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Effects of Maternal Low Protein Diet on Expression of Drug Transporters in the Blood-Brain Barrier of Adult Offspring

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Abstract

Adverse uterine environment, manifested as low birth weight (LBW), has been shown to predispose individuals to hypertension, diabetes, and obesity by mechanisms that are just beginning to be understood. One of the mechanisms is the dysregulation of the expression or function of drug transport proteins, such as the organic anion transporter (OAT) family, which are crucial for the transport of various endogenous and exogenous compounds into and out of all organs, especially the brain. Hence, we examined the status of select drug transporters in the blood-brain barrier (BBB), using a LBW rat model. Maternal low protein diet (LPD) during gestation and lactation is a widely used animal model to induce LBW. Indoxyl sulfate, a substrate for Oat3, is found in lower concentrations in the brain tissue of LBW rats and higher concentrations in the serum, as analyzed by HPLC. In support of these data, an increase in the protein expression of Oat3, an efflux transporter, was observed in the LBW group. On the contrary, the BBB mRNA expression of Oat3, and other drug transporters Oatp1c1, Oatp1a4, and P-gp in LBW rats was found to be decreased compared to normal birth weight rats. Most notably, we found an almost 100-fold decrease in the expression of Oat3 in low birth weight male rats. In summary, large scale differences in the expression and function of drug transporters in the brains of LBW individuals could not only affect the action of exogenous pharmaceutical agents, but also the ability of the brain to maintain homeostasis by balancing the concentrations of endogenous compounds.

Introduction

Individuals with low birth weight (LBW) have been shown to have increased risk of developing several chronic diseases. A forerunner in the research of the LBW condition, Dr. David Barker, has established a correlation between LBW and the adult onset of heart disease, hypertension, and metabolic syndrome[1,2,3]. Others have also observed the deleterious effects of LBW such as a study of monozygotic twin pairs in Denmark, which revealed a greater risk of developing diabetes in the twin with lower birth weight[4].

LBW is defined as weighing less than 2,500 grams or 5.5 pounds at birth[5]. There are several reasons why an infant might weigh less than this standard. Premature birth obviously contributes to the number of infants born with this characteristic, but insults to fetal development leading to growth restriction during pregnancy can also cause this effect. The World Health Organization in its 2004 report on LBW cited that infants are more likely to be born to women living in deprived socioeconomic conditions, women whom are undernourished and in poor health themselves, and women who do physically demanding labor. In short, the maternal characteristics associated with LBW are mostly attributable to conditions of poverty.

LBW is a condition which is typically more prevalent in developing rather than developed countries. The proportion of infants born with LBW in developed countries is 7% compared to developing countries where the rate is 16.5%, more than two times higher[5]. Interestingly, LBW is more common in the United States than in other developed countries[6], with 8% of infants born LBW in 2002[5] which is slightly higher than the average for developed countries.

Several animal models have been developed to study LBW. Because LBW is so closely linked to maternal nutrition, several of the models restrict a key nutrient in the maternal diet. Studies have demonstrated that LBW can be induced by intrauterine glucocorticoid exposure, ligation of the uterine artery, or by maternal diets that are restricted in protein, iron, or total calorie intake[7]. The protein restriction model has been verified by our lab to decrease birth weight in rats, making the maternal low protein diet (LPD) a good model to study the LBW condition[8].

Programming is the term used to describe "…the process whereby a stimulus or insult at a critical period of development has lasting or lifelong effects." [9] This "programming effect" has been witnessed in the morphological and functional differences in the organs of LBW animals. Burns, et. al. found that maternal protein restriction in rats resulted in offspring with impaired glucose tolerance and livers which contained half the number of lobules[10]. Additionally, Zimanyi, et. al. observed that maternal protein restriction produced LBW in offspring, decreased offspring kidney size, and reduced the number of nephrons by 30%[11].

Although many organs are modified by fetal programming, there is evidence that indicates that the brain is protected from insult in utero. This "brain protective effect" might be mediated by redistribution of blood flow. Campbell, et. al. observed increased carotid and cerebral blood flow and vasodilatation in mature fetal sheep in response to asphyxia that was produced by occlusion of the umbilical cord or by impeding ventilation by stopping the respiratory pump[12]. This was corroborated by work by Rudolph, et. al. which showed that under conditions of hypoxic stress, circulation in fetal sheep is redirected to provide well-oxygenated blood to the brain and heart[13].

There is also evidence, however, that the brain might not be as impermeable to in utero insult as previously thought. Maternal LPD resulted in decreased blood vessel density in the cerebral cortex (measured both as vessel length and vessel perimeter), and this effect was not recoverable with a normal diet[14]. In addition, Plagemann, et. al. observed aberrations in neuron number as well as changes in the expression of neuropeptide Y in the hypothalamic nuclei of the offspring of maternal LPD rats[15]. Finally, Cherala, et. al. observed that when dosed with the sleep-inducing barbiturate, hexobarbital, LBW female rats exhibit a significantly shorter sleep time[16]. Taken together, these studies indicate that fetal programming results in morphological and physiological changes in the brain.

