frontiers in GENETICS

ORIGINAL RESEARCH ARTICLE
published: 29 August 2011
doi: 10.3389/fgene.2011.00052

Perinatal exogenous nitric oxide in fawn-hooded hypertensive rats reduces renal ribosomal biogenesis in early life

Sebastiaan Wesseling[1†], Paul B. Essers[2†], Maarten P. Koeners[1], Tamara C. Pereboom[2], Branko Braam[3,4], Ernst E. van Faassen[5], Alyson W. MacInnes[2] and Jaap A. Joles[1]*

[1] Department of Nephrology and Hypertension, University Medical Center, Utrecht, Netherlands
[2] Hubrecht Institute, KNAW and University Medical Center Utrecht, Utrecht, Netherlands
[3] Division of Nephrology and Immunology, Department Medicine, University of Alberta, Edmonton, AB, Canada
[4] Department of Physiology, University of Alberta, Edmonton, AB, Canada
[5] Department of Nephrology, Leiden University Medical Center, Leiden, Netherlands

Edited by:
Jeff Schwartz, Griffith University, Australia

Reviewed by:
Mark Chappell, Wake Forest University School of Medicine, USA
Tamara Paravicini, The University of Queensland, Australia
Kirk Peter Conrad, University of Florida, USA

***Correspondence:**
Jaap A. Joles, Department of Nephrology and Hypertension F03.223, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, Netherlands.
e-mail: j.a.joles@umcutrecht.nl

†*Sebastiaan Wesseling and Paul B. Essers have contributed equally to this work.*

Nitric oxide (NO) is known to depress ribosome biogenesis *in vitro*. In this study we analyzed the influence of exogenous NO on ribosome biogenesis *in vivo* using a proven antihypertensive model of perinatal NO administration in genetically hypertensive rats. Fawn-hooded hypertensive rat (FHH) dams were supplied with the NO-donor molsidomine in drinking water from 2 weeks before to 4 weeks after birth, and the kidneys were subsequently collected from 2 day, 2 week, and 9 to 10-month-old adult offspring. Although the NO-donor increased maternal NO metabolite excretion, the NO status of juvenile renal (and liver) tissue was unchanged as assayed by EPR spectroscopy of NO trapped with iron-dithiocarbamate complexes. Nevertheless, microarray analysis revealed marked differential up-regulation of renal ribosomal protein genes at 2 days and down-regulation at 2 weeks and in adult males. Such differential regulation of renal ribosomal protein genes was not observed in females. These changes were confirmed in males at 2 weeks by expression analysis of renal ribosomal protein L36a and by polysome profiling, which also revealed a down-regulation of ribosomes in females at that age. However, renal polysome profiles returned to normal in adults after early exposure to molsidomine. No direct effects of molsidomine were observed on cellular proliferation in kidneys at any age, and the changes induced by molsidomine in renal polysome profiles at 2 weeks were absent in the livers of the same rats. Our results suggest that the previously found prolonged antihypertensive effects of perinatal NO administration may be due to epigenetically programmed alterations in renal ribosome biogenesis during a critical fetal period of renal development, and provide a salient example of a drug-induced reduction of ribosome biogenesis that is accompanied by a beneficial long-term health effect in both males and females.

Keywords: nitric oxide, ribosomal biogenesis, microarray, polysome profiling, perinatal, epigenetic, kidney

INTRODUCTION

Plasticity of organogenesis provides an opportunity for interventions in a specific window of early development that may have long-term beneficial or detrimental effects on adult health and disease (McMillen and Robinson, 2005). One critical regulation of such plasticity is protein synthesis. Upstream factors affecting protein synthesis include tight regulations at multiple stages of ribosome biogenesis. For example, it is well known that epigenetic silencing of ribosomal DNA (rDNA) regularly occurs, even in proliferating cells (McStay and Grummt, 2008; Sanij and Hannan, 2009). One exogenous factor that has been shown to affect rDNA and ribosome biogenesis is nitric oxide (NO). Exposure of cells to high levels of NO, using either NO-donors, or inducing expression of inducible NO synthase (iNOS), results in inhibition of the 80S ribosomal complex (Kim et al., 1998) and enhanced rRNA cleavage resulting in a reduction of both 60S and 80S ribosomal particles (Cai et al., 2000).

Hypertension is associated with decreased NO availability (Wilcox, 2005). The fawn-hooded hypertensive rat (FHH) is a genetic model of hypertension susceptible to progressive renal injury. In FHH hypertension is aggravated and the development of renal injury is accelerated when NOS is chronically inhibited, revealing partial NO dependency of the adult FHH phenotype (Van Dokkum et al., 1998). Renal transplantation under different conditions has shown that blood pressure regulation is intricately linked to the kidney (Smallegange et al., 2004; Crowley et al., 2005), and we hypothesized that this is also the case in the perinatal phase (Koeners et al., 2008a). Recently, we observed that perinatal supplementation of FHH dams with molsidomine, an NO-releasing

Abbreviations: FHH, fawn-hooded hypertensive rat; NO, nitric oxide.

prodrug (Feelisch, 1998; Singh et al., 1999), persistently lowered the blood pressure and attenuated the development of renal injury in male and female FHH (Koeners et al., 2008b).

Long-term regulation of blood pressure is determined by the relationship between renal perfusion pressure and NaCl excretion, and this relationship is facilitated by renal NO availability (Cowley, 2008; Garvin et al., 2011). Thus our interest is directed at mechanisms in the kidney that link availability of NO in early development to regulation blood pressure in adult life. Conceivably temporal changes in the regulation of renal protein synthesis via ribosomal control of gene translation could constitute such a link (Kasinath et al., 2006). For instance, compensatory renal hypertrophy involves a global increase in polysome profiles within less than 1 day after uninephrectomy (Chen et al., 2005). Based on the known effects of high levels of NO on the ribosomal elements in cultured cells, we hypothesized that ribosome biogenesis *in vivo* in the neonatal FHH kidney may also be regulated by NO availability. This perinatal regulation of ribosome biogenesis may then affect kidney organogenesis in a manner that impacts the long-term regulation of blood pressure and renal integrity.

Here we demonstrate that the perinatal administration of NO results in a dramatic biphasic change of ribosomal protein gene expression in FHH rats at 2 days and 2 weeks of age. This results in decreased post-translational levels of certain ribosomal proteins, and a remarkable reduction of assembled ribosome structures at the 2-week point. Intriguingly, we did not find an increase in renal NO content at 2 weeks in the offspring of NO-donor-treated rats. Our results suggest that the increased availability of NO in gestation epigenetically alters renal ribosome biogenesis during a critical period of renal development. In conjunction with previously published findings, we conclude that this effect by NO may alter renal organogenesis in a manner that alleviates the hypertension phenotype normally experienced by FHH rats.

