Maternal diet programs embryonic kidney gene expression

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Maternal diet programs embryonic kidney gene expression. Physiol Genomics 22: 48–56, 2005. First published April 12, 2005; 10.1152/physiolgenomics.00167.2004.—Human epidemiological data associating birth weight with adult disease suggest that organogenesis is “programmed” by maternal diet. In rats, protein restriction in pregnancy produces offspring with fewer renal glomeruli and higher systemic blood pressures than controls. We tested the hypothesis that maternal diet alters gene expression in the metanephros, the precursor of the definitive mammalian kidney. We demonstrated that maternal low-protein diet initiated when pregnancy starts and maintained to embryonic day 13, when the metanephros consists of mesenchyme surrounding a once-branched ureteric bud, is sufficient to significantly reduce glomerular numbers in offspring by about 20%. As assessed by representation difference analyses and real-time quantitative polymerase chain reactions, low-protein diet modulated gene expression in embryonic day 13 metanephros. In particular, levels of prox-1, the ortholog of Drosophila transcription factor prospero, and cofilin-1, a regulator of the actin cytoskeleton, were reduced. During normal metanephrogenesis, prox-1 protein was first detected in mesenchymal cells around the ureteric tree and thereafter in nascent nephron epithelia, whereas cofilin-1 immunolocalized to bud derivatives and condensing mesenchyme. Previously, we reported that low-protein diets increased mesenchymal apoptosis cells when metanephrogenesis began and thereafter reduced numbers of precursor cells. Collectively, these studies prove that the maternal diet programs the embryonic kidney, altering cell turnover and gene expression at a time when nephrons and glomeruli have yet to form. The human implication is that the maternal diet ingested during pregnancy modulates the development of the metanephros and other organs (40, 42). This idea is indirectly supported by human epidemiological data linking low birth weight with adult diseases such as hypertension as well as Type 2 diabetes mellitus and coronary heart disease (3, 61); such associations might be explained if fetal differentiation could be “programmed” by the maternal diet. Indeed, in rats, protein restriction in pregnancy produces offspring with fewer glomeruli and higher systemic blood pressures than controls (32, 33, 56). Furthermore, in humans, there is a positive correlation between birth weight and glomerular number (24).

What causes a person to be born with fewer nephrons than normal (renal hypoplasia)? There are probably at least three general causes: 1) mutations of nephrogenesis genes such as PAX2 and HNF1B (4; 51); 2) impairment of fetal urinary flow caused by physical obstruction in the lower urinary outflow tract, a phenotype that can be modeled in experimental animals (46, 59); and 3) teratogens (45). Recently, a fourth cause has been suggested, namely, that the maternal diet ingested during pregnancy modulates the development of the metanephros and other organs (40, 42). What biological events underlie embryonic kidney programming? During normal metanephric kidney development, subsets of cells are deleted by apoptosis (12, 18, 57), a mechanism that probably limits the number of nephrons generated and/or the number of cells in each nephron. We previously demonstrated that maternal low-protein diets (LPD; 9% and 6% protein vs. 18% in controls) fed to rats from the day of conception until birth, which occurs around day 21 of pregnancy, led to glomerular deficits of about 25% when measured 2 wk after birth (56). LPD were associated with increased apoptosis in E13.0 metanephric mesenchyme, as assessed by counting pyknotic and in situ end-labeled nuclei. At this stage, the ureteric bud has branched once, branch tips are surrounded by condensed mesenchyme, which will later undergo epithelial transformation into nephrons, and the ureteric stalk is surrounded by loose mesenchyme, called stroma. By contrast, there was no difference in apoptosis between dietary groups at E15.0, when several rounds of ureteric bud branching and formation of primitive nephrons (vesicles, comma- and S-shapes) has already occurred. The total number of metanephric cells was similar (about 2 × 10⁶) in all dietary groups at E13.0,
with a 100-fold increase in control metanephroi over the next 48 h. At E15.0, however, total cell numbers were significantly reduced in metanephroi exposed to maternal LPD. These observations support the hypothesis that LPD alters the trajectory of metanephric growth by modulating cell turnover at the inception of organogenesis.