One explanation for the decreased hexobarbital sleep time in LBW female rats, as observed by Cherala et. al., might be that hexobarbital is metabolized more quickly in LBW rats. The hexobarbital sleep time test is a measure of the function of the P450 enzyme superfamily. Cherala et. al., found that there were no differences in the activity of hexobarbital hydroxylase and no differences in the amount of CYP2C12 protein in the liver, which is one of the principal P450 enzymes for hexobarbital metabolism[16]. In light of this discovery, it is possible that hexobarbital is not metabolized more quickly and thus less is made available to induce sleep in LBW female rats, but perhaps less hexobarbital is reaching their brains, or alternatively, more hexobarbital is effluxed out of the brain, although an equivalent amount of the compound is in the circulation.

Pharmacokinetics concerns itself with the movement of substances into, around, and out of the body and is studied by observing drug absorption, distribution, elimination, metabolism, and excretion[17]. Endogenous and exogenous compounds enter and exit various compartments of the body by a variety of mechanisms including diffusion (both active and passive), and

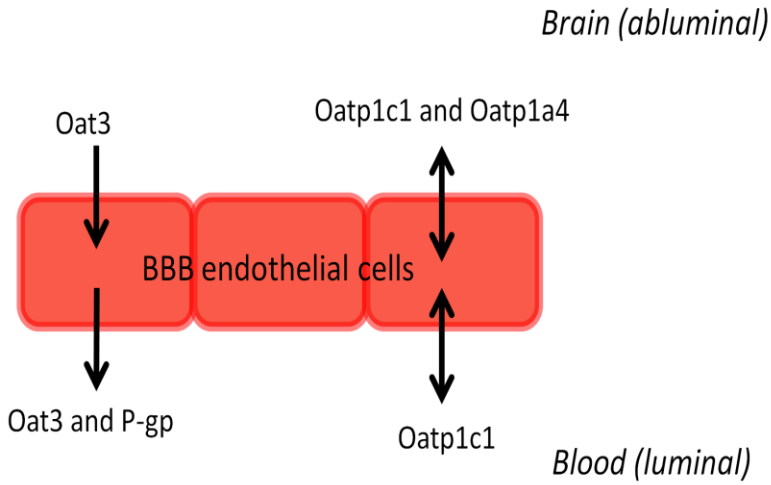
facilitated transport into different cell types by membrane-bound transport proteins, often referred to as drug transporters. Additionally, a very diverse complement of enzymes metabolizes compounds into different forms which help these substances to either be utilized or removed from the body. LBW has been associated with changes in the morphology and physiology of the liver and kidneys; key organs in pharmacokinetic study, which play important roles in how pharmacologically active compounds enter the body, are introduced to the circulation, and are a excreted[10,11,18].

So how might LBW affect pharmacokinetics in the brain? One mechanism is by modifying the expression or function of transporters at the blood-brain barrier (BBB). The BBB is critical for maintaining the correct ion concentrations in the interstitial space, supplying essential nutrients to the brain, and flushing out waste products into the circulation[19]. The BBB is comprised of the brain microvascular endothelium, forming an interface between the systemic circulation and the interstitial space of the brain which contains more than 100 billion capillaries[20]. The endothelial cells of the BBB form tight junctions with one another, permitting the diffusion of only small molecules with high lipid solubility and low molecular mass[21]. Transport proteins expressed on both the abluminal and luminal membranes of BBB endothelial cells selectively bind substrates for either influx into the interstitial space of the brain or efflux into the systemic circulation.

Drug transporters are critical to the role of the BBB in maintaining homeostasis and protecting the brain from toxic accumulation of xenobiotics[22]. One of the families of transport proteins that are expressed in the BBB is the organic anion transporter (OAT) family. The proteins Oat3, Oatp1c1, Oatp1a4, and P-glycoprotein (P-gp) were chosen as representative organic-anion transporters which vary in their localized expression (abluminal or luminal

membrane of BBB endothelial cells) and in their directional transport (influx or efflux). Figure 1 shows a schematic which represents the localized expression and directional transport of each protein.

Figure 1: Diagram of representative organic-anion transporters and their expression loci in BBB endothelial cells.



Sources: Roberts (2008), Urquhart (2009), Bauer (2008).

These transporters are responsible for the flux of a variety of different substrates into ant out of the brain interstitial space. Table 1 shows several examples of substrates of each transporter.

Table 1: Examples of substrates for each transporter

Transporter	**Substrates**
OAT3	Indoxyl sulfate[26], para-aminohippurate[24]
P-gp	Some chemotherapy drugs[24], cyclosporin A[24]
OATP1C1	Thyroxine[27]
OATP1A4	Digoxin[27]

The present study was undertaken to determine whether the LBW condition affects the expression and, or function of transporters in the brain tissue. Indoxyl sulfate, a uremic toxin, was measured in cerebral cortex and in serum to assess the function of OATs in the BBB, namely Oat3. Protein and mRNA expression experiments were

conducted to assess whether altered transport of solutes is due to altered function or aberrant expression.

Methods

Diets

Modified isocaloric versions of the AIN76A purified diet were purchased in pellet form from Purina Test Diets (Richmond, IN). The control and low protein diets contained 19% and 8% casein, respectively. Detailed compositions of both diets are available in the literature[8].

Experiments with dams

This study was approved by the Institutional Animal Care and Use Committee of the Oregon Health & Science University, Portland, OR. Virgin female Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) were mated by housing one male rat with two female rats. Day 1 of pregnancy was assigned upon observation of sperm in the daily morning vaginal smears, at which time rats were randomly assigned to the two diet groups; control or low protein. Each treatment group consisted of 5-7 pregnant rats, and these rats received their assigned diet throughout pregnancy and lactation.