MATERIALS AND METHODS

ANIMAL EXPERIMENT

Fawn-hooded hypertensive rat were from our own colony, derived from the original colony at Erasmus University Rotterdam (FHH/EUR) maintained by Dr. A. Provoost. FHH dams were supplied with molsidomine (Sigma-Aldrich, Zwijndrecht, Netherlands) in drinking water (120 mg/L) 2 weeks before to 4 weeks after birth. Control FHH mothers and their offspring received regular tap water. All offspring from 4 weeks of age received regular tap water and regular chow (Special Diets Services, Witham, Essex, England). Offspring were sacrificed at 2 days, 2 weeks, 36 weeks (males), and 42 weeks (females). The adult ages were chosen when renal injury in males and females was similar. Kidneys were isolated and snap-frozen (for microarray analysis), kept on ice (for Western blotting and polysome profiling), or fixed in formaldehyde (for immunohistochemistry). Note that although functional and morphological data from the adult rats have been published previously (Koeners et al., 2008b), all microarray data and all data pertaining to renal ribosomal proteins in adult kidneys is novel. Directly after weaning of the pups, the dams were placed in metabolic cages without food but with access to water with 2% glucose and 24-h urine was collected on antibiotic/antimycotic solution (Sigma-Aldrich) to prevent degradation of NO metabolites. NO metabolites were determined as described (Bongartz et al., 2010). Sentinel animals were housed under the same conditions and regularly monitored for infections by nematodes, pathogenic bacteria, and antibodies for rodent viral pathogens (International Council for Laboratory Animal Science, Nijmegen, Netherlands). The Utrecht University Board for studies in experimental animals approved the protocol.

MICROARRAY

For an overview and extensive explanation of microarray data processing, please see Appendix. In short, a piece of snap-frozen kidney was put in 1 mL TRIzol (Invitrogen, Breda, Netherlands) containing 100–150 mg 1 mm glass beads (BioSpec Products, Bartlesville, OK, USA) and immediately homogenized in 30 s using a mini-beadbeater (BioSpec). The total RNA was isolated according to the manufacturer's instructions. Total RNA was purified using NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany). Samples were then put on Illumina BeadChips (RatRef-12) by ServiceXS[1] (Leiden, Netherlands). Kidneys from 2 days, 2 weeks, and adult FHH of both genders were used (at least $n = 5$/group). All samples were randomly placed on different arrays in order to minimize variation between BeadChips and between arrays.

After calculating the average intensity per probe, all arrays were Log_2-transformed and Quantile normalized. The arrays were grouped and the average intensity was calculated. The significance of the differences in intensity between the groups was calculated using Cyber t-test. This final data containing normalized data, average intensity per group and statistical significance between groups were used in data evaluation. The data are submitted as MIAME-complaint to GEO[2] under accession number GSE27725.

The number of genes differentially expressed in the molsidomine samples were counted per age in each gender. These were then compared in order to elucidate whether there were genes persistently affected by molsidomine. The 40 genes that were most differentially regulated (20 up and 20 down) by molsidomine were collected at each age for each gender.

All genes encoding for ribosomal proteins were collected. The differentially expressed genes encoding for ribosomal proteins were compared at each age. In order to determine whether the effect of molsidomine on ribosomal genes was stronger than on general gene expression profiles, the ratio of differentially regulated ribosomal genes to the whole ribosomal gene population was compared to the ratio of total differentially expressed genes with whole microarray data in a size test.

WESTERN BLOT ANALYSIS

Fresh kidney samples were lysed on ice in lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100; all from Sigma-Aldrich) plus protease inhibitors (Santa Cruz Biotech, #29130) and subjected to centrifugation at 14K rpm at 4°C for 10 min. Protein content in the supernatants was quantified using Biorad Protein Assay. 6× Laemmli loading buffer was added to 50 μg samples which were then boiled for 5 min and loaded on a 10% SDS/PAGE gel. Transfers to PVDF membranes (Millipore, #IPVH00010) were done overnight at 15 V at 4°C, blocked in

[1] www.servicexs.com
[2] http://www.ncbi.nlm.nih.gov/geo/

5% milk/TBST solution for 1 h at RT, and subjected to blotting with αL36a (Abnova, #H00006173-M02) or α-actin (Santa Cruz Biotech, #1616) at dilutions of 1:200 in blocking buffer overnight at 4˚C. Either α-mouse (L36a) or α-rabbit (actin) HRP-conjugated secondary antibodies (GE Healthcare, #NXA931 and NA934) were used at a dilution of 1:5000 in TBST for 20 min at RT. Blots were washed 3× in TBST for 10 min at RT. Detection was done with the ECL Advance Western Blot Detection Kit (GE Healthcare, #RPN2135). Quantifications were performed using a GS-800 densitometer (Biorad, Veenendaal, Netherlands) and Quantity One software (Biorad).

POLYSOME PROFILING

The kidneys from FHH pups from control dams or dams treated with molsidomine were collected at age 2 days and 2 weeks, maintained fresh on ice, and processed for polysome profiling on the same day. For polysome profiling of adult tissue frozen kidney tissue was used. Comparisons were only performed between treated and control rats of both genders at each age. Livers from 2-week-old pups were used to determine tissue-specificity.

All steps of this protocol were performed at 4˚C or on ice. Gradients of 17–50% sucrose (11 ml) in gradient buffer (110 mM KAc, 20 mM $MgAc_2$, and 10 mM HEPES pH 7.6) were prepared on the day prior to use. Kidneys were lysed in 500 μl polysome lysis buffer (gradient buffer containing 100 mM KCl, 10 mM $MgCl_2$, 0.1% NP-40, 2 mM DTT, and 40 U/ml RNasin; Promega, Leiden, Netherlands) using a dounce homogenizer. The samples were centrifuged at 1200 g for 10 min to remove debris and loaded onto sucrose gradients. The gradients were ultracentrifuged for 2 h at 40,000 rpm in an SW41Ti rotor (Beckman-Coulter, USA). The gradients were displaced into a UA6 absorbance reader (Teledyne ISCO, USA) using a syringe pump (Brandel, USA) containing 60% sucrose. Absorbance was recorded at an OD of 254 nm. All chemicals came from Sigma-Aldrich unless stated otherwise.

