume more EGF and have decreased urinary EGF excretion. These data also suggest that EGF is closely related to fetal growth and may play some important roles in fetal growth.

Key words : EGF, IUGR, HFD, fetal rat liver, EGF receptor

Epidermal growth factor (EGF) is a polypeptide (molecular weight 6,045) which has various influence on normal and neoplastic tissues of ectodermal and mesodermal origin (1). Studies using primary cultured adult and fetal rat hepatocytes have shown that EGF stimulates the synthesis of DNA, glycogen, polyamine, and ornithine decarboxylase which regulates polyamine synthesis (2, 3). Moreover, EGF has a greater effect than many other hormones which increase during pregnancy; estrogen, progesterone, insulin, human chorionic gonadotropin, *etc.* (3). There are many studies demonstrating the presence of EGF receptors in developing fetuses (4-7). These data suggest that EGF plays an important roles in fetal growth. We have also reported (8) that the urinary EGF levels in intrauterine growth retarded (IUGR) and heavy-for-date (HFD) infants were lower than those in appropriate-for-date (AFD) infants. In this investigation, we analyzed EGF receptors in fetal rat liver to reveal the machanisms that control EGF levels during pregnancy.

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Materials and Methods

Animals. Wistar rats were used in this experiment. Animals were fed *ad libitum* on Oriental Laboratory Chow (MF) and water, and housed at a constant temperature (20°-24°C) with a 12 h light/dark cycle. For the experimental IUGR groups, food was withheld from mothers for 120 h before delivery on day 21, but were given free access to water. Heavy birth weight rats were selected for use as the HFD models.

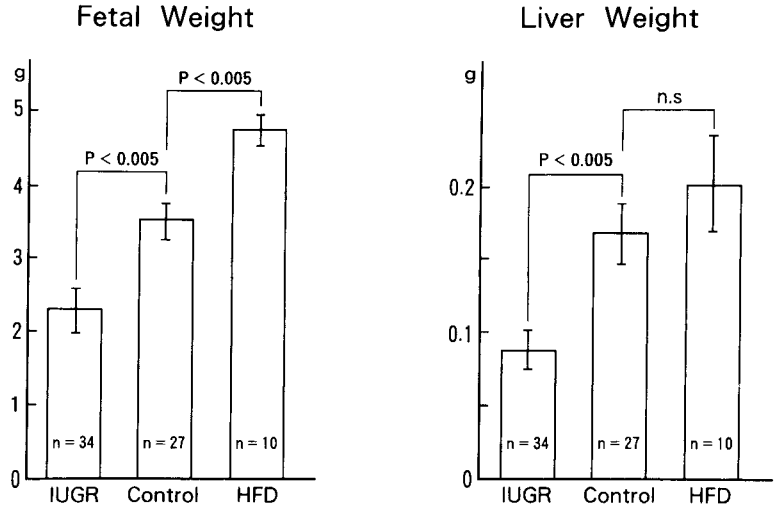
Preparation of subcellular fractions for the EGF receptor assay. Pregnant rats at day 21 were anesthetized with nembutal (5 mg/100 g) and the abdomens were opened. Adult and fetal rat livers were removed and cut into small pieces on ice. Membranes were prepared according to the method of Koppelman and Dufau (9). Tissues were homogenized with ice-cold buffer A (18 g of NaCl, 10.2 g of KCl, 1.14 g of Na_2HPO_4, 0.2 g of KH_2PO_4 and 100 mg of trypsin inhibitor/L, 0.1 % bovine serum albumin with a pH of 7.4) in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 300 g for 15 min at 4°C; then the supernatant was decanted and recentrifuged at 25,000 g for 20 min at 4°C. The resulting pellet was suspended in buffer A and adjusted to 3 mg protein/ml. Protein was measured by the method of Lowry *et al.* (10) using bovine serum albumin as the standard.

Iodination of mouse EGF. Mouse EGF was purchased from Biomedical Technologies (Stoughton, MA). Na-^{125}I was purchased from Nordion Inc. (Ontario, Canada). Mouse EGF was iodized with chloramine T and was purified using a Sephadex G-25 Fine column. The specific activity of labeled EGF was 300–400 nCi/ng EGF.

Binding studies. The total assay volume was 0.15 ml; consisting of 0.1 ml liver cytosol (3 mg protein/ml) and 0.05 ml of ^{125}I-EGF (0.026–4.92 ng) with or without 400 ng of non-radioactive EGF (an approximately 100-fold excess of labeled EGF). Nonspecific binding was assumed to be the measured amount of labeled hormone bound to the receptor material in the presence of a 100-fold excess of unlabeled EGF. After 2 h of incubation at 20 °C, the assay mixture was filtered through a Whatman GF/C filter to separate bound from unbound ligands. The filter was then counted using an Aloka ARC 1000 apparatus. The specific binding data were transformed by the Scatchard method (11) using a computer program. Student's *t*-test was used for statistical analysis of the data.