Experiments with offspring

Upon birth, litter size and sex of pups was noted. All litters were randomly culled to 12 pups (6 male and 6 female) on the day of birth and further randomly culled to 8 pups (4 males and 4 females) on day 4 after birth. Offspring from both groups were weaned on day 28 after birth and were housed in iso-sexual groups according to perinatal diet treatment. Different dietary treatments were administered only during gestation and lactation, and all pups were weaned onto a non-purified diet. On day 120 after birth, a single blood draw from the medial saphenous vein was performed on two male and two female offspring from each litter, serum was isolated within

30 minutes of blood collection, and stored at -80°C.Two male and two female offspring from each litter in both groups were randomly chosen and sacrificed on days 90 and 150 after birth. Brain was collected from all sacrificed animals, weighed, snap-frozen in liquid nitrogen and stored at -80°C.

RNA isolation

Cortex tissue (100-200mg) was homogenized with mortar and pestle over liquid nitrogen, and total RNA was extracted from sample using the TRIZOL method according to the manufacturer's protocol and stored at -80°C. Purity and concentration of the RNA product was assessed by measuring A260/230 and A260/280 values, respectively (Nanodrop 1000, Thermo-Scientific, Wilmington, DE).

cDNA synthesis

cDNA was generated using iScript cDNA synthesis kit (BioRad, Hercules, CA). Briefly, approximately 1µg of RNA was added to reaction mixture containing iScript reaction mix, iScript reverse transcriptase, and nuclease-free water to a final reaction volume of 20µl. cDNA synthesis was conducted using the BioRad MyiQ thermocycler. Concentration of cDNA was assessed by measuring A260/280 value (Nanodrop 1000), and samples were stored at -20°C.

Real-time polymerase chain reaction (RT-PCR)

cDNA (10-20 ng) was added to a reaction mixture containing iQ SYBR Green supermix (BioRad, Hercules, CA) and forward and reverse primers; (250nM concentration, see table 2) to a final reaction volume of 20 µL. PCR amplification was performed in the BioRad MyiQ Thermocycler as follows: 2 minutes at 95˚C, followed by 40 cycles of 95˚C for 20 seconds and 50˚C for 30 seconds. RT-PCR was conducted in triplicate for each sample. Each RT-PCR plate

was run with a negative control (water), and a positive control (a randomly selected control sample, which was the same for every plate).

Table 2: Primer sequences for PCR study.

Gene	Accession #		Sequence
GAPDH	NM_017008	Forward	GTGGTGCCAAAAGGGTCAT
		Reverse	ATTTCTCGTGGTTCACACCCA
HPRT1	XM_217584	Forward	GCCCCAAAATGGTTAAGGTTG
		Reverse	TCCACTTTCGCTGATGACACA
GUSB	NM_017015	Forward	TCACTCGACAGAGAAACCCCA
		Reverse	CTCTGGTTTCGTTGGCAATCC
TBP	NM_001004198	Forward	CGGTTTGCTGCAGTCATCAT
		Reverse	GTGCACACCATTTTCCCAGA
β-Actin	NM_031144	Forward	GCCAACACAGTGCTGTCTG
		Reverse	CACATCTGCTGGAAGGTGG
Oat3	NM_031332.1	Forward	AGCCGTGCTTGGATGGCTGGAT
		Reverse	GCTGCACACCAAGTCCCACTCT
P-gp	NM_012623.2	Forward	CCGCATTCTGCCGAGCGTTACT
		Reverse	AGACGTCATCTGTGAGCCGGGT
Oat p1a4	NM_131906.1	Forward	AGTCTCAGCATGGAGAGGACCGT
		Reverse	ACACACTCTGAGGGGTCTTGTGT
Oatp1c1	NM_053441.1	Forward	TGACTTCTACCTCCAGGCCGGGAT
		Reverse	CCGGCAGGTTGCGCTGAATT

PCR data analysis

The efficiency (E) of primer pairs was determined using a 10x serial dilution of a control cDNA sample[28]. The slope of the relationship between Log transformation of dilution factor and cycle threshold (Ct) values at each dilution factor was calculated. The 'E' of each primer pair was then calculated as 10^(-1/slope). Reference gene was chosen by determining housekeeping gene which changed least in expression between diet, age, and sex. In brain, *GAPDH* varied least across treatment groups and was used as the reference gene. Fold differences in the expression of a reference gene between groups were calculated as $E^{(\Delta Ct_{(control-LPD)})}$. Theoretical effects on

target gene expression were calculated by the method adapted from Pfaffl method[29]. A substantial change in gene expression is considered to be a fold difference of more than two.

Plasma membrane isolation

Cerebral cortex was homogenized according to Naud, with some modifications[30]. Briefly, approximately 500mg cerebral cortex was homogenized with a dounce homogenizer in 8mL buffer containing 250mM sucrose, 5mM Tris/HEPES, and 0.1mM PMSF. An aliquot of total homogenate was taken at this point and stored at -80°C for later HPLC analysis. To isolate plasma membrane fraction, the remaining homogenate was centrifuged at 9000xg for ten minutes at 4°C. The supernatant removed and then centrifuged at 33,000xg for 60 minutes at 4°C. The supernatant was aspirated and the pellet, containing plasma membranes, was resuspended in saline buffer (1x PBS, 0.1mM PMSF) and stored at -80°C.