TISSUE NO CONTENT

Endogenous NO levels in kidney and liver tissues of FHH pups were assayed at 2 weeks by NO trapping with iron-dithiocarbamate (Fe-DETC) complexes, as previously described (Koeners et al., 2007; Van Faassen et al., 2008). Briefly, the yields of paramagnetic NO-Fe(II)-DETC complexes (mononitrosyl-iron complexes, MNIC) in tissues was quantified with EPR spectroscopy of intact frozen tissue sections at 77K. The tissues were reduced at room temperature with dithionite (50 mM for 15 min) to remove the overlapping EPR signal from paramagnetic Cu(II)-DETC complexes as commonly found in biological materials.

IMMUNOHISTOCHEMISTRY

Kidneys were fixed overnight at room temperature in 4% formaldehyde. The tissue was embedded in paraffin and 4 μm sections were made on silanized glass slides. The slides were baked at 58˚C overnight, deparaffinated, and rehydrated. Endogenous peroxidase was blocked using citric acid. For antigen retrieval, the sections were boiled for 20 min in citrate buffer (pH 6) and allowed to cool slowly. The sections were then blocked in 1% BSA (w/v) in PBS and incubated with rabbit-anti-pH3 (Santa Cruz Biotech, #1791) overnight at 4˚C. The sections were then incubated in anti-rabbit Powervision PO (Immunologic, #DPVR110 HRP) for 30 min at RT and developed using DAB. Finally the sections were counterstained in hematoxylin, dehydrated, and enclosed in pertex. The quantification was performed as follows: At 20× magnification random fields were chosen, taking care not to include the edges of the tissue. The number of positive cells was counted in three fields of two sections per kidney. The average of these six counts was used for analysis.

STATISTICS

For statistics in microarray, please refer to the methodology. For other measurements the values are expressed as means ± SEM. Data were compared with unpaired *t*-test, one-way ANOVA, and two-way ANOVA where appropriate followed by *post hoc* test Student-Newman–Keuls. $P < 0.05$ is considered significant.

RESULTS

BIOMETRICAL DATA

Biometrical data of FHH offspring and the number of rats studied are collected in **Table 1**. Note that adult kidney weight and tail-cuff blood pressure data, which were published previously (Koeners et al., 2008b), are included in the table for the sake of convenience. Molsidomine treatment decreased the kidney weight relative to body weight in 2-day-old females ($P < 0.05$) but not at older ages. However, in both male and female 2-week-old FHH rats relative kidney weight was unchanged. The kidneys of adult males exposed to perinatal molsidomine weighed less than controls, probably in association with reduced injury (Koeners et al., 2008b). Perinatal molsidomine decreased systolic blood pressure in adult FHH offspring (Koeners et al., 2008b). NO metabolites were determined in a 24-h collection of urine from FHH dams to substantiate the direct effects of molsidomine in their pups. Indeed, maternal urine NOx was increased by molsidomine ($n = 4$) vs. controls ($n = 4$) from 1.6 ± 0.1 to 2.6 ± 0.2 μmol/(100 g BW)/d ($P < 0.01$).

TISSUE NO CONTENT

Although the NO-donor increased maternal NO metabolite excretion, the NO status of 2-week-old renal (and liver) tissue was unchanged as assayed by EPR spectroscopy of NO trapped with iron-dithiocarbamate complexes (**Table 2**). Unfortunately, the NO trapping procedure is not possible in 2-day-old pups.

MICROARRAY

Perinatal treatment with molsidomine significantly affected transcription of hundreds of genes at 2 days and at older ages (see **Figure A1** in Appendix). The data also clearly shows that the transcriptional effect of molsidomine differs between ages. Few genes remained differentially expressed at all ages and those that did displayed bidirectional expression between ages.

The 40 most differentially expressed genes (20 induced and 20 reduced) were nearly all different between males and females at the same age. Several genes encoding for ribosomal proteins were present in the top 20 genes in males at all ages, however were less present in the top 20 of females (see **Tables A1A–F** in Appendix).

Table 1 | Biometrical data of control FHH and FHH during molsidomine (2 days and 2 weeks) or after perinatal molsidomine (adult).

	Males		Females	
	Controls	**Molsidomine**	**Controls**	**Molsidomine**
2 DAYS				
Number of pups/number of litters	12/7	12/7	12/6	19/10
RKW/BW (mg/g)	4.9 ± 0.1	4.8 ± 0.1	5.3 ± 0.1	4.9 ± 0.1[#]
2 WEEKS				
Number/litters	15/8	17/10	13/8	18/10
RKW/BW (mg/g)	5.2 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.5 ± 0.1
ADULT				
Number/litters	23/10	13/4	24/10	16/5
RKW/BW (mg/g)	3.8 ± 0.1	3.4 ± 0.03[#]	4.4 ± 0.1	4.3 ± 0.1
Systolic blood pressure (mmHg)	158 ± 3	139 ± 4[#]	145 ± 5	118 ± 5[#]

[#]P < 0.05 vs. controls of same gender. Biometrical data in adults published previously (Koeners et al., 2008b) are included for the sake of convenience.

Table 2 | Nitric oxide yields (pmol MNIC/mg tissue) determined by EPR in kidneys and liver in of 2-week-old control FHH and 2-week-old FHH offspring of dams treated with molsidomine.

	Males		Females	
	Controls	**Molsidomine**	**Controls**	**Molsidomine**
2 WEEKS				
Number/litters	4/2	7/4	4/2	8/4
NO yield in kidney (left and right)	0.49 ± 0.03	0.53 ± 0.01	0.50 ± 0.04	0.49 ± 0.02
NO yield in liver	1.30 ± 0.08	1.39 ± 0.04	1.35 ± 0.08	1.32 ± 0.04

MNIC, mononitrosyl-iron complexes.