Results

The experimental IUGR fetuses had a 38 % decrease in body weight and a 44 % decrease in liver weight, while the HFD fetuses showed a 31 % increase in body weight and a 15 % increase in liver weight (Fig. 1). EGF binding to control fetal rat liver increased markedly during the 3 days before birth. The number of receptors in the fetal rat livers of day 21 was approximately five times greater than day 19 (Fig. 2). This pattern closely resembles the curve



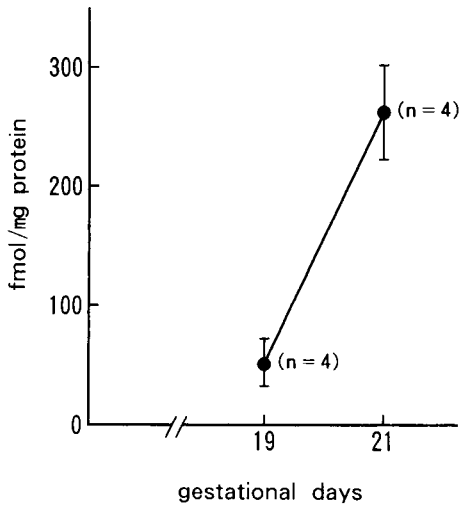


Fig. 1 (Upper) Fetal body weight and liver weight of three groups at day 21. IUGR: intrauterine growth retardation; HFD: heavy-for-dates. Values are mean ± SD.

Fig. 2 (Lower) Epidermal growth factor (EGF) bindings to normal fetal rat liver at day 19 and day 21 of gestation. EGF binding to fetal rat liver increased markedly during 3 days before birth. Values are mean ± SD.

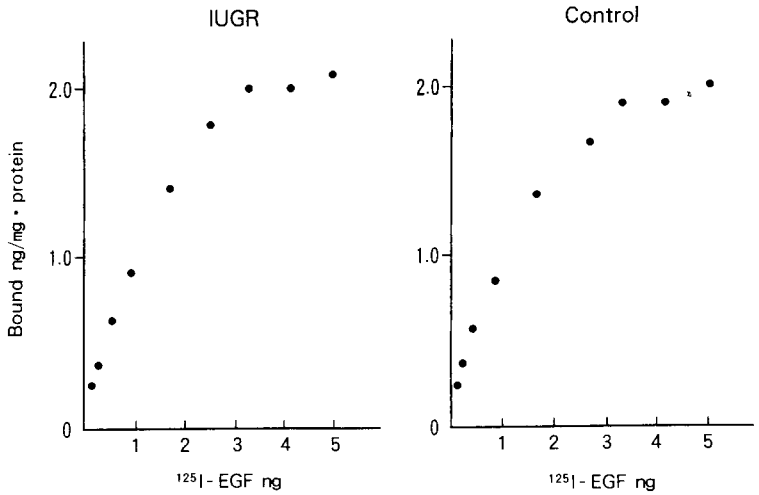


Fig. 3 EGF binding to the livers of control maternal rats and of maternal rats with IUGR fetuses at day 21 of gestation. EGF, IUGR: See Figs 1, 2. Values are mean of 4 samples.

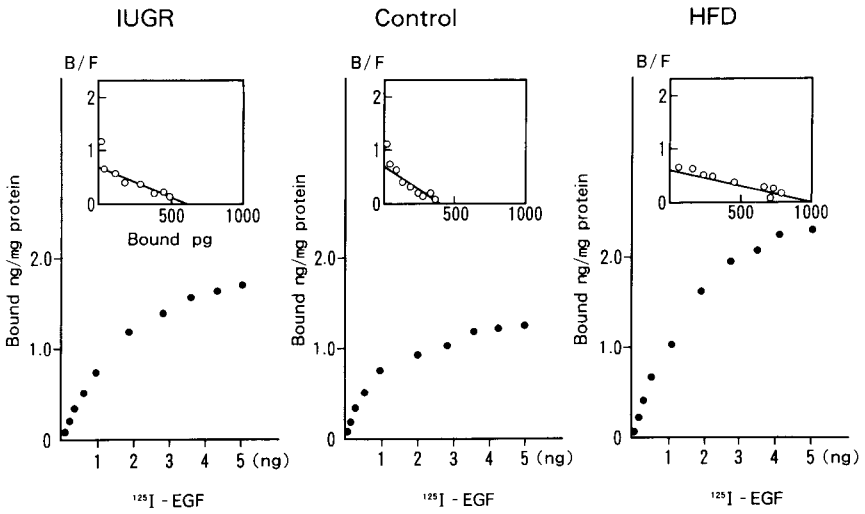


Fig. 4 EGF binding to the liver of IUGR, control and HFD fetal rats at day 21 of gestation. EGF bindings to IUGR and HFD fetal rat liver were significantly increased compared with binding to control fetal rat liver. EGF, IUGR, and HFD: See Figs. 1, 2. Values are mean of 4 samples.