Polyacrylimide gel electrophoresis

Crude plasma membrane samples were assayed for protein concentration using the Bradford method and equal concentrations of protein were subjected to western blotting. Briefly, samples were reduced with laemmli sample buffer (Bio-Rad, Hercules, CA) and denatured in a 100°C water bath for 3 min. For brain plasma membranes, 20µL of each sample was loaded onto a 10% polyacrylimide gel and subjected to electrophoresis at 150mV for 60 minutes.

Western blotting

Samples were then transferred onto a nitrocellulose membrane, blocked with blocking buffer (LiCor Biosciences, Lincoln, NE), and incubated with a 1:1000 concentration of primary rabbit anti-rat OAT-3 antibody (Alpha-Diagnostic, San-Antonio, TX). The membrane was then incubated with 1:15000 concentration IRDye 680RD secondary goat anti-rabbit antibody (LiCor Biosciences, Lincoln, NE) before being imaged on Odyssey Imaging System (Licor Biosciences,

Lincoln, NE). For quantification, band intensity was quantified using NIH ImageJ software, and normalized to a control sample run on every gel.

Quantitation of indoxyl sulfate

A modified high-performance liquid chromatography (HPLC) was used to quantify indoxyl sulfate in brain tissue and serum, as described by Tsutsumi et.al[31]. Briefly, 10 µL of total brain homogenate was treated with acetonitrile, vortexed, and centrifuged at 8,000xg for 10min. Two µL of the supernatant was injected onto a 4.6mmX100mm, 2.6µm Kinetex C_{18} analytical column (Phenomenex, Torrance, CA) maintained at 34^{o}C and fitted with Waters Symmetry C_{18} guard column (3.9mm X 20mm; 5µm particles). The mobile phase consisted of 84/16 v/v of acetate buffer (0.2M, pH 4.5)/acetonitrile at a flow rate of 1.0 mL/min. Indoxyl sulfate was monitored using fluorescence detector at 280 nm (excitation) and 375 nm (emission). The fluorescence reading was converted into indoxyl sulfate concentrations using a standard curve (0-200 ng/mL). The intra- and inter-day coefficients of variation were <5%.

Data analysis

Differences in brain and serum indoxyl sulfate and brain Oat3 expression were calculated using t-test for treatment group comparisons. Statistical analysis conducted at α=0.05 (SigmaPlot v11.0, San Jose, CA). To assess differences in reference gene expression, two-way ANOVA was conducted with Student-Newman-Keuls post-hoc analysis on the cycle threshold (Ct) values (SigmaPlot v11.0, San Jose, CA). Transporter Ct values were normalized to *GAPDH*, and fold differences greater than two are accepted as significant.

Results

There was significantly more indoxyl sulfate in the serum of female LPD rats than in the serum of female control rats at day 150 (see figure 2). Males followed a similar trend, but the

difference was not significant. Additionally, there was a significantly decreased concentration of indoxyl sulfate in the brain tissue of LPD rats of both sexes compared to control rats at day 150 (see figure 3).

Figure 2: Indoxyl sulfate in serum.

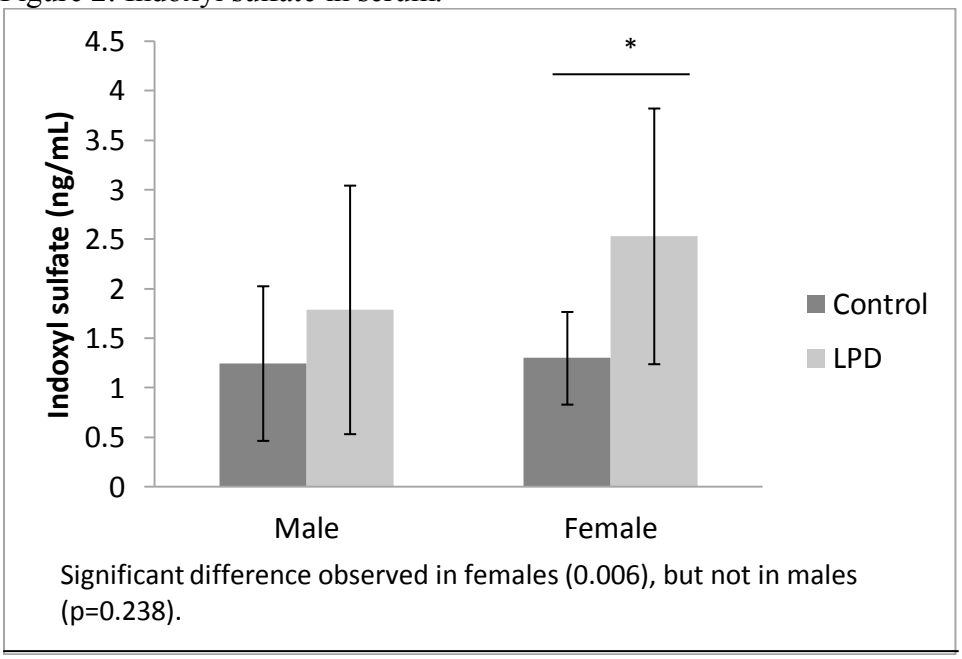
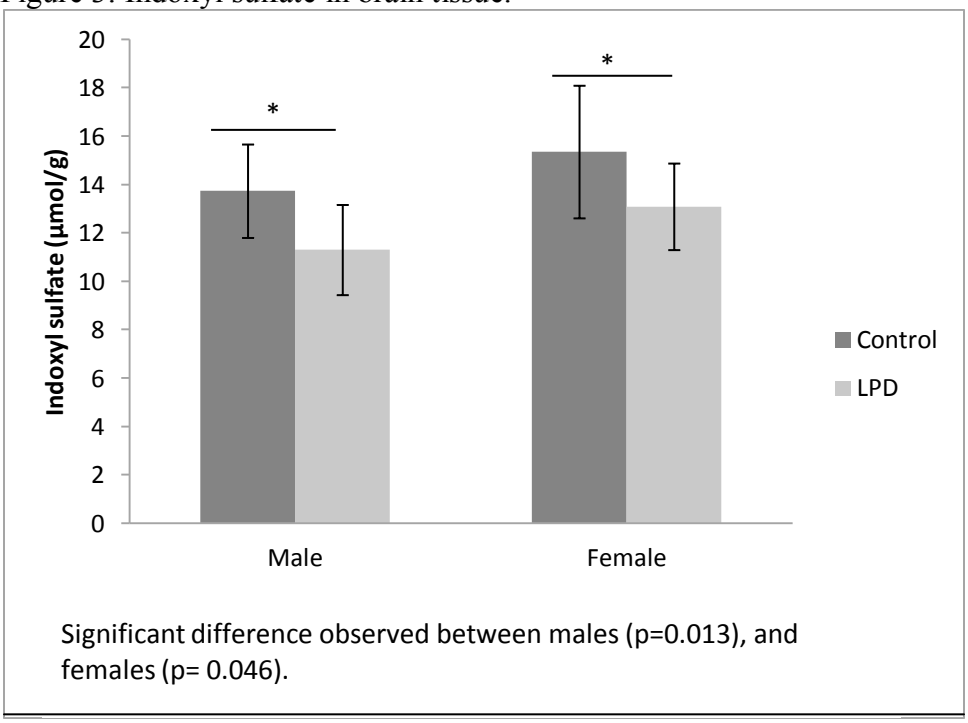
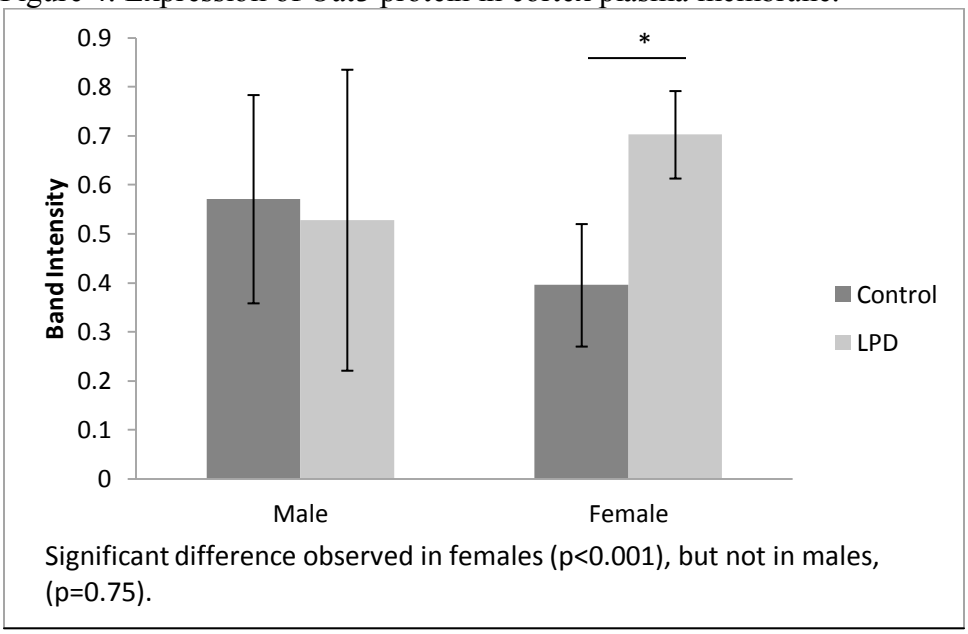


Figure 3: Indoxyl sulfate in brain tissue.



Western blot analysis of protein expression showed that female LPD rats expressed significantly more Oat3 protein in the cortex plasma membrane fractions than their control diet counterparts. Males, however, expressed slightly less (see figure 4).

Figure 4: Expression of Oat3 protein in cortex plasma membrane.



The expression in the brain of several commonly used reference genes was compared so as to select a reference gene whose expression is unchanged by the low-protein diet model. The expression of *HPRT1* was decreased in LPD individuals of both sexes at day 150. LPD females at day 90 also express less *HPRT1*. However, males at day 90 exhibit the opposite pattern, and the LPD group expresses more *HPRT1* than the control group. Significant differences were also found in the expression of *BA*. At day 90, both male and female LPD express more *BA* than their control counterparts, but this effect did not occur in females at day 150, and male LPD at day 150 still actually express significantly less *BA* than controls. See table 3.