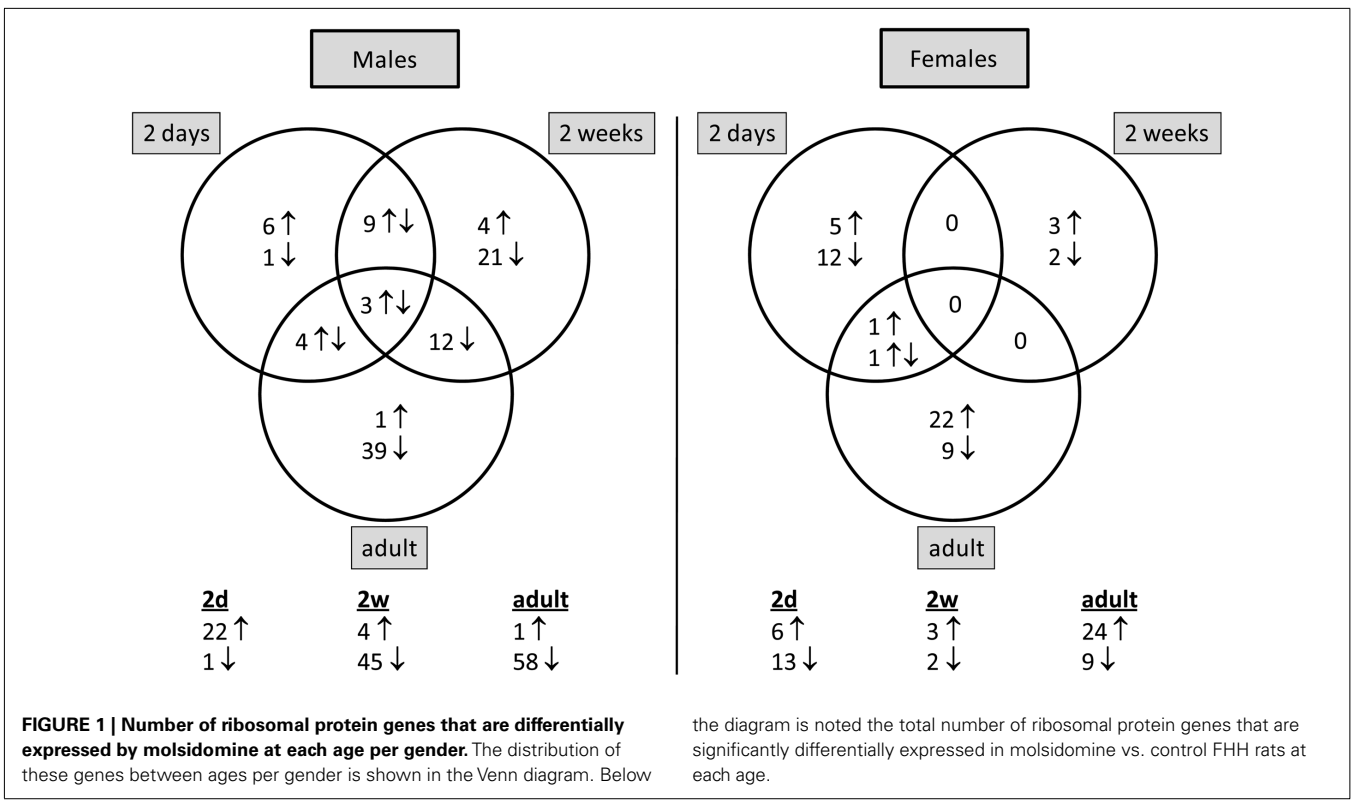


FIGURE 1 | Number of ribosomal protein genes that are differentially expressed by molsidomine at each age per gender. The distribution of these genes between ages per gender is shown in the Venn diagram. Below the diagram is noted the total number of ribosomal protein genes that are significantly differentially expressed in molsidomine vs. control FHH rats at each age.

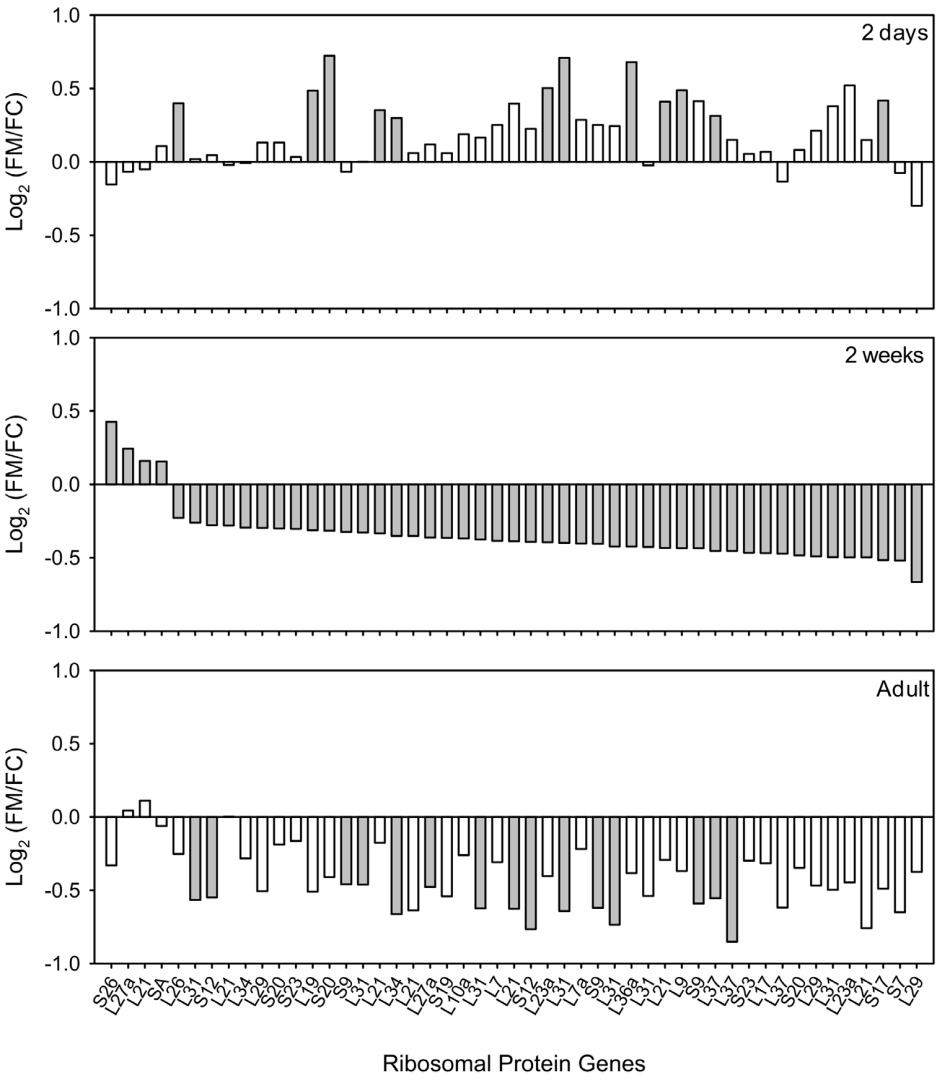


FIGURE 2 | Ribosomal protein genes in kidneys of FHH males differentially expressed by molsidomine at 2 weeks and whether these genes were also regulated at other ages. All genes that are significantly differentially expressed by molsidomine at 2 weeks are ranked (middle panel). Some of these genes were also regulated at 2 days (upper panel) and in adults (lower panel). The ratio of the genes is expressed as Log2(FM/FC), where FM and FC are the normalized intensities of gene expression in the molsidomine and control groups, respectively. Significant differential expression is indicated by a closed bar, non-significant differential expression by an open bar.