In the present study, we addressed two questions: 1) does maternal LPD have detrimental effects on final glomerular numbers if experimental diets are instigated at the start of pregnancy and continued to just E13.0 rather than the end of gestation, and 2) does LPD modulate gene expression in the E13.0 metanephroi? The results of these experiments demonstrate that the maternal diet programs transcription in the embryonic kidney at a time when nephrons and glomeruli have yet to form.

**MATERIALS AND METHODS**

Animal protocols were approved by the United Kingdom Home Office. All reagents were from Sigma Chemical (Dorset, UK) unless otherwise stated.

**Dietary manipulations and glomerular counting.** Female Wistar rats (Harlan; Bicester, UK) were time mated by exposure to males between 14.00 and 17.00 hours. At 9.00 hours the next day, rats with vaginal plugs were selected for further study, and this time was designated E0.0. Females were immediately randomly supplied one of three diets (Dyets; Bethlehem, PA) containing either control diet (18% protein by weight) or LPD (9% or 6% protein by weight). The constituents of the 18%, 9%, and 6% protein diets were as follows: casein (180, 90, and 60 g/kg), maize starch (425, 485, and 505 g/kg), sucrose (213, 243, and 253 g/kg). All diets additionally contained methionine (5 g/kg), corn oil (100 g/kg), vitamin mix AIN-76 (5 g/kg), mineral mix (20 g/kg), choline chloride (2 g/kg), and cellulose (50 g/kg) as previously described (56); all diets had the same caloric, vitamin, and mineral contents. We used an identical protocol to that of Langley-Evans et al. (33); using the same rat strain, these investigators demonstrated that food intake was equal among the different diets. Rats were given free access to water through the study, although water intake was not formally quantified. All groups received standard Chow from the manufacturer until 2 wk after birth, with one of the following five variations before birth: 1) 18% protein throughout pregnancy; 2) 9% protein throughout pregnancy; 3) 6% protein throughout pregnancy; 4) 9% protein until E13.0, followed by 18% protein to term; and 5) 6% protein until E13.0, followed by 18% protein to term. Offspring were killed at 2 wk of age, 1 wk after the end of the nephrogenic period, and glomerular numbers were determined after acid digestion using the protocol of Welham et al. (56), modified to use 0.1 M HCl. A multilevel statistical model was used to analyze data to negate the possibility of pseudoreplication (19) as previously described (56).

**Dietary manipulations performed for molecular and histological analyses.** Pregnant rats were supplied either 18%, 9%, or 6% protein diet from E0 until either E13.0 or E15.0. At E13.0 and E15.0, dams were killed, and embryos were removed and kept at 4°C. Metanephroi were snap frozen in liquid nitrogen for biochemical/molecular analyses or fixed in 4% paraformaldehyde. Representational difference analysis (RDA) was performed as previously described (23). In brief, E13.0 kidneys (on average, 35 kidneys from 5 litters for each diet) were digested in Tri-reagent, and total RNA and protein were isolated. mRNA was isolated using Oligotex beads (Qiagen; Crawley, UK) from which cDNA was synthesized. After DpnII (NEB; Hertfordshire, UK) digestion, cDNA pools were ligated with 12- and 24-mer adapter pair ends (5’-GATCTGGTGTA-3’ and 5’-AGCCACGCTCTGAGCA-CAAGGCAACTGTTCATG-3’), and multiplexed using PCR to produce representative cDNA populations. “Driver” and “tester” populations from both the 18% protein group (18D and 18T, respectively) and the 9% LPD group (9D and 9T, respectively) were produced for use in reciprocal subtractive hybridizations in which each driver was used in excess relative to the tester to quench as many of the common sequences as possible, thus minimizing the possibility of false positives. After the first round of hybridization, the difference product was used as the tester for the next hybridization. The new tester populations were religated to new adapter ends (5’-GATCTGGTTCATG-3’ and 5’-AGCCACGCTCTGAGCA-CAAGGCAACTGTTCATG-3’, respectively) and subtractively hybridized against the driver from the opposite dietary group once more. Thus the two hybridizations performed were 18T versus 9D and 9T versus 18D. The former was designed to isolate sequences downregulated by maternal LPD, whereas the latter isolated sequences upregulated by maternal LPD. After two rounds of subtractive hybridization after increasing the stringency of tester concentration versus driver concentration (first round, driver-to-tester ratio is 100:1; second round, driver-to-tester ratio is 800:1), another pair of primer ends was used (5’-GATCTGGTTCATG-3’ and 5’-AGCCACGCTCTGAGCA-CAAGGCAACTGTTCATG-3’), and the final difference product from each hybridization was isolated. Each difference product was ligated into BamHI-digested K5+ Bluestrip vectors and propagated in E. coli (Invitrogen, Paisley, UK). Products were sequenced on an ABI automated sequencer (Applied Biosystems; Foster City, CA), and BLAST searches were carried out. Only sequences with more than 100 identified bases and with a sequence homology of 95% or greater with known sequences were selected as bona fide matches.

**Real-time PCR.** Because of the small quantities of RNA available from E13.0 metanephroi (each metanephros contains about 2 × 10⁴ cells) (56), sequences identified by RDA as being either up- or downregulated by maternal LPD were validated using quantitative (real-time) PCR with SYBR green. Total RNA pooled from E13.0 metanephroi from dams fed either 18%, 9%, or 6% protein diets was converted into cDNA (here, for the 18% and 9% protein groups, we used contralateral organs to those used in the RDA above). For each diet, we pooled organs from three to four litters due to the small quantities of tissue available and the requirement to determine expression of a number of genes. GAPDH was used as a standard against which the expression of individual genes was factored to control for possible differences arising from unequal template concentrations. GAPDH standards were created using forward and reverse primers designed to produce products of 139 bp (5’-TTGCCACCTCAGAGACTGTGGG-3’ and 5’-TAAGAGCTCTCCCGTGTCACCACGCTCT-3’). Known concentrations of this product were diluted to produce a range of standards. For the genes that were apparently differentially expressed according to the RDA results, primers were designed to produce PCR products of between ~100 and 250 bp (primer sequences and relative nucleotide positions are shown Table 2). Individual PCRs were performed using annealing temperatures appropriate for the primer sets. The relative transcript levels between different diets, which are quoted in RESULTS, represent the average measured ratios from three sets of real-time PCRs on each dietary sample. In addition to genes identified as differentially expressed by the RDA screen, both Bax and Bcl2 were also assayed by real-time PCR to assess the levels of expression of known apoptosis-related transcripts.

**Western blot analysis and immunohistochemistry.** Ten micrometers of protein from each dietary group, constituting pools from several litters in each group, were electrophoresed through a 12% SDS polyacrylamide gel and transferred onto nitrocellulose membranes, as previously described (56). Blots were probed with primary antibodies against prox-1 (ReliaTech; Braunschweig, Germany) and cofillin-1 (Chemicon; Temecula, CA) at a concentration of 1:500. The cofillin-1 antibody used in Western blot analysis recognizes both the phosphorylated (inactive) and dephosphorylated (active) forms of cofillin-1. Five-micrometer sections of paraffin-embedded E13.0 and E15.0 metanephroi were dewaxed and probed with antibodies against prox-1 and cofillin-1 (in addition to the cofillin-1 antibody described earlier, we also used an antibody that exclusively recognized the phosphorylated form of cofillin-1; Chemicon) using previously published im...
RESULTS

Maternal diets and final glomerular number. To determine the effects of maternal LPD on final nephron number in offspring, we first examined protocols in which dietary protein content was controlled throughout pregnancy (Fig. 1). Offspring of dams fed 18% protein from E0.0 to term possessed $3.0 \times 10^3 \pm 0.7 \times 10^3$ (means $\pm$ SE) glomeruli/kidney; offspring exposed to a 9% LPD throughout gestation possessed $3.0 \times 10^3 \pm 1.7 \times 10^3$ fewer glomeruli/kidney than controls (about 20% fewer, $P < 0.001$), and those exposed to maternal 6% LPD throughout pregnancy possessed $7.1 \times 10^3 \pm 1.6 \times 10^3$ glomeruli/kidney fewer than controls (about 23% fewer, $P < 0.001$). There was no significant difference between the number of glomeruli in kidneys of 9% versus 6% LPD offspring ($P = 0.716$). The relationships between dietary groups are similar to our previously published data (56), although the absolute numbers in this study are somewhat higher, probably explained by the more gentle dissociation method used here. Strikingly, when glomerular numbers were quantified in offspring from dams exposed to LPD restricted to the period between E0.0 and E13.0, we observed glomerular deficits of similar magnitude compared with the offspring of dams in which LPD was continued beyond E13.0 to the end of pregnancy. For example, offspring of the group exposed to 9% LPD until E13.0 only possessed $5.8 \times 10^3 \pm 1.4 \times 10^3$ glomeruli/kidney fewer than controls (about 19% fewer, $P < 0.001$), and those from the similarly timed 6% LPD group possessed $7.5 \times 10^3 \pm 1.3 \times 10^3$ glomeruli/kidney fewer (about 24% fewer, $P < 0.001$).

Effects of LPD on metanephric gene expression assessed by RDA. The above results suggest that LPD has somehow altered the trajectory of rat nephrogenesis by the day that the metanephros forms. Consistent with this, we (56) previously documented a increased metanephric mesenchymal apoptosis at E13.0 in embryos of mothers exposed to LPD initiated at the start of pregnancy. We reasoned that this programming effect might be correlated with alternations of metanephric gene expression and therefore performed an RDA screen comparing E13.0 metanephroi between control and LPD-exposed animals. By sequencing 31 colonies with inserts in the 18T versus 9D hybridization and 18 colonies with inserts in the 9T versus 18D hybridization and excluding “spurious” products appearing in both sets, several transcripts were isolated by this screen as being either up- or downregulated by maternal LPD (Table 1) (9, 21, 25, 28, 29, 34, 39, 47, 52, 55, 62), with some appearing a number of times.

Effects of LPD on metanephric gene expression assessed by real-time PCR. Several of these genes isolated in the RDA screens code for proteins that fulfil the criteria of being potentially implicated in differentiation and organogenesis (e.g., transcription factors, adhesion molecules, proteins that affect the cytoskeleton, etc.). These were selected for measurement in E13.0 metanephroi using real-time PCR in both 9% and also 6% LPD versus control diets. The results are shown in Table 2.

Immunohistochemistry. From genes found to be differentially expressed, we focused on prox-1, which codes for a homeobox transcription factor (47), and cofilin-1, which codes for an actin depolymerizing protein (55). With the use of E13.0 metanephroin from control animals, immunoprobing for prox-1 demonstrated a signal in stromal cells around the stalk of the ureteric bud (Fig. 2, A–C). As expected for a transcription factor protein, the signal was nuclear. A very faint signal was also seen in a few nuclei in ureteric bud branches and condensing mesenchyme. At E15.0, prox-1 immunoreactivity was detected in the nephrogenic cortex where subsets of nuclei were positive both in mesenchyme and also in epithelia of ureteric bud branches and nephron precursors such as vesicles (Fig. 2, D and E). We immunolocalized cofilin-1 using antibodies that recognize either both the phosphorylated (inactive) and dephosphorylated (active) forms and an antibody that recognizes only the phosphorylated form. With the use of the former antibody in sections of control E13.0 metanephroi (Fig. 3, A and B), cytoplasmic immunoreactivity was detected in condensing mesenchyme. Cofilin-1 protein was also detected in the ureteric stalk and its first branches; in these epithelia, cofilin-1 immunoreactivity was prominent in the zone between the nucleus and the apical (lumenal) surface. There was no...
significant signal in stromal cells, the compartment shown to express prox-1 at this stage. With the use of the antibody specific for the phosphorylated form of cofilin-1, a similar pattern was observed (Fig. 3C). At E15.0, cofilin-1 was immunolocalized in deeper sections of the ureteric tree (i.e., mature collecting ducts), where there was a subapical accentuation of the signal (Fig. 3D). In the nephrogenic cortex, there was little signal above background (data not shown). Western blot analysis confirmed the expression of prox-1 (83 kDa) and cofilin-1 (18 kDa) in lysates of pools of E13.0 and E15.0 metanephroi (data not shown); formal quantification of proteins was not performed in this study.

Expression of apoptosis-related genes Bax and Bcl-2. Expressions of the proapoptotic gene Bax and the antiapoptotic gene Bcl-2 were measured in the various dietary groups. With the use of real-time PCR of E13.0 metanephroi, there was a step-wise increase in the expression of both genes with 9% and 6% LPD (Table 3); the proportional increase was greater for the proapoptotic gene Bax versus the antiapoptotic gene Bcl-2. The increase in Bax expression relative to controls was 560% for 9% LPD and 1,269% for 6% LPD. A similar pattern was observed for Bcl-2, where 9% LPD showed an increase of 286% and 6% LPD showed an increase of 527% compared with controls. Comparison of the ratio of Bax to Bcl-2 demonstrated that this was roughly doubled versus controls in 9% LPD (97% increase) and 6% LPD (141% increase) metanephroi.

## DISCUSSION

The phenomenon of metabolic programming has received considerable interest over the past few years. Studies examining the permanent effects of intrauterine exposure to a maternal LPD indicate that many physiological functions may be altered including immune function (30), glucose homeostasis (48), and, in particular, blood pressure, as alluded to in the Introduction. Previously, we (56) noted that rat E13.0 body and placental weights and also total numbers of cells within E13.0 metanephroi were unaffected by maternal LPD. We consider the present finding, namely, that maternal LPD alters meta-

### Table 2. Real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tubulin</td>
<td>NM_022985</td>
<td>Cytoskeletal component</td>
<td>34</td>
</tr>
<tr>
<td>Calmodulin-1</td>
<td>NM_051690</td>
<td>Intracellular calcium sensor</td>
<td>28</td>
</tr>
<tr>
<td>Cofilin-1</td>
<td>NM_017147</td>
<td>Actin depolymerization enzyme</td>
<td>55</td>
</tr>
<tr>
<td>Kinectin-1</td>
<td>NM_00487</td>
<td>Present on cytoplasmic face of membranous vessels; function unclear</td>
<td>62</td>
</tr>
<tr>
<td>Lactate dehydrogenase A</td>
<td>NM_017025</td>
<td>Catalyzes conversion of lactate to pyruvate</td>
<td>29</td>
</tr>
<tr>
<td>Nap1L1 (nucleosome assembly protein 1-like 1)</td>
<td>NM_053861</td>
<td>Putative participant in DNA replication</td>
<td>52</td>
</tr>
<tr>
<td>Prox-1</td>
<td>XM_223067</td>
<td>Homebox transcription factor</td>
<td>47</td>
</tr>
<tr>
<td>Translation initiator factor-4, γ-2</td>
<td>XM_341906</td>
<td>Translation repressor</td>
<td>25</td>
</tr>
<tr>
<td>Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, θ-polypeptide</td>
<td>XM_013053</td>
<td>Activation protein of tyrosine 3-monooxygenase, which catalyzes the conversion of L-tyrosine to L-dihydroxy-phenylalanine; this conversion is lacking in albinism</td>
<td>9</td>
</tr>
<tr>
<td>Cadherin-11</td>
<td>XM_341639</td>
<td>Glycoprotein mediating Ca$^{2+}$-dependent cell-cell adhesion</td>
<td>9</td>
</tr>
<tr>
<td>Smt3h2</td>
<td>NM_133594</td>
<td>Homolog of yeast SMT3 gene, which suppresses mutations in MIF2, a centromeric protein</td>
<td>39</td>
</tr>
<tr>
<td>Topoisomerase (DNA) II binding protein</td>
<td>XM_343460</td>
<td>Negative regulator of transcription factor Miz1. Transactivation of Miz1 allows upregulation of p21Cip1 upon UV irradiation</td>
<td>21</td>
</tr>
</tbody>
</table>

Transcripts are classified as either down- or upregulated by maternal 9% low-protein diet (LPD). RDA, representational difference analysis. *Transcript for which there is currently no known rat sequence and thus refers to the mouse transcript.
nephric gene expression at E13.0, especially interesting. In addition, we (56) have reported that maternal LPD increased apoptosis at the start of metanephrogenesis and thereafter was associated with a reduction in numbers of precursor cells. Collectively, the experimental animal data prove that the maternal diet programs the embryonic kidney, altering metanephric cell turnover and gene expression at a time when nephrons and glomeruli have yet to form and at a time point when overall “growth” of the conceptus is overtly normal. The human implication is that the maternal diet ingested between conception and 5- to 6-wk gestation contributes to the variation in glomerular numbers that are known to occur between and in healthy and hypertensive populations.

In the present study, we used a dietary protocol that varied the protein content of the synthetic diet supplied to pregnant rats. If this was to have been the sole manipulation, then the total energy content of the diet would be diminished in the LPD versus normal diets. To enable conclusions to be drawn regarding the consequences of a dietary protein restriction as opposed to a global energy reduction, the energy content of the diets had to be restored; to this end, we increased the quantity of carbohydrates in the diet. The increase in carbohydrate content in the LPD was therefore rather modest, and we consider it to be an unlikely cause of the phenomena we reported in this study; although a confounding effect cannot be excluded, we know of no independent published data that prove that such a modest increase of carbohydrates perturbs embryonic growth.

The current study also suggests that several molecules, some not previously reported to be expressed in the metanephros, might be important in kidney development.

In the present analyses of 9% LPD versus the control diet, data from the quantitative PCR analyses confirmed the up- and downregulation of genes identified as differentially expressed.
in the RDA screen; furthermore, quantitative PCR analyses of a more severe diet restriction, i.e., 6% LPD, showed similar up- or downregulation of the same set of genes. With respect to these studies, it should be noted that mRNA used for the RDA and quantitative PCR analyses were derived from separate pools of embryonic organs for each diet.

Metanephric prox-1 transcripts were downregulated by LPD. Prox-1 is the mammalian homolog of prospero, a *Drosophila* homeobox transcription factor (35, 47). In fly neuroblast development, prospero localizes in nuclei of ganglion mother cells, causing them to exit the cell cycle and terminally differentiate. In murine development, prox-1 plays a similar role in retinal progenitor cells (17). Prox-1 is also expressed outside the nervous system. In embryonic mice, prox-1 is expressed in endoderm, which will give rise to the liver and pancreas, and null mutant mice have defective hepatocyte migration into the septum transversum and small livers (54). In mice, the gene also has a key role in inducing embryonic vasculature to form lymphatic channels (22), and prox-1 is also expressed in epithelia during taste bud differentiation (41). Recently, a role for the zebrafish homolog in differentiation of the intrarenal (adrenal) primordium has been demonstrated (37). Prox-1-null mutant mice die around E14, but the state of the metanephros, which begins to form at E11.0, has not been reported. Apart from a study (63) showing, as assessed by Northern blot analysis, that prox-1 is expressed in the human fetal kidney, ours is the first report that this gene is expressed in the metanephros. We detected prox-1 in several populations of rat kidney cells. At E13.0, the protein was prominent in “stromal” cells around the ureteric bud stalk; this is likely to be a heterogeneous population of loose mesenchymal cells comprising interstitial and vascular cell precursors. Two days later, at E15.0, a different picture emerged, with subsets of prox-1-positive nuclei in mesenchyme as well as epithelia of ureteric bud branches and nephron precursors. These patterns allow one to speculate that, during metanephrogenesis, prox-1 might enhance the growth of both the interstitial compartment and also epithelial cells. With regard to the former hypothesis, it is well established that metanephric stroma actively supports the growth of adjacent tubules (20). Apoptosis is prominent in this compartment during normal development and is upregulated by LPD (56), and it is possible that prox-1 may protect these cells from dying. Proliferation is downregulated as renal mesenchyme forms nephrons (58), and, with regard to these nascent epithelia, we speculate that prox-1 downregulates proliferation and induces differentiation, analogous to its role in the nervous system.

Metanephric cofilin-1 transcripts were downregulated by LPD. Cofilin-1 is a member of a small family of proteins (cofilin-1, cofilin-2, and ADF) that regulate actin filament turnover (2, 55). The active form, which is dephosphorylated, is found outside the nucleus and is a key modulator of actin filament dynamics. In the developing kidney, cofilin-1 is expressed in the metanephric mesenchyme, where it plays a role in the migration and morphogenesis of kidney tubule cells. The downregulation of cofilin-1 by LPD may affect the actin filament dynamics, thereby impacting the growth and differentiation of kidney epithelial cells.

**Table 3. Real-time PCR for apoptosis-related molecules**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Percent Difference in Expression Vs. 18% Protein Controls</th>
<th>Primer Pairs</th>
<th>Relative Transcript Levels between Different Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9% LPD</td>
<td>6% LPD</td>
<td>5u-Sequence-5u</td>
</tr>
<tr>
<td>Bax</td>
<td>560</td>
<td>1,269</td>
<td>5u6-TCGATTGAGACAGGAGGCTGG-3u5</td>
</tr>
<tr>
<td></td>
<td>5432</td>
<td>5432</td>
<td>5u5-GCGATCTCTTGGCGCTGTGAT-3u5</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>286</td>
<td>527</td>
<td>5u5-GCGATCTCTTGGAGGACAGGCA-3u5</td>
</tr>
<tr>
<td></td>
<td>5432</td>
<td>5432</td>
<td>5u5-GCGATCTCTTGGAGGACAGGCA-3u5</td>
</tr>
</tbody>
</table>

Relative transcript levels between different diets represent the average measured ratios from three sets of real-time PCR reactions on each dietary sample. The primers used in the real-time analysis are shown.
binds to F-actin and enhances the rate of actin subunit dissociation, and this form is found in the lamellipodia of migrating cells (15). LIM kinase inactivates cofilin-1 by phosphorylating the protein on serine 3 (2); of note, the human LIM gene is deleted in some individuals with Williams syndrome (2), and this disorder is associated with diverse urinary tract malformations including renal hypoplasia (49). In the adult kidney, cofilin has been detected in cultured human glomerular mesangial/pericyte cells (16), and, in vivo, ischemic renal injury in rats correlates with destabilization of the cortical actin network within proximal tubule cells together with ADF activation and relocation to the apical domain (1). Both cofilin-1 and ADF have been reported to be expressed in the E14 mouse embryonic kidney, as assessed by in situ hybridization (55), although the specific cell types expressing the gene were not detailed. In the present rat study, at E13.0, we found that cofilin-1 was immunolocalized in condensing renal mesenchymal cells and also found in the apical domain of ureteric bud branch tips. Both of these tissues are undergoing profound cell reorganization (i.e., condensation and lumen formation in the mesenchyme/nephron lineage and branching in the ureteric bud lineage). Given its role in actin-based cell motility, cofilin may well be playing a role in these cell types. Recently, Chua et al. (11) provided data to show that cofilin is involved in apoptosis induction; however, at least in E13.0 rat metanephrors, cofilin protein did not appear to localize to loose stroma/loose mesenchyme, which is a “hotspot” for LPD-upregulated apoptosis (56). To test the hypotheses that cofilin-1 or prox-1 might play active roles in renal organogenesis, functional experiments will be needed, probably using siRNA to reduce levels of these proteins in organ cultures of wild-type metanephroi (14). Furthermore, biochemical experimental are required to assess how prox-1 might fit into the cascade of transcription factors that control nephrogenesis (7).

The present RDA screen identified several other genes that were up- or downregulated by maternal LPD. While space does not allow a detailed description of all of them, it is notable that lactate dehydrogenase enzymes have been detected in murine metanephror (29) and cadherin-11 has been reported to be expressed within mesenchyme of renal and other organ rudiments (10); of note, using a cDNA microarray analysis, cadherin-11 was identified as a gene upregulated in uninduced metanephroi (21). Internal to this report, it was notable that p53 gene methylation status was altered in these experimental animals. Intriguingly, altered methylation of the human prox-1 gene occurs in some hematological malignancies (44). Taken together, it would be interesting to perform biochemical experiments to ascertain whether the maternal LPD alters the methylation status and transcriptional rate of genes such as prox-1 and cofilin-1.

How might gene expressions be altered by maternal diet? Glucocorticoids mediate embryonic programming because dexamethasone administration in pregnant rats causes renal hypoplasia and hypertension in offspring (8), and LPD reduces placental 11β-hydroxysteroid dehydrogenase, an enzyme protecting the conceptus from maternal glucocorticoids, and up-regulate fetal biochemical markers of glucocorticoid exposure (31). The developing urinary tract expresses glucocorticoid receptors (27), and it would be informative to study the effects of glucocorticoids on the expression of the genes identified in the present RDA screen. Experimental intrauterine growth retardation is another scenario associated with increased renal apoptosis and glomerular deficits (50); interestingly, the same study reported that p53 gene methylation status was altered in these experimental animals. Intriguingly, altered methylation of the human prox-1 gene occurs in some hematological malignancies (44). Taken together, it would be interesting to perform biochemical experiments to ascertain whether the maternal LPD alters the methylation status and transcriptional rate of genes such as prox-1 and cofilin-1.


46. Winyard PJD, Risdon RA, Sams VR, Dressler GR, and Woolf AS. The PAX2 transcription factor is expressed in cystic and hyperproliferative
56 MATERNAL DIET PROGRAMS EMBRYONIC KIDNEY GENE EXPRESSION