Table 3: Effect of maternal LPD on brain mRNA expression of select reference genes in day 150, and 90 old male and female offspring.

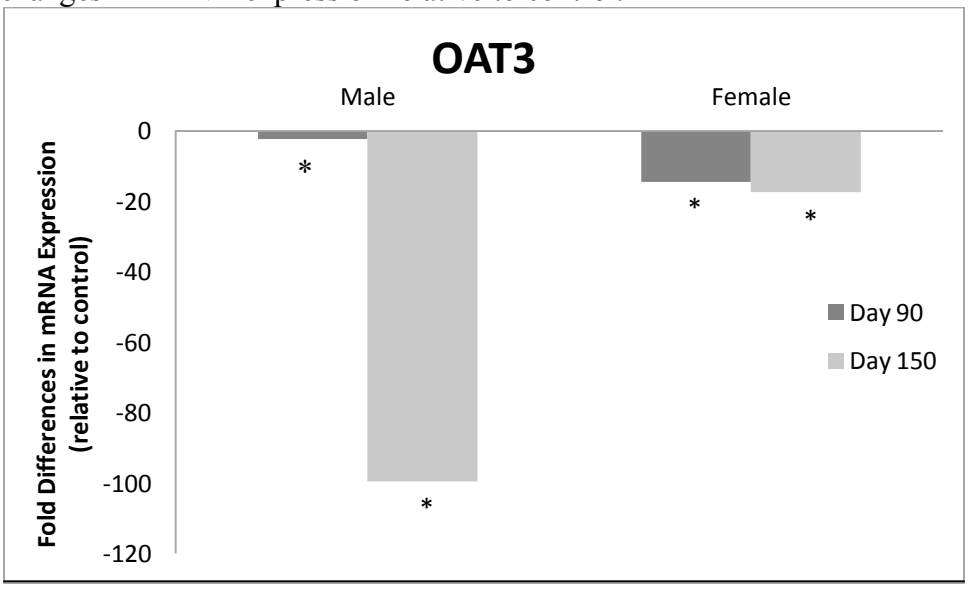
Age	Gene	Females			Males		
		Control	LPD	Fold Diff.[1]	Control	LPD	Fold Diff.
Day 150	GAPDH	22.60± 0.67 (2.95)[2]	23.96± 0.27 (1.10)	-2.19	22.59± 0.67 (2.98)	24.26± 0.18 (0.73)	-2.62
	HPRT1	22.10± 0.32 (1.47)	31.77± 7.10 (22.33)	-303.54 **	23.06± 0.37 (1.58)	35.99± 6.94 (19.27)	-2086.50 **
	GUSB	26.47± 0.24 (0.89)	27.21± 0.93 (3.41)	-1.60	26.70± 1.16 (4.36)	26.97± 0.38 (1.41)	-1.19
	BA	18.03± 0.25 (1.37)	22.32± 2.69 (12.06)	-13.26	18.76± 0.58 (3.11)	24.16± 0.58 (2.42)	-25.88 *
Day 90	GAPDH	23.08± 1.02 (4.41)	25.12± 0.48 (1.91)	-3.25	24.91± 0.47 (1.90)	22.45± 0.19 (0.83)	4.14
	TBP	25.63± 0.24 (0.94)	27.18± 0.38 (1.41)	-2.24	26.78± 0.54 (2.03)	25.40	2.22
	HPRT1	22.60± 0.42 (1.84)	35.90± 7.10 (19.78)	-2598.11 **	34.88± 8.00 (22.94)	22.27± 0.23 (1.03)	1724.18 **
	GUSB	26.80± 0.46 (1.70)	27.74± 0.33 (1.18)	-1.80	27.85± 0.35 (1.25)	26.43± 0.47 (1.79)	2.43
	BA	19.12± 0.85	24.92± 0.37	-32.73	23.72± 1.68	17.91± 0.42	33.07

		(4.47)	(1.49)	*	(7.08)	(2.37)	*

[1] Fold differences in the expression of gene in LPD group offspring, compared to the control group offspring; [2] The Ct values are expressed as mean±S.D. (%CV); * $p<0.05$; ** $p<.001$

GAPDH was selected as the reference gene for Q-PCR analysis of mRNA expression of organic anion transporters Oat3, P-gp, Oatp1c1, and Oatp1a4. Analysis revealed large differences between expression levels of transporter mRNA in LBW individuals compared to controls. In Oat3 mRNA expression, there was a sex-dependent decrease in expression; the female LBW group at day 90 showed a 14-fold decrease in expression compared to a 2-fold decrease in the males. However, there was an almost 100-fold decrease in the expression of this transporter by males at day 150. The magnitude of this decrease was smaller in females (approximately 17-fold) at day 150 (see figure 4).

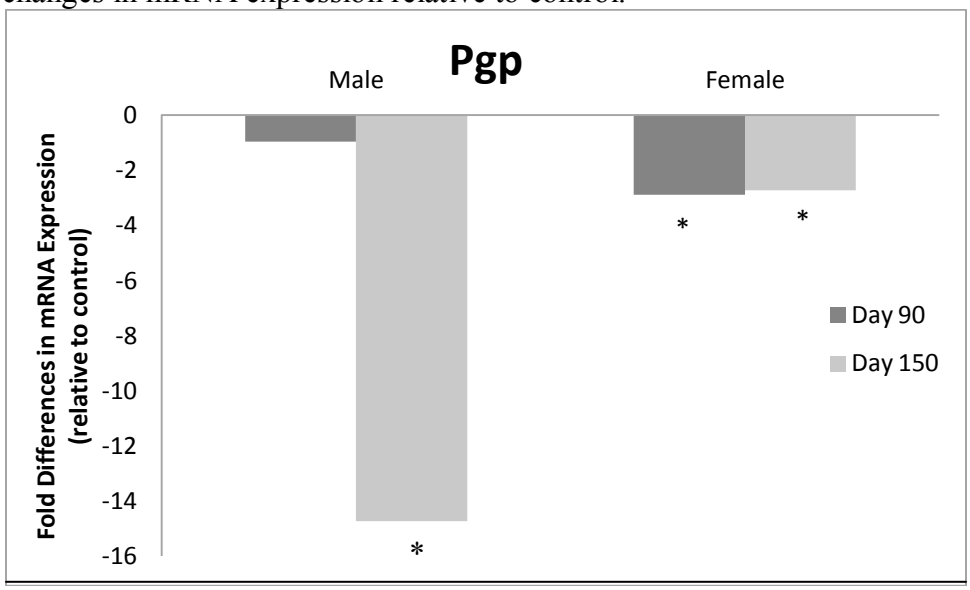
Figure 4: Expression of OAT3 mRNA in male and female LBW groups. Represented as fold changes in mRNA expression relative to control.



P-gp mRNA analysis revealed a similar trend to the expression of Oat3. At day 90, the female LBW group shows a more pronounced decrease in P-gp mRNA expression than the male LBW group, but at day 150, the males show a much more marked decrease in expression

compared to the controls (14-fold decrease in males compared to 2-fold decrease in females) (see figure 5).

Figure 5: Expression of P-gp mRNA in male and female LBW groups. Represented as fold changes in mRNA expression relative to control.



PCR analysis of transporters Oatp1c1 and Oatp1a4 showed similar patterns of expression in LBW males. Generally, there is a small decrease in transporter expression at day 90, followed by a much larger-scale decrease in expression at day 150 (see figures 6 and 7). However, this trend is not observed in the females. Day 90 LBW females express 13-fold less Oatp1c1 than controls at day 90, but then at day 150, they recover some expression and show only a 10-fold decrease in expression. The opposite is true for female expression of Oatp1a4. At day 90, there is a 1-fold decrease in expression relative to the controls, followed by a 4-fold decrease in expression at day 150.

Figure 6: Expression of Oatp1c1 mRNA in male and female LBW groups. Represented as fold changes in mRNA expression relative to control.

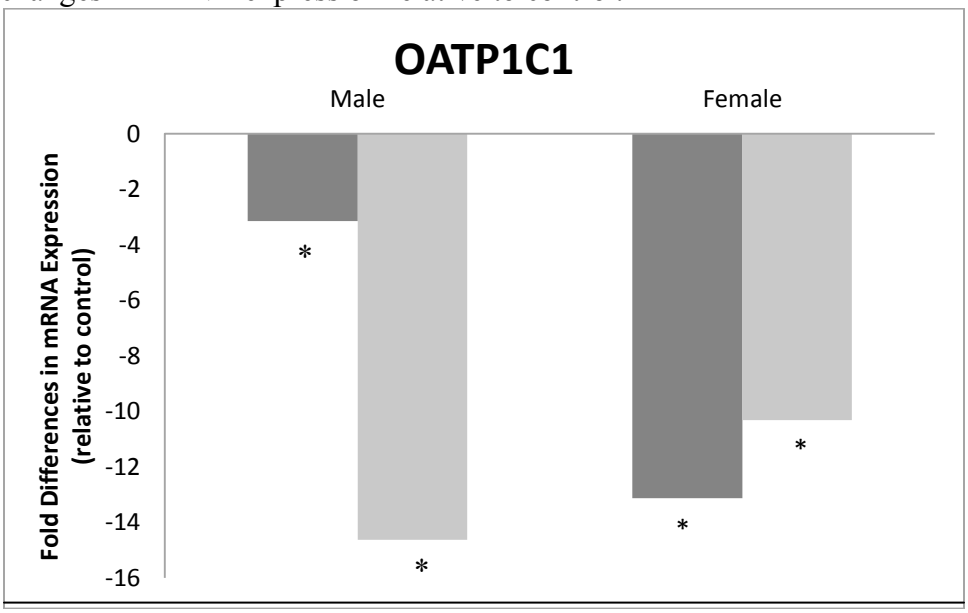
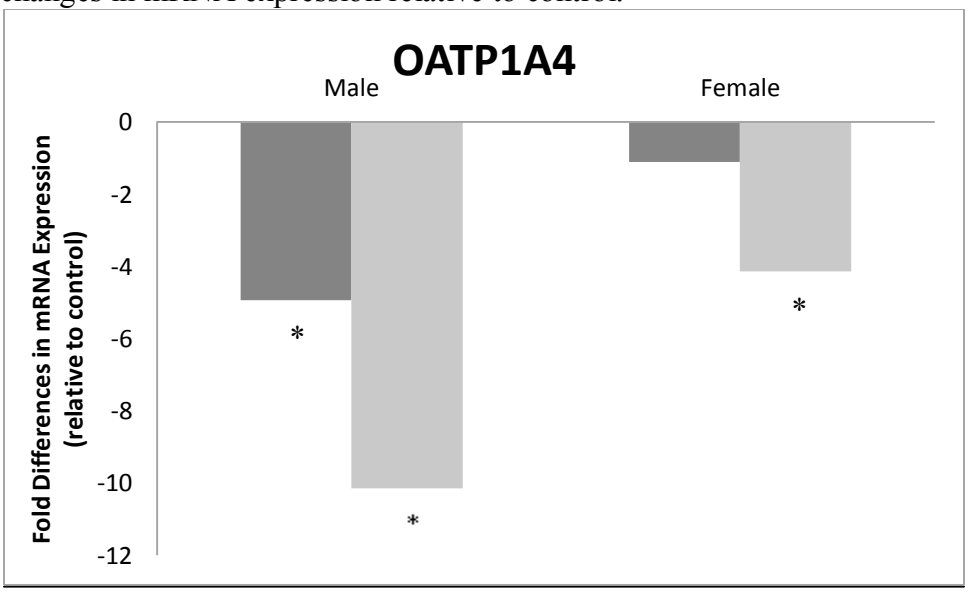


Figure 7: Expression of Oatp1a4 mRNA in male and female LBW groups. Represented as fold changes in mRNA expression relative to control.



Discussion

The LPD treatment did not affect the expression of *GAPDH* in the brain. Selection of a housekeeping gene with stable expression across treatment groups for normalization of PCR studies is important in order to discern changes in the gene transcript expression of genes of

interest[32]. Because of the stable expression of *GAPDH,* this gene is an ideal internal control for gene expression studies in brain tissue of our LPD model.

The functional data provided by analysis of indoxyl sulfate concentrations in brain and serum show that there is less of this compound accumulating in the brain tissue in LBW subjects and more is present in the serum, suggesting either less influx transport or greater efflux transport. As indoxyl sulfate is a substrate for Oat3, an efflux transporter, we might expect to see an increase in the protein expression of Oat3 in the plasma membranes of BBB endothelial cells.

By western blot analysis, this is exactly the trend that is observed. There is more Oat3 protein in the plasma membrane fraction of brain homogenate from LBW subjects. However, when the mRNA expression of Oat3 and other OATs is analyzed, a general decrease in mRNA expression is observed in LBW subjects compared to controls.

There are several reasons why this might occur. It is possible that the LBW phenotype induces extra stability of mRNA transcripts so that a greater number of proteins can be translated from each transcript.

Lucas et. al. illuminates possible mechanisms by which maternal protein restriction might act to induce LBW and organ-specific effects. The first is relatively simple: that nutrient restriction might fail to fuel growth processes[33]. This might explain several of the reductions in cell number in LBW organs. As mentioned above, maternal LPD was shown to decrease kidney size in offspring and also decrease nephron number by 30%[11]. Additionally, maternal LPD has been shown to reduce blood vessel density in the cerebral cortex[14].

But when normalized to organ size, differences in the expression of transporters still exist. Lucas suggests that nutrients could possibly act directly or indirectly on receptors in sensitive tissues, and the responses initiated in these cells could lead to adaptive gene expression,

or perhaps the nutitional environment could lead to adaptive clonal selection which would allow for proliferation of the cell type best suited to the limited nutrient density[33]. It may be for these reasons that programming effects are seen later in life, as far as 150 days after birth in our rodent model and late into adulthood in humans. In a study conducted on individuals whom were in-utero while their mothers suffered the Dutch Hunger Winter, it was found that periconceptional exposure to conditions of famine influenced the methylation status of the insulin-like growth factor II (IFG2) gene in offspring[34]. Epigenetic modifications that persist later in life could be a mechanism of regulatory gene expression induced by maternal LPD.

The programming effects observed in this study were not uniform between sexes, however. The maternal LPD rat model has been shown to decrease the serum concentrations of luteinizing hormone (LH) and testosterone in male offspring[35]. Buist, et. al. observed that the expression of kidney Oat1 and liver Oat3 were decreased in gonadectomized male rats, and female gonadectomized rats showed decreases in Oat1, Oat3, and kidney Oat2[35]. Given that male and female rats show different expression patterns of transporters and drug-metabolizing enzymes to begin with[36], it is clear that sex hormone regulation might be a cause for the differences between the sexes in mRNA and protein expression that observed in the current study.

Heart disease, diabetes, and hypertension are chronic diseases which require pharmaceutical intervention. As the LBW phenotype is associated with development of these conditions, it is highly likely that while these individuals constitute a small portion of the overall population; they will comprise a large portion of the population requiring medication long-term for chronic disease. Changes in the transporters that mediate the brain's ability to maintain homeostasis could have a very large impact on how LBW individuals process pharmaceutical

agents. Over-expression of efflux transporters could mean that certain neuroactive drugs do not reach the correct concentrations in the brain because they are flushed out at a much faster rate than anticipated. Alternatively, over-expression of influx transporters could cause accumulation in the brain of endogenous or exogenous compounds to a concentration that is harmful to brain tissue.

It is important to understand how the LBW phenotype might affect drug transport proteins in order for clinicians to make informed decisions about drug dosage that will optimize treatment and minimize harm.

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