of fetal body weight gain for the same period. A comparison of EGF binding to the livers of maternal rats in the control group (444 ± 66 fmol/mg protein, dissociation constant (Kd) 1.60 ± 0.49 nM) and maternal rats with IUGR fetuses (500 ± 74, 1.87 ± 0.56) is shown in Fig. 3. There were no differences in the EGF binding and Kd between these two groups (Table 1).

A comparison of hepatic EGF binding in control, IUGR and HFD fetal rats is shown in Fig. 4. EGF binding to IUGR rat liver (380 ± 57 fmol/mg protein) and HFD rat liver (545 ± 112) was significantly higher than that of control fetal rat liver (258 ± 47), ($p < 0.05$ and $p < 0.05$, respectively). There was no significant difference in the Kd between IUGR and control rats, but the Kd of HFD rats was higher than that of control rats ($p < 0.05$) (Table 2).

Table 1 Receptor number and binding affinity in maternal rat livers at day 21 of gestation.

Group	Receptor number (fmol/mg protein)	Dissociation constant (Kd) (nM)
IUGR[a]	500 ± 74 (n = 4)	1.87 ± 0.56
Control	444 ± 66 (n = 4)	1.60 ± 0.49

There were no differences in the EGF binding and dissociation constants between the two groups. Values are mean ± SD.
a: Intrauterine growth retardation.

Table 2 Receptor number and binding affinity in IUGR, control, and HFD fetal rat livers at day 21 of gestation.

Group	Receptor number (fmol/mg protein)	Dissociation constant (Kd) (nM)
IUGR[a]	380 ± 57.8* (n = 4)	1.49 ± 0.45
Control	258 ± 47.3 (n = 4)	1.07 ± 0.20
HFD[b]	545 ± 112* (n = 4)	2.31 ± 0.67*

Values are mean ± SD.
*: $p < 0.05$ *vs.* control.
a: Intrauterine growth retardation.
b: Heavy-for-dates.

Discussion

In this study, we analyzed EGF receptor expression in fetal rat liver to investigate mechanisms that control EGF level. We found that hepatic EGF bindings in IUGR and HFD fetal rats were significantly greater than in the control group. This result demonstrated an inverse relationship to neonatal urinary EGF levels, in which EGF concentrations in the first-voided urine were higher in AFD infants than in IUGR and HFD infants (8).

EGF binding to fetal rat liver increased markedly during the three days before birth. Gruppso (12) reported that there was insufficient specific EGF binding in 17-day-old fetal rat liver to permit Scatchard analysis and that EGF receptor numbers increased markedly from days 17 -21 of gestation. This change of EGF receptor binding is in agreement with rapid fetal weight increase of this period and suggests that EGF may be closely associated with fetal growth mediated by EGF receptors in the fetal liver. IUGR fetal rats may compensate for an undernorished condition by increasing hepatic EGF receptors. Gruppso (12) commented that IUGR due to maternal fasting did not significantly affect hepatic EGF receptor numbers in fetal rats, which differs from our results. This difference may be due to differences in the degree of IUGR. Their fetal weight loss was approximately 10 %, while ours was 44 %.

Increased EGF binding to HFD fetal rat liver may suggest that HFD rats consume more of the total supply of EGF, and this may be the reason why EGF concentrations were low in HFD infants' first voided urine. The Kd of HFD rats was slightly higher than that of the control group. This result may be due to the lower affinity of binding derived from the increase of EGF binding to receptors.

The placenta also has many EGF. receptors. Lawrence *et al.* (13) found that EGF binding to membranes prepared from IUGR fetuses was greater than the control binding, and suggested this might serve to enhance nutrient uptake. EGF binding to rat placenta increases slightly from days 19-21 of gestation (13), but the degree of this increase is very small compared to that of fetal rat liver. Thus, EGF seems to be much more active in the liver than in the placenta during the period of rapid increase of fetal body weight.

EGF is closely associated with fetal body weight changes and may control fetal growth by acting on EGF receptors in the liver. Further examination of EGF receptor mRNA and hormonal or nutritional influences on EGF receptor expression may help to reveal the role of EGF in fetal growth more concretely.

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Received July 27, 1992; accepted September 2, 1992.