The present study is focused on the changes in ribosomal protein gene expression. Remarkably, these ribosomal protein genes in males were differentially induced by molsidomine at 2 days, then differentially reduced by molsidomine at both 2 weeks and in adults (see **Figures 1** and **2**; the collection of genes are shown in **Table A2** in Appendix). These changes in ribosomal protein gene expression were significant at all ages ($P < 0.05$) and this effect was specific for ribosomal genes only in males (vs. all genes in the microarray; $P < 0.001$).

PROTEIN EXPRESSION OF L36a

In order to determine if the changes in ribosomal protein gene expression suggested by the microarray analysis could be verified at the protein level, we analyzed the expression of ribosomal protein L36a at 2 days and 2 weeks. **Table A2** in Appendix shows that genes coding for the ribosomal protein L36a are subject to some of the most significant up-regulation at 2 days and down-regulation at 2 weeks (and in adults) in molsidomine-treated FHH males. Note that more than one ribosomal protein L36a gene from different chromosomes is listed on **Table 2**. Western blot analysis of kidney samples of FHH rats demonstrated that ribosomal protein L36a protein tends to be down-regulated by molsidomine in 2 week males (**Figure 3**). Quantification of three independent experiments verified that this change in ribosomal protein L36a protein expression occurred only in molsidomine-treated males at 2 weeks (**Figure 3**). These results suggest that despite the increase in ribosomal protein gene expression seen in 2-day-old molsidomine-treated FHH males, this increases does not manifest

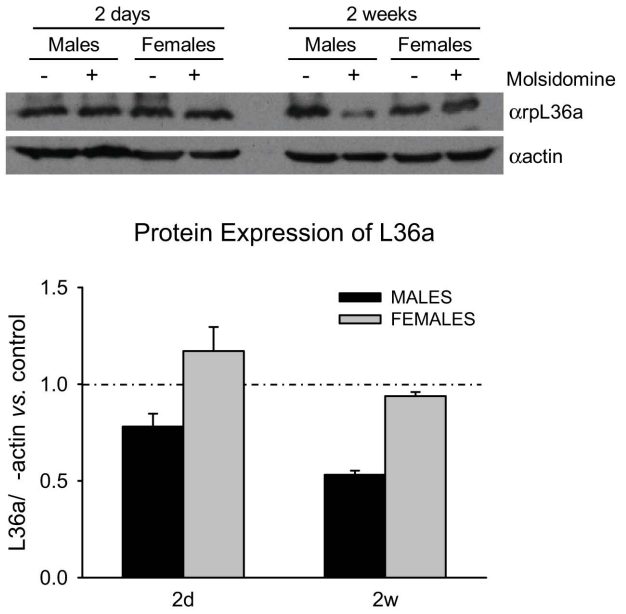


FIGURE 3 | Western Blot on ribosomal protein L36a. Western blot analysis was performed on kidneys lysates from 2 day and 2 week control and molsidomine FHH rats of both genders using an antibody against ribosomal protein L36a (upper panel). The measured band intensity of ribosomal protein L36a was normalized by α-actin of the same sample (ribosomal protein L36a/actin). The molsidomine effect on ribosomal protein L36a/actin is expressed relative to the age- and gender matched control, shown in graph by the dotted line (lower panel). The reduction in 2 week molsidomine males is borderline significant ($P = 0.1$). All 3 replicates are biological.

as an increase in ribosomal protein levels (see also **Figure 4**). In contrast, the western blot results suggest that the decrease in ribosomal protein gene expression at 2 weeks in molsidomine-treated rats indeed does affect L36a proteins levels, as also supported by the profiles of **Figure 4**.

RIBOSOMAL EVALUATION

Polysome profiling was performed on kidneys and the effects on the peaks representing the small ribosomal subunit (40S), large ribosomal subunit (60S), and monosome (80S) were determined (**Figure 4**). Two days after birth and in adults, molsidomine had no effect on polysome profiles, but 2 weeks after birth all peaks were significantly reduced by molsidomine in both males and females ($P < 0.01$). This effect may be specific to the kidney, as no effect of molsidomine was observed in males at 2 weeks of age in liver polysome profiles (**Figure 5**). Northern blotting was performed on total RNA from 2 week FHH kidneys in order to determine rates of rRNA processing (**Figure A2** in Appendix). However, no differences were observed in processing using probes binding to either the external transcribed spacer (ETS) or the internal transcribed spacer 1 (ITS1).

IMMUNOHISTOCHEMISTRY

In order to determine if molsidomine resulted in a mitotic index change, we subjected kidneys from FHH rats to staining with a phospho-specific histone-3 (pH3) antibody (**Figure 6**). We saw no difference in the number of pH3-positive cells on molsidomine-treated FHH rat kidney slices compared to controls. As expected the number of pH3-positive cells decreased as the age of the rats increased. No pH3-positive cells were observed in adults (data not shown).

DISCUSSION

Nitric oxide donors are known to inhibit proliferation of mesangial and other glomerular cells *in vitro* (Rupprecht et al., 2000). Little is known about the effects of NO-donors on early growth and nephrogenesis. Recently we observed that administration of NO-donors during early development ameliorates the long-term phenotype in the FHH rat model of progressive hypertension-linked renal injury (Koeners et al., 2008b). Assuming that the direct effects of the NO-donor in early development were related to an as of yet undefined aspect of renal development, the present study focused on a global analysis of ribosomal proteins as a key step in the post-transcriptional regulation of protein synthesis.

The prominent presence of ribosomal protein genes, especially in FHH males, on the lists of the most differentially expressed genes as a result of perinatal NO administration led us to examine how these changes in gene expression affected the protein levels of an individual ribosomal protein as well as the structures of mature, assembled ribosomes. Interestingly, when we measured the protein level of one of the most significantly differentially expressed gene in FHH males, ribosomal protein L36a, we found that only at 2 weeks of age did the change in gene expression correlate with a change in protein expression. This is in contrast to the ribosomal protein L36a protein levels at 2 days, which show no increase despite a substantial increase in ribosomal protein L36a gene expression at that age in males. This discrepancy may be due to the ribosome biogenesis machinery being saturated at 2 days of age and unable to incorporate higher levels of ribosomal proteins. The tight regulation of this biogenesis may likely be degrading excess ribosomal proteins at the protein level or blocking translation of ribosomal proteins at the mRNA level, although at present our data cannot distinguish between these two possibilities. No change in ribosomal protein L36a protein expression was observed in FHH females at 2 weeks of age, but this is not surprising given that no significant gene expression change of ribosomal protein L36a is seen in these animals. However, given the following data, it is likely that there is a reduction of one or more key ribosomal proteins at the protein level in FHH females at 2 weeks. Conceivably by the time the FHH rat has reached adulthood the kidney cells have adjusted the half-lives and/or degradation rates of certain ribosomal proteins in order to reach the normal number of mature ribosome structures.

The most significant finding of this study was that at 2 weeks after birth, i.e., at the end of nephrogenesis (Marquez et al., 2002), perinatal NO administration resulted in a global reduction of ribosome structures in both male and female FHH rats. All of the peaks representing major ribosome structures were found to be substantially decreased in molsidomine-treated FHH rats at 2 weeks of age. This global reduction in NO-treated females at 2 weeks was surprising because only two ribosomal protein genes, coding for ribosomal protein L16 and ribosomal protein L21, were

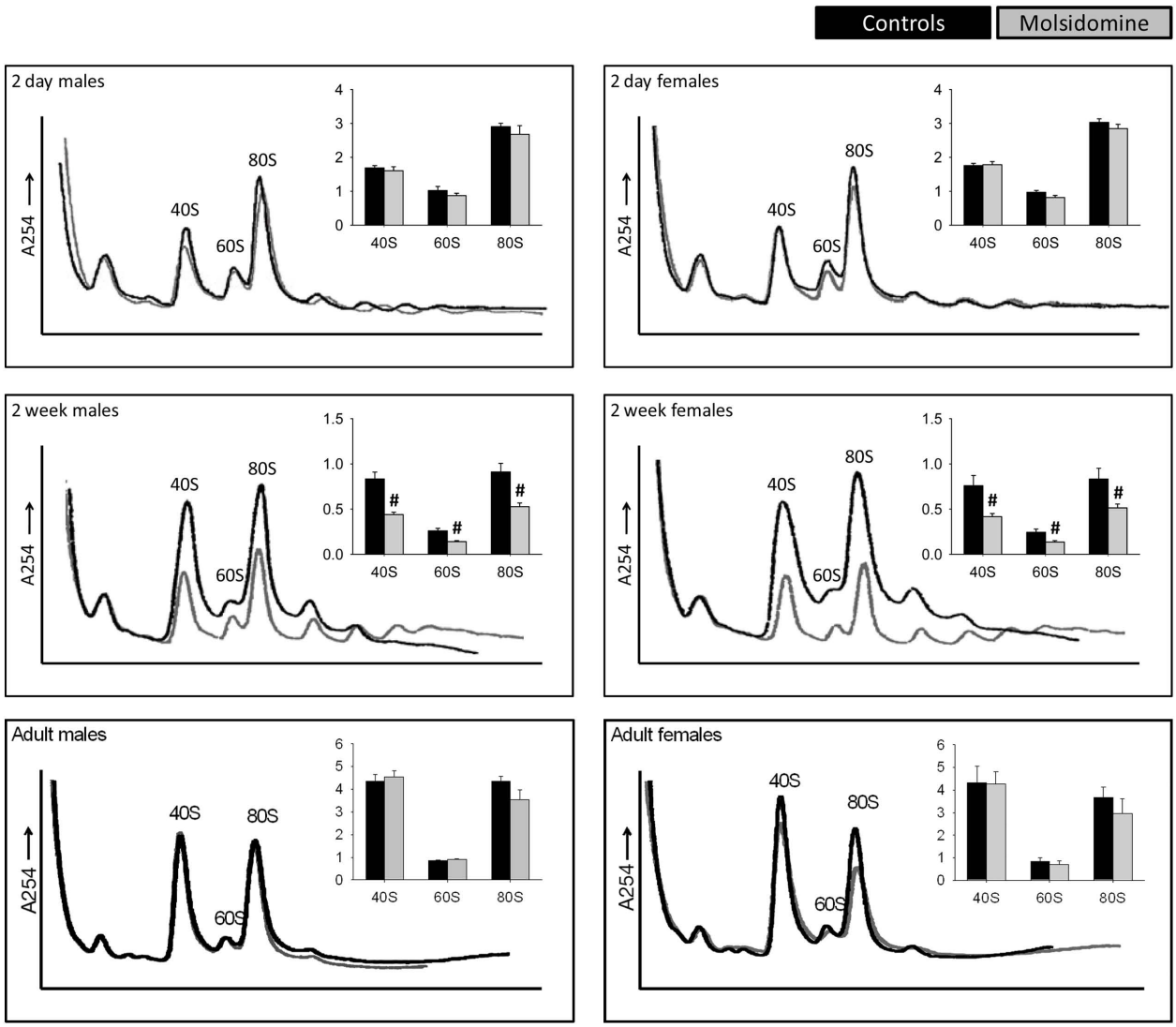


FIGURE 4 | Polysome profiles of kidneys. Kidneys from control 2 day old (males $n = 5$, females $n = 6$), 2 week old ($n = 8$ in both genders), and adult ($n = 4$ in both genders) and molsidomine-treated 2 day old (males $n = 4$, females $n = 6$), 2 week old ($n = 9$ in both genders) and adult ($n = 5$ in both genders) FHH rats were profiled to measure the total number of assembled ribosome structures. The peaks of 40S, 60S, and 80S were normalized against the left-most peak and the results shown in the corresponding histograms. $^{\#}P < 0.01$ vs. untreated of the same peak.

significantly reduced. Ribosomal protein L16 gene expression was not reduced by molsidomine in FHH males at 2 weeks, but strikingly 6 out of the 45 genes that were significantly reduced coded for ribosomal protein L21. This suggests that ribosomal protein L21 may be a key ribosomal protein in the biogenesis of the 60S large subunit and may also play an unappreciated role in the biogenesis of the 40S subunit. Additionally, ribosomal protein L21 appears to be important in the development of craniofacial organs (Xie et al., 2009) and a missense mutation in L21 leads to hereditary hypotrichosis simplex in humans (Zhou et al., 2011). Our findings suggest that ribosomal protein L21 may have an as yet unrecognized role in the development of blood pressure control mechanisms of the kidney.

To our knowledge, this is the one of the most striking examples of a drug-induced decrease in ribosome biogenesis in an animal model to date that is not accompanied by deleterious effects. For example, rapamycin, a powerful inhibitor of the mTOR pathway that directly regulates ribosome biogenesis, when injected into rats had only a slight effect on polysome profiles of liver tissue (Reiter et al., 2004). However, doses of rapamycin as low as 1 ng/mL have been shown to negatively affect cell function and contribute to cell death, for instance in rodent islet cells (Bell et al., 2003; Tanemura et al., 2009). Moreover, many vertebrate and invertebrate models of deficiency of a single ribosomal protein due to gene deletion, knockdown, or missense mutations often show severe phenotypes (Caldarola et al., 2009), and

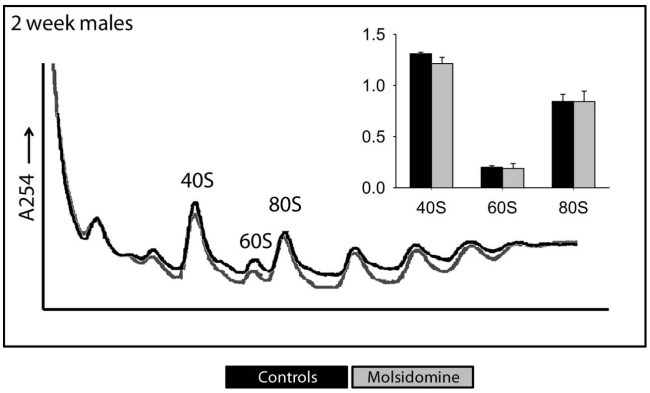
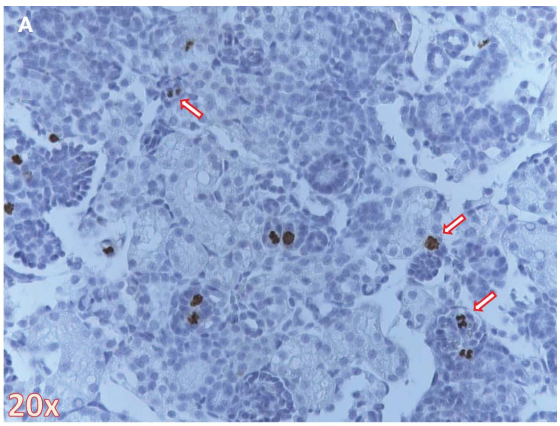


FIGURE 5 | Polysome profiles of livers. Livers from 2-week-old FHH males were profiled to measure the total number of assembled ribosome structures. The peaks of 40S, 60S, and 80S were normalized against the left-most peak and the results shown in the corresponding histograms. Black and gray lines are from the control and molsidomine groups, respectively.



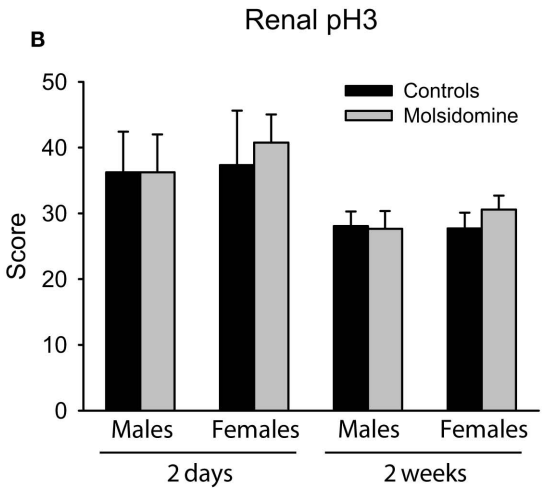


FIGURE 6 | Phospho-histone H3 (pH3) immunohistochemistry. Histology was performed on the kidneys from 2 day, 2 week and adult control and molsidomine FHH rats of both genders for pH3. An example of positive staining for pH3 in 2 day female molsidomine-treated FHH rat is shown in **(A)**. Several arrows indicate cells in mitosis staining positive for pH3. The number of positive cells at 2 days and 2 weeks is shown in **(B)**. Note that data in adults is not shown as all stained sections were completely negative for pH3.

models of biallelic loss of every ribosomal protein gene (with the one exception of ribosomal protein L22; Anderson et al., 2007) results in lethality. In contrast, our data suggest that a decrease of ribosome biogenesis during a critical period of nephrogenesis results in permanent physiologic changes to the kidney, which in turn ameliorate the hypertension phenotype late in life. Interestingly, the effects of exogenous NO on ribosomal biogenesis did not affect the liver, underlining the crucial role of NO in the developing cardiovascular system (Bustamante et al., 1996). An obvious change linked to a decrease in ribosome biogenesis would be a reduction in proliferation. However, immunohistochemistry did not reveal a change in proliferation. Note that although SIN-1, the active metabolite of molsidomine, can generate peroxynitrite *in vitro*, it appears to function solely as a NO-donor at *in vivo* oxygen concentrations (Singh et al., 1999). Indeed, recently we supplemented molsidomine to rescue cardiac function in rats with cardiorenal failure and found no increase in 3-nitrotyrosine in heart, kidney, or liver (Bongartz et al., 2010).

The mechanism of action of NO administered perinatally to FHH rats on ribosomal protein gene expression and the subsequent reduction of ribosome structures remains unclear. Indeed, direct measurement of whole kidney NO content at 2 weeks failed to show any effect of maternal NO-donor treatment, suggesting that the decrease in ribosome biogenesis at 2 weeks is programmed by an earlier event. Possibly maternal molsidomine intake results in increased placental transfer of NO adducts and increased fetal renal NO content. Alternatively, indirect effects on the placental circulation could play a role. Indeed, although previous studies (Cai et al., 2000) suggest a link between NO, rRNA synthesis and proliferation, we were unable to establish any differences in pre-rRNA levels in our model that would support direct effects of exogenous NO on rRNA production (**Figure A2** in Appendix). This suggests that the effect of NO at 2 weeks more likely lies within epigenetically programmed transcriptional changes of ribosomal protein genes, as demonstrated by the microarray results. Note that in a previous study we did find an increase in offspring kidney NO content when spontaneously hypertensive rat (SHR) dams were treated with citrulline (Koeners et al., 2007). Subsequently siblings of these SHR had a lower blood pressure than control SHR. The results of the present study suggest that mechanisms underlying the antihypertensive effects of perinatal administration of the NO-donor molsidomine in rats with genetic hypertension could be quite different.

It has been previously established that perinatal NO administration alleviates the hypertension phenotype in FHH rats (Koeners et al., 2008b). The present study suggests a potential mechanism underlying this phenotype alleviation. At this stage we have no direct proof that the remarkable ribosomal changes we observe provide the causal mechanism for the beneficial effects of maternal

NO on the blood pressure and renal function of the offspring. However, our data demonstrate a novel possibility that long-term amelioration of hypertension by NO in gestation may induce epigenetic changes that affect the postnatal transcription of ribosomal protein genes and a reduction of ribosome structures during a critical period of nephrogenesis.

ACKNOWLEDGMENTS

Expert assistance: Paula P. Martens (UMC Utrecht), Jeroen Korving, and Harry Begthel (Hubrecht Institute). This study was financially supported by the Dutch Kidney Foundation (NSN C06.2168) and Royal Netherlands Academy of Arts and Sciences (KNAW).

REFERENCES

Anderson, S. J., Lauritsen, J. P., Hartman, M. G., Foushee, A. M., Lefebvre, J. M., Shinton, S. A., Gerhardt, B., Hardy, R. R., Oravecz, T., and Wiest, D. L. (2007). Ablation of ribosomal protein L22 selectively impairs alphabeta T cell development by activation of a p53-dependent checkpoint. *Immunity* 26, 759–772.

Bell, E., Cao, X., Moibi, J. A., Greene, S. R., Young, R., Trucco, M., Gao, Z., Matschinsky, F. M., Deng, S., Markman, J. F., Naji, A., and Wolf, B. A. (2003). Rapamycin has a deleterious effect on MIN-6 cells and rat and human islets. *Diabetes* 52, 2731–2739.

Bongartz, L. G., Braam, B., Verhaar, M. C., Cramer, M. J., Goldschmeding, R., Gaillard, C. A., Steendijk, P., Doevendans, P. A., and Joles, J. A. (2010). The nitric oxide donor molsidomine rescues cardiac function in rats with chronic kidney disease and cardiac dysfunction. *Am. J. Physiol. Heart Circ. Physiol.* 299, H2037–H2045.

Bustamante, S. A., Pang, Y., Romero, S., Pierce, M. R., Voelker, C. A., Thompson, J. H., Sandoval, M., Liu, X., and Miller, M. J. (1996). Inducible nitric oxide synthase and the regulation of central vessel caliber in the fetal rat. *Circulation* 94, 1948–1953.

Cai, C. Q., Guo, H., Schroeder, R. A., Punzalan, C., and Kuo, P. C. (2000). Nitric oxide-dependent ribosomal RNA cleavage is associated with inhibition of ribosomal peptidyl transferase activity in ANA-1 murine macrophages. *J. Immunol.* 165, 3978–3984.

Caldarola, S., De Stefano, M. C., Amaldi, F., and Loreni, F. (2009). Synthesis and function of ribosomal proteins – fading models and new perspectives. *FEBS J.* 276, 3199–3210.

Chen, J. K., Chen, J., Neilson, E. G., and Harris, R. C. (2005). Role of mammalian target of rapamycin signaling in compensatory renal hypertrophy. *J. Am. Soc. Nephrol.* 16, 1384–1391.

Cowley, A. W. Jr. (2008). Renal medullary oxidative stress, pressure-natriuresis, and hypertension. *Hypertension* 52, 777–786.

Crowley, S. D., Gurley, S. B., Oliverio, M. I., Pazmino, A. K., Griffiths, R., Flannery, P. J., Spurney, R. F., Kim, H. S., Smithies, O., Le, T. H., and Coffman, T. M. (2005). Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system. *J. Clin. Invest.* 115, 1092–1099.

Feelisch, M. (1998). The use of nitric oxide donors in pharmacological studies. *Naunyn Schmiedebergs Arch. Pharmacol.* 358, 113–122.

Garvin, J. L., Herrera, M., and Ortiz, P. A. (2011). Regulation of renal NaCl transport by nitric oxide, endothelin, and ATP: clinical implications. *Annu. Rev. Physiol.* 73, 359–376.

Kasinath, B. S., Mariappan, M. M., Sataranatarajan, K., Lee, M. J., and Feliers, D. (2006). mRNA translation: unexplored territory in renal science. *J. Am. Soc. Nephrol.* 17, 3281–3292.

Kim, Y. M., Son, K., Hong, S. J., Green, A., Chen, J. J., Tzeng, E., Hierholzer, C., and Billiar, T. R. (1998). Inhibition of protein synthesis by nitric oxide correlates with cytostatic activity: nitric oxide induces phosphorylation of initiation factor eIF-2 alpha. *Mol. Med.* 4, 179–190.

Koeners, M. P., Braam, B., and Joles, J. A. (2008a). Blood pressure follows the kidney: perinatal influences on hereditary hypertension. *Organogenesis* 4, 153–157.

Koeners, M. P., Braam, B., Van Der Giezen, D. M., Goldschmeding, R., and Joles, J. A. (2008b). A perinatal nitric oxide donor increases renal vascular resistance and ameliorates hypertension and glomerular injury in adult fawn-hooded hypertensive rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R1847–R1855.

Koeners, M. P., Van Faassen, E. E., Wesseling, S., De Sain-Van Der Velden, M., Koomans, H. A., Braam, B., and Joles, J. A. (2007). Maternal supplementation with citrulline increases renal nitric oxide in young spontaneously hypertensive rats and has long-term antihypertensive effects. *Hypertension* 50, 1077–1084.

Marquez, M. G., Cabrera, I., Serrano, D. J., and Sterin-Speziale, N. (2002). Cell proliferation and morphometric changes in the rat kidney during postnatal development. *Anat. Embryol.* 205, 431–440.

McMillen, I. C., and Robinson, J. S. (2005). Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol. Rev.* 85, 571–633.

McStay, B., and Grummt, I. (2008). The epigenetics of rRNA genes: from molecular to chromosome biology. *Annu. Rev. Cell Dev. Biol.* 24, 131–157.

Reiter, A. K., Anthony, T. G., Anthony, J. C., Jefferson, L. S., and Kimball, S. R. (2004). The mTOR signaling pathway mediates control of ribosomal protein mRNA translation in rat liver. *Int. J. Biochem. Cell Biol.* 36, 2169–2179.

Rupprecht, H. D., Akagi, Y., Keil, A., and Hofer, G. (2000). Nitric oxide inhibits growth of glomerular mesangial cells: role of the transcription factor EGR-1. *Kidney Int.* 57, 70–82.

Sanij, E., and Hannan, R. D. (2009). The role of UBF in regulating the structure and dynamics of transcriptionally active rDNA chromatin. *Epigenetics* 4, 374–382.

Singh, R. J., Hogg, N., Joseph, J., Konorev, E., and Kalyanaraman, B. (1999). The peroxynitrite generator, SIN-1, becomes a nitric oxide donor in the presence of electron acceptors. