Differential hepatic lobar gene expression in offspring exposed to altered maternal dietary protein intake

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Zhang, J unlong, and Christopher D. Byrne Differential hepatic lobar gene expression in offspring exposed to altered maternal dietary protein intake. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G128–G136, 2000.—Increased plasma fibrinogen concentrations are a recognized risk factor for coronary heart disease, and increased fibrinogen levels in adults are associated with parameters of reduced early growth. We studied fibrinogen gene expression in adult offspring of dams fed either a 20% (control) or an 8% protein diet [maternal low-protein (MLP) rats] during pregnancy and lactation and determined whether any effects were consistent between left and right liver lobes, since the fetal liver has a unique blood supply that produces differential stimuli to the left and right lobes. In MLP offspring, there was a reduction in all three fibrinogen mRNA copy numbers in the left liver lobe (left vs. right lobes for α-, β-, and γ-fibrinogen (×10⁶ copies/ng total RNA): 8.04 vs. 23.16, P < 0.001; 4.74 vs. 13.07, P < 0.001; and 4.61 vs. 16.38, P = 0.007, respectively), with a parallel reduction in fibrinogen concentration in the left liver lobe (8.53 ± 0.33 vs. 10.41 ± 0.65 arbitrary units, P = 0.014, left and right lobes, respectively). No such effect was observed in offspring of control dams. To investigate the underlying mechanism, glucocorticoid receptor function and mRNA levels were studied, since expression of fibrinogen genes is regulated by glucocorticoid hormones. The binding affinity of the high-affinity glucocorticoid receptor was reduced only in the left liver lobe of the MLP offspring (P = 0.02, left vs. right), with a parallel reduction in this lobe in glucocorticoid receptor mRNA level (P = 0.006, left vs. right). In conclusion, maternal dietary protein restriction reduces fibrinogen gene expression, fibrinogen protein, and mRNA level and binding affinity of glucocorticoid receptors only in the left liver lobe of the adult offspring.

A proposed mechanism linking early growth retardation with adult disease involves changes in concentrations of fetal and placental hormones (4), and it has been suggested that exposure of the fetus to excess maternal glucocorticoid hormone underpins the epidemiological findings (35, 46). Small babies have increased levels of plasma cortisol in adulthood (48), and placental 11β-hydroxysteroid dehydrogenase (11β-HSD2) plays a crucial role in regulating fetal exposure to maternal glucocorticoid hormone (11). Animal studies have shown that rats exposed to malnutrition during pregnancy had reduced placental 11β-HSD2 activity (33), producing hypertensive glucose-intolerant offspring with increased basal and stress-induced glucocorticoid secretion (52). Furthermore, administration of an 11β-HSD antagonist to pregnant rats leads to a significant reduction in average birth weight, with subsequent hyperglycemia later in life (35). In support of these findings, high levels of maternal glucocorticoid in animals (induced by administration of dexamethasone) reduced birth weight of the offspring in rats (13), sheep (31), and lambs (30). Moreover, rat offspring were also hypertensive in this model system.

Increased plasma fibrinogen concentration is an important and independent risk factor for coronary heart disease (17, 23, 25, 32, 34, 41, 57). Epidemiological studies have suggested that adult fibrinogen concentrations are influenced by early development, because plasma fibrinogen concentrations decrease with increasing weight at 1 yr of age (7). Plasma fibrinogen levels are predominantly determined by factors regulating fibrinogen gene expression, and expression of all three fibrinogen genes is known to be regulated by glucocorticoid hormones (50). We have shown that postweaning dietary protein restriction reduces plasma fibrinogen concentrations (59), which is related to reduced glucocorticoid receptor function (unpublished observations). However, to date it is uncertain whether maternal dietary protein restriction alone has any independent long-term consequences on adult plasma fibrinogen concentrations in the offspring, despite normal nutrition in these offspring from weaning.

Any effect of maternal dietary protein restriction on liver development of the fetus may be mediated by the unique fetal hepatic blood supply. The fetal hepatic circulation is different from that of adults because of the presence of the ductus venosus, which closes shortly after birth. Maternally-derived nutrients reaching the fetus from the placenta arrive via the umbilical vein.

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The fetal left liver lobe receives a greater blood supply than the right liver lobe because of the differential blood supply between the left and right lobes derived from the umbilical vein (24). Thus the left liver lobe should normally receive a greater supply of nutrients, growth factors, and hormones, although to date it is uncertain whether differential lobar blood supply has any subsequent effect on liver development or lobar gene expression in the developing fetus. Because we had shown that adult dietary protein intake affects fibrinogen gene expression (59), we reasoned that maternal dietary protein intake may have a lasting impact on fibrinogen gene expression in the developing offspring. Furthermore, because the fetal left liver lobe may be more severely affected by the mother’s nutritional state during gestation because of the unique fetal hepatic blood flow during development (24), we hypothesized that there might be a difference in fibrinogen gene expression and protein concentration between left and right liver lobes in the offspring of maternal low-protein (MLP) rats.

The aims of this study were to investigate whether in the offspring 1) hepatic fibrinogen gene expression could be modulated by maternal dietary protein restriction, 2) there was differential fibrinogen expression between left and right liver lobes, and 3) any effect of dietary protein restriction on fibrinogen gene expression was associated with altered glucocorticoid receptor function, because glucocorticoid receptor activity regulates fibrinogen gene expression.

MATERIALS AND METHODS

Experimental Animals

Male Wistar rats were used in the study. The MLP rats were born to mothers fed a low-protein (8% casein) diet during pregnancy and lactation. The control rats were born to dams fed a normal (20% casein) diet. Both control and MLP rats were weaned at day 21 onto a normal 20% casein diet until adulthood (3 mo). The low-protein diets were isocaloric with the 20% protein diets and matched for minerals, vitamins, corn starch, cellulose, and soybean oil. The control diet contained 55.2 g of cerelose, and the low-protein diet contained 68.2 g of cerelose per 100 g total feed. Rats were fasted overnight and anesthetized before blood was obtained by cardiac puncture for plasma fibrinogen measurement. Then rats were killed, and the livers were removed for further study.

Measurements of Plasma Fibrinogen

Plasma fibrinogen was measured by the clotting method of Clauss (16). The coagulation time was measured using an Amelung KC10 coagulometer linked to a computer that calculated the fibrinogen content of each sample by reference to a standard curve generated at the start of each assay run. Citrated blood (1.9 vol/vol) was centrifuged at −1,750 g for 10 min for preparation of plasma. The standard (IMMONO Reference Plasma 100%) was diluted serially in Owren's buffer to provide a three-point standard curve at 1:5, 1:10, and 1:20. Duplicate 0.2-ml aliquots of each dilution were transferred to KC10 cuvettes. Clot formation was initiated by adding 0.1 ml of thrombin [50 U/ml bovine thrombin (Sigma)], and time taken for clot formation was noted. Test samples were diluted 1:10 in Owren's buffer and dotted as the standard. The clotting times of the test samples were automatically converted to fibrinogen content by comparison to the standard curve.

Preparation of Total RNA

All analytical chemicals for total RNA preparation were purchased from Sigma. RNA was prepared as described (15). Liver tissue (−100 mg) from both the left and right lobes of control and MLP rats was homogenized on ice. The yield of total RNA was between 1.5 and 3 mg/g liver tissue. Total RNA was quantified by spectrophotometry, and its quality was checked by formaldehyde-agarose gel electrophoresis. Only samples with a ratio of 28S to 18S ribosomal RNA of 2:1 were accepted for analyses of mRNA copy number.

Preparation of Oligonucleotides

Three primer-binding site sequences (1, 5, and 3) were identified for the preparation of oligonucleotides to undertake RT-PCR (61). All primers were synthesized by Genosys Europe (Cambridge, UK). Synthesized primers were dissolved in TE buffer (10 mM Tris·HCl, 1 mM EDTA, pH 8.0) to a stock concentration of 80 µM.

The primers for the α-fibrinogen gene (18) were primer 1 (sense sequence), 5′-TAATACGACTCATAAGGGAGGTGTTAGTTGAGAAG-3′; primer 5 (antisense sequence), 5′-TTAATGCTTCCACTCTGGG-3′; and primer 3 (antisense sequence), 5′-TTAATGCTTCCACTCTGGGAGTACCTGTTG-3′. Final amplification with primers 1 and 5 resulted in two PCR products, 564 bp from the target RNA and 454 bp from the standard RNA. The 110-bp difference between the two products could be separated on an agarose gel.

Primers for the β-fibrinogen gene (22) were primer 1 (sense sequence), 5′-TAATACGACTCATAAGGGAGGTGTTAGTTGAGAAG-3′; primer 5 (antisense sequence), 5′-CACCCCAAGTATGATCTGCC-3′; and primer 3 (antisense sequence), 5′-CACCCCAAGTATGATCTGCCGAGA-3′. Final amplification with primers 1 and 5 resulted in two PCR products, 497 bp from the target RNA and 386 bp from standard RNA.

Primers for the γ-fibrinogen gene (45) were primer 1 (sense sequence), 5′-TAATACGACTCATAAGGGAGGTGTTAGTTGAGAAG-3′; primer 5 (antisense sequence), 5′-TCATGGGTTAGTAACAGTGCTTACCTGTTG-3′; and primer 3 (antisense sequence), 5′-TCATGGGTTAGTAACAGTGCTTACCTGTTG-3′. Final amplification with primers 1 and 5 resulted in two PCR products, 434 bp from the target RNA and 303 bp from standard RNA.

Primers for the glucocorticoid receptor (42) were primer 1 (sense sequence), 5′-TAATACGACTCATAAGGGAGGTGTTAGTTGAGAAG-3′; primer 5 (antisense sequence), 5′-TGAGTGTTGCTATAATCTGCTCC-3′; and primer 3 (antisense sequence), 5′-TGAGTGTTGCTATAATCTGCTCC-3′. Final amplification with primers 1 and 5 resulted in two PCR products, 434 bp from the target RNA and 344 bp from the standard RNA.

Conditions for cDNA Synthesis

The reaction conditions for cDNA synthesis of standard RNA, quality control RNA, and RT-PCR assays were the same as described previously (58, 59, 61). RNAs were denatured at 70°C for 5 min and added to a reverse transcription solution containing 0.5 mM dNTPs, 5 µM antisense primer (PCR primer 5), or random hexamers when synthesizing cDNA for standard RNA or quality control RNA (see Preparation of
Standard RNA and Quality Control RNA, 0.3 U/µl Super RT (HT Biotechnology, Cambridge, UK), 1 U/µl RNase inhibitor (Promega), 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM dithiothreitol (DTT), and 10 mM MgCl2 in a final volume of 20 µl. The cDNA synthesis reaction was performed at 42°C for 60 min. The reaction was stopped by denaturing at 95°C for 5 min.

Conditions for PCR Amplification

PCR amplification was performed in a total volume of 25 µl/reaction using Ready-To-Go PCR beads (Promega), which contained ~1.5 units Taq, 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 1.5 mM MgCl2, and 0.2 mM dNTPs and stabilizers, including BSA. Approximately 1.25 µl of freshly synthesized cDNA and 0.4 pmol/µl sense and antisense primers (primer 1 and primer 5) were added to each of the PCR beads, and four individual PCR beads were prepared for each of the cDNA to be amplified. Each amplification was performed with 1 min of denaturation at 95°C, 1 min of extension at 72°C, and a final 7-min extension time at the end of the PCR reaction. The annealing time was 1 min, and the temperatures used were 55°C for α-fibrinogen, 56°C for β-fibrinogen, and 57°C for γ-fibrinogen genes. Amplification was carried out for 20–23 cycles for each cDNA, with the four PCR reactions for all three genes. For the glucocorticoid receptor, the amplification conditions were the same, except that annealing temperature was 57°C and the cycle numbers were 25–28 cycles.

Preparation of Standard RNA and Quality Control RNA

One microgram of total RNA was reverse transcribed into cDNA (as described in Conditions for cDNA Synthesis, except random hexamers were used), then amplified by PCR with primer 1 and primer 3 for synthesis of standard RNA, or primer 1 and primer 5 (61) for synthesis of quality control RNA (in a total volume of 50 µl for each reaction). Amplified PCR product was checked on a 2% (wt/vol) agarose gel, and the specific band was cut off under a long-wave ultraviolet lamp. The gel containing the desired DNA was dissected into small cubes (~1 mm), added with 1 ml of sodium perchlorate (4.5 × vol), and incubated at 55°C for 5–10 min until the gel cubes were completely melted. The specific DNA bands for either standard RNA synthesis or quality control RNA synthesis were purified according to the instructions provided by the manufacturer (Scotlab). The purified DNA was dissolved in diethyl pyrocarbonate-H2O and added to DTT (10 mM), rNTPs (0.5 mM), 1 × T7 RNA polymerase buffer (40 mM Tris-HCl, pH 7.9, 6 mM MgCl2, 2 mM spermidine, and 10 mM NaCl; Promega), RNase (1 U/µl) inhibitor, T7 RNA polymerase (1 unit/µl; Promega), and T7 primer (1.25 µM) to a total volume of 20 µl. The reaction was incubated at 37°C for 1 h, and then the DNA was digested by incubation with RNase-free DNase (1.0 U/µl) at 37°C for 30 min. The synthesized standard RNA or quality control RNA were extracted with chloroform-isomyl alcohol, precipitated with 100% (vol/vol) ethanol and washed with 70% (vol/vol) ethanol. The standard RNA or quality control RNA pellets were vacuum dried separately, individually dissolved in diethyl pyrocarbonate-H2O, and quantified by spectrophotometer.

Quantitative Competitive RT-PCR

To determine mRNA copy number in a single RNA sample, four separate cDNA synthesis reactions were undertaken with increasing amounts of total RNA. Each cDNA synthesis reaction was divided into a further four aliquots after adding the PCR master mix, and each aliquot was amplified a certain number of cycles (20, 21, 22, or 23 cycles for fibrinogen; see Conditions for PCR Amplification). Consequently, results from 16 reactions were obtained from each total RNA sample that was examined. Data were analyzed and mRNA copy numbers were calculated as described previously (59).

Quantification of RT-PCR Products

Quantification of RT-PCR products was as described previously (61). Briefly, RT-PCR products were analyzed on a 2% agarose gel containing ethidium bromide. DNA bands were photographed using an Eagle Eye II video system (Stratagene). The image was exported in a TIF file, and DNA bands were quantified using NIH Image 1.55 software for Macintosh. The quantification value of the bands in the gel was designated the optical density (pixels). Measurements of optical density using the above software were linear up to 120 pixels. In each case, optical densities from target template (Dt) and standard templates (Ds) were obtained. The ratio of Dt to Ds was determined, and, since the number of copies of standard RNA added to the reaction was known (1 mol = 6 × 10²³ molecules (Avogadro’s constant)), the number of copies of target RNA could be calculated. The mRNA level was expressed as the number of mRNA copies per nanogram of total RNA.

ELISA for Measurement of Total Fibrinogen

Preparation of liver lysate. Rat liver (~0.1 g) from the left and right lobes was homogenized in 1 ml of buffer containing 50 mM HEPES, 150 mM NaCl, 1% Triton X-100, 1 mM Na3VO4, 30 mM NaF, 10 mM Na3P2O7, 10 mM EDTA, 1 µg/ml Antipain, 1 µg/ml pepstatin, 2.5 mM benzamidine, and 0.5 mM phenylmethylsulfonyl fluoride. Homogenized liver lysates were centrifuged at 13,000 g for 5 min at 4°C, and the pellets of cell debris were discarded. In preliminary experiments, the Triton X-100 in the homogenization buffer was found to interfere with the ELISA even at very low concentrations [0.0005% (vol/vol)]. Therefore, the liver lysate was purified before ELISA.

Purification of liver lysate solution. Ten microliters of the supernatant was diluted with 90 µl of PBS, put into a sample reservoir of a Microcon-30 column (Amicon, Beverly, MA), and centrifuged at 6,500 g for 20 min at 4°C. The solution was discarded and the sample reservoir was washed twice with 200 µl PBS and centrifuged at 6,500 g for 20 min. Proteins were collected by inverting the sample reservoir and were centrifuged at 3,000 g for 3 min. The collected proteins were dissolved in 100 µl of PBS and quantified according to the modified Lowry method (36).

ELISA. Each well of a 96-well microtiter plate (Nunc, Maxisorp) was coated with 100 µl of polyclonal antibody against rat fibrinogen [raised in rabbit, diluted 1:750 in 0.1 M NaHCO3 (pH 9.2); Nordic Immunological] at 4°C overnight and blocked with 200 µl of blocking buffer (PBS-1% (wt/vol) BSA) for 1 h at room temperature, washed three times with washing buffer (PBS-0.1% (vol/vol) Tween 20). Samples (100 µl) diluted with sample buffer (PBS-3% (wt/vol) BSA) were incubated for 2 h at room temperature. The plate was washed four times with 200 µl of washing buffer, and then 100 µl of rat fibrinogen polyclonal antibody [raised in goat, conjugated with peroxidase, diluted 1:2,000 with PBS-1% (wt/vol) BSA, and 0.1% Tween 20; Nordic Immunological] was added per well for 2 h at room temperature. The plate was then washed four times with 200 µl of washing buffer and twice with PBS, before 200 µl of 0.1 M citrate buffer (pH 4.0) containing 0.25 µl of 0.3% H2O2 was added for 1 h at room temperature. Absorbance at 405 nm was measured. The intra-assay coeffi-
Table 1. Body weight, liver weight, and plasma fibrinogen concentrations in rats born to mothers fed with different diets during gestation and lactation

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>MLP</th>
<th>P</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>479.80 ± 37.7</td>
<td>420.33 ± 42.12</td>
<td>0.011</td>
<td>6</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>16.46 ± 2.06</td>
<td>13.78 ± 1.71</td>
<td>0.002</td>
<td>6</td>
</tr>
<tr>
<td>Plasma fibrinogen, mg/dl</td>
<td>154.60 ± 8.70</td>
<td>145.80 ± 8.40</td>
<td>0.2</td>
<td>12</td>
</tr>
</tbody>
</table>

Values are means ± SE. MLP, maternal low protein.

dient of variation (c.v.) was 6.8% (n = 30), and the interassay c.v. was 7.9% (n = 8).

Human fibrinogen protein (Sigma) was used as a standard because rat fibrinogen was not available. Since it was unknown whether anti-rat fibrinogen antibodies bound with equal affinities to the human fibrinogen protein used as the standard, we have expressed the fibrinogen protein results as arbitrary units.

Statistical Analyses

All statistical calculations were performed using Statworks statistical software. Differences in mean mRNA copy number of fibrinogen genes, glucocorticoid receptor gene, and liver weight between the left and right liver lobes of the same rat were examined by paired Student's t-test. Difference in mean plasma fibrinogen concentration between control and low-protein rats were examined by unpaired Student's t-test. Results are presented as means ± SE unless otherwise indicated.

RESULTS

Effects of Maternal Dietary Protein Restriction on Rat Liver, Body Weight, and Plasma Fibrinogen Concentrations

Although both control and MLP rats were fed an identical normal 20% protein diet from weaning (at day 21) until adulthood (3 mo), the body and liver weight of MLP rats were reduced by 12.4% and 16.3%, respectively, compared with the control rats (Table 1). Plasma fibrinogen concentrations tended to be lower in the MLP rats, although this difference was not significant (Table 1).

Effects of Maternal Dietary Protein Restriction on Fibrinogen Protein Concentrations

We determined whether there was a difference in fibrinogen protein concentration between left and right liver lobes in control and MLP rats because it has been suggested that the fetal left liver lobe may be more affected by the mother's nutritional state during gestation because of the unique fetal hepatic blood flow during development (24).

Hepatic fibrinogen protein concentrations were measured by ELISA and are presented as arbitrary units. Fibrinogen protein concentration was lower in the left liver lobe than that in the right liver lobe in MLP rats (Table 2). In contrast, fibrinogen protein concentration was similar between the left and right liver lobes in control rats (Table 2). Thus these data suggest that maternal diet has a long-term effect on fibrinogen protein synthesis, differentially affecting the left liver lobe in the offspring.

Table 2. Fibrinogen protein concentrations and mRNA copy numbers between the left and right liver lobes

<table>
<thead>
<tr>
<th>Genes</th>
<th>Left Lobe</th>
<th>Right Lobe</th>
<th>P</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-FBG mRNA copy numbers</td>
<td>17.96 ± 3.03</td>
<td>20.13 ± 2.67</td>
<td>0.6</td>
<td>5</td>
</tr>
<tr>
<td>β-FBG mRNA copy numbers</td>
<td>11.21 ± 1.23</td>
<td>13.77 ± 2.40</td>
<td>0.37</td>
<td>5</td>
</tr>
<tr>
<td>γ-FBG mRNA copy numbers</td>
<td>11.67 ± 1.45</td>
<td>17.20 ± 2.80</td>
<td>0.07</td>
<td>7</td>
</tr>
<tr>
<td>MLP rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-FBG mRNA copy numbers</td>
<td>8.04 ± 1.24</td>
<td>23.16 ± 1.21</td>
<td>&lt;0.001</td>
<td>5</td>
</tr>
<tr>
<td>β-FBG mRNA copy numbers</td>
<td>4.74 ± 0.53</td>
<td>13.07 ± 0.96</td>
<td>0.001</td>
<td>5</td>
</tr>
<tr>
<td>γ-FBG mRNA copy numbers</td>
<td>4.61 ± 0.39</td>
<td>16.38 ± 2.33</td>
<td>0.007</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Copy numbers are ×10⁶ copies/ng total RNA. AU, arbitrary units; FBG, fibrinogen.
classes of glucocorticoid receptors, designated as high-
affinity glucocorticoid receptors (GRh) and low-affinity
binding sites (LAGS), were present in both the control and MLP rats, differing in both binding affinities and binding capacities (Fig. 2).

There was no difference in either binding affinities or binding capacities of GRh or the LAGS between the left and right liver lobes in the control rats (Table 3). However, the binding affinity of GRh was markedly reduced in the left liver lobe in MLP rats, although the binding capacity of GRh was similar between the left and right liver lobes (Table 3). In contrast to GRh, there was no difference in both binding affinity and capacity of the LAGS between the left and right liver lobes in MLP rats (Table 3). These data suggest that maternal dietary protein restriction has a long-term effect, differentially reducing the binding affinity of GRh in the left liver lobe without affecting either binding affinity or capacity of the LAGS.

Effects of Maternal Low-Protein Diet on the mRNA Levels of Glucocorticoid Receptor

Having observed a differential effect of maternal dietary protein restriction on glucocorticoid receptor-binding affinity in the left liver lobe, we determined whether mRNA levels of glucocorticoid receptor were affected by this diet. Glucocorticoid receptor mRNA...
levels were measured by quantitative competitive RT-PCR (Fig. 3). Glucocorticoid receptor mRNA levels were similar between left and right liver lobes in the control rats [18.11 ± 6.56 vs. 18.25 ± 6.85 (×10^3 copies/ng total RNA) in left and right lobes, respectively; \( P = 0.69; n = 6 \)]. In support of the fibrinogen data, glucocorticoid receptor mRNA levels in MLP offspring were markedly reduced in the left compared with the right liver lobe [3.38 ± 0.23 vs. 7.64 ± 0.87 (×10^3 copies/ng total RNA) in the left and right liver lobes, respectively; \( P = 0.006; n = 6 \)]. Thus these data suggest that maternal dietary protein restriction has a parallel long-term effect on glucocorticoid receptor mRNA levels in the offspring and that this effect is more severe in the left rather than right hepatic lobes.

**DISCUSSION**

The novel result of this study is that a simple modification of the maternal diet during gestation and lactation produced a permanent effect on fibrinogen gene expression, particularly affecting the left hepatic lobe in the adult offspring. This effect occurred despite a normal diet in the offspring from weaning until they were examined as adults at 3 mo. Fibrinogen protein concentration and all three fibrinogen mRNA levels were markedly reduced in the left liver lobe compared with the right liver lobe in the MLP offspring, and this effect was associated with altered glucocorticoid receptor affinity and expression. In contrast to these findings, no such effect was observed in offspring born of dams fed a normal diet throughout gestation and weaning.

We have shown the presence of two classes of glucocorticoid receptors that differ in both binding affinities and capacities in both groups of rats. Whereas our data show a long-term effect of maternal dietary protein restriction on binding affinity of the high-affinity GRh receptor in the left lobe, in contrast, maternal dietary protein restriction had no such effect on LAGS. Furthermore, we have shown that maternal dietary protein restriction was associated with markedly reduced GRh mRNA levels of the offspring and that, in parallel with the fibrinogen mRNA data, this effect was also most severe in the left lobe.

**Table 3. Comparison of glucocorticoid receptor functions and mRNA levels between the left and right liver lobes**

<table>
<thead>
<tr>
<th></th>
<th>Left Lobe</th>
<th>Right Lobe</th>
<th>( P ) Values</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_{dH} ), nM</td>
<td>30.8 ± 4.1</td>
<td>29.7 ± 3.9</td>
<td>0.68</td>
<td>6</td>
</tr>
<tr>
<td>( B_{maxH} ), fmol/mg</td>
<td>118.7 ± 30.0</td>
<td>107.0 ± 29.0</td>
<td>0.42</td>
<td>6</td>
</tr>
<tr>
<td>( K_{dL} ), nM</td>
<td>237.0 ± 77.0</td>
<td>289.0 ± 98.0</td>
<td>0.70</td>
<td>5</td>
</tr>
<tr>
<td>( B_{maxL} ), fmol/mg</td>
<td>675.0 ± 21.0</td>
<td>640.0 ± 78.0</td>
<td>0.86</td>
<td>5</td>
</tr>
<tr>
<td>mRNA levels</td>
<td>18.11 ± 6.56</td>
<td>18.65 ± 6.85</td>
<td>0.69</td>
<td>6</td>
</tr>
<tr>
<td>MLP rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_{dH} ), nM</td>
<td>45.2 ± 6.9</td>
<td>25.7 ± 4.8</td>
<td>0.02</td>
<td>2</td>
</tr>
<tr>
<td>( B_{maxH} ), fmol/mg</td>
<td>103.6 ± 11.0</td>
<td>113.6 ± 17.0</td>
<td>0.63</td>
<td>6</td>
</tr>
<tr>
<td>( K_{dL} ), nM</td>
<td>168.3 ± 25.0</td>
<td>197.0 ± 37.0</td>
<td>0.45</td>
<td>6</td>
</tr>
<tr>
<td>( B_{maxL} ), fmol/mg</td>
<td>601.0 ± 14.3</td>
<td>746.0 ± 193</td>
<td>0.25</td>
<td>6</td>
</tr>
<tr>
<td>mRNA levels</td>
<td>3.38 ± 0.23</td>
<td>7.64 ± 0.87</td>
<td>0.006</td>
<td>6</td>
</tr>
</tbody>
</table>

\( K_{dH}, K_{dL} \): dissociation constant for high-affinity glucocorticoid receptors (GRh) and low-affinity glucocorticoid-binding sites (LAGS) respectively (in nM); \( B_{maxH}, B_{maxL} \): maximum binding capacity for GRh and LAGS, respectively. mRNA levels are presented as copies (×10^3) per nanogram of total RNA.

![Image](image-url)
A Long-Term Effect of Maternal Dietary Protein Restriction on Gene Expression in the Adult Offspring

The phenomenon linking long-term outcome in adults with early fetal development was first observed in epidemiological studies. For example, increased death from coronary heart disease (9, 10) and the presence of high blood pressure (3), impaired glucose tolerance, hyperinsulinemia (38), insulin resistance (49), non-insulin-dependent diabetes (26, 27), and dyslipidemia (6, 37) have all been shown to be associated with reduced birth weight. In keeping with these findings, it has been suggested that the metabolic syndrome should be referred to as “the small-baby syndrome” (5). A long-term, more generalized effect of maternal dietary protein restriction on body weight and liver weight has been reported previously (19). Even though we observed no marked difference in plasma fibrinogen concentrations between MLP offspring and the control rats, a permanent effect of maternal dietary protein restriction on hepatic fibrinogen protein concentration occurred in the left liver lobe of the MLP offspring. The reduction in fibrinogen gene expression in the left liver lobe is specific, since there was no significant reduction in weight of the left liver lobe (data not shown). The data in this study support the hypothesis that adult concentrations of fibrinogen are influenced by the growth of the liver in fetal life (39). Although this hypothesis was derived from epidemiological observations, it demonstrated an association between increased plasma fibrinogen concentrations and low birth weight (39). The data in our study did not support a relationship between reduced birth weight and increased plasma fibrinogen concentration (data not shown), in contrast to the epidemiological observations. The reasons for this discrepancy are complex and are probably due to the fact that many factors cause reduced birth weight. For example, polygenic influences resulting in insulin resistance in the normal population are likely to result in lower birth weight (29). Mutations in the glucokinase gene of the fetus (28), maternal malnutrition such as low vitamin C intake (40), and abnormal placental function (47, 54, 56) have been shown to be associated with reduced birth weight. Furthermore, altered hormone action such as increased fetal exposure to glucocorticoids (53), maternal hypothyroidism (44), and maternal insulin-like growth factor I (12) is associated with altered birth weight. Therefore, individuals in the epidemiological studies may have low birth weight due to many different factors. Thus the relationship between reduced birth weight and plasma fibrinogen concentrations in adults may vary depending on the specific factor responsible for causing low birth weight. In our study, a manipulation of the maternal diet, with increased carbohydrate content and reduced protein content, reduced birth weight (data not shown) but did not result in reduced plasma fibrinogen concentration, although a reduction in fibrinogen gene expression occurred. We have shown elsewhere that plasma fibrinogen concentration can be reduced by a change in adult dietary carbohydrate and protein contents (59). Thus we suggest that the observation of similar levels of plasma fibrinogen between MLP offspring and control rats may be due to the fact that identical diets were fed to offspring in both groups from weaning. Furthermore, reduced expression in fibrinogen mRNA levels in the left liver lobe, not resulting in an overall reduction in plasma fibrinogen concentration, may be due to a compensatory increase in fibrinogen synthesis in the other two liver lobes, namely, the caudate and quadrate lobes. Unfortunately, we did not have an opportunity to measure fibrinogen expression in these lobes.

Mechanisms of Regulation of Fibrinogen Gene Expression

We have shown previously that dietary protein restriction introduced only after weaning in the offspring reduced glucocorticoid receptor function (unpublished observation) and fibrinogen gene expression despite these offspring being subjected to an entirely normal diet during gestation and weaning (59). Consistent with these findings, the presented results show that maternal dietary protein restriction alone affected both the glucocorticoid receptor mRNA levels and glucocorticoid receptor-binding affinity only in the left liver lobe of the offspring. Thus these data suggest that reduced binding affinity in the left liver lobe may reduce binding of receptors to glucocorticoid hormones and in turn alter the activity of genes regulated by this important regulator of gene expression. Differentially reduced expression of fibrinogen genes in the left liver lobe is therefore likely to be mediated, at least in part, by reduced glucocorticoid receptor function in this lobe, since transcription of the fibrinogen genes is increased by glucocorticoid hormones (50). Thus reduced fibrinogen expression in the left liver lobe of the offspring may be due in part to reduced glucocorticoid receptor function in this lobe, induced by maternal dietary protein restriction.

Differential Reduction in the Left Liver Lobe May Be Attributable to the Unique Hepatic Fetal Blood Flow

Because the differential effect on gene expression between liver lobes in the offspring was induced by modulation of the pregnant mother’s diet, this result suggests that the effect of maternal dietary protein restriction is mediated by altered hepatic development during fetal life. The fetal hepatic blood supply is different from that of the adult liver because the fetal liver receives most of its oxygenated hemoglobin, nutrients, growth factors, and hormones via the umbilical venous circulation and not from the hepatic artery and portal circulation. Umbilical venous blood is distributed preferentially to the left liver lobe because of a potent ductus venosus (43), and therefore the left fetal liver lobe receives blood richer in the various stimuli from the mother (24). Consequently, expression of nutrition-sensitive genes may be differentially and more severely affected in the left, rather than right, hepatic lobes exposed to altered maternally-derived protein.
nutrients. Glucocorticoid receptor expression is necessary for regulation of gene expression during fetal life (14), and therefore, in keeping with our results, it is possible that changes in glucocorticoid receptor expression have an important impact on regulation of gene expression during fetal development. Perhaps even more importantly, the effect on gene expression persists into adulthood despite the introduction of a normal diet from weaning.

We speculate that development of the left hepatic lobe and expression of genes within this lobe may be more susceptible to altered stimuli from the mother and that this effect is mediated by the differential blood supply to left and right fetal hepatic lobes during development.

In conclusion, we have shown that maternal dietary protein restriction has a permanent and marked effect on fibrinogen expression, particularly in the left liver lobe. Our results suggest that this effect is in part mediated by reduced glucocorticoid receptor expression and function.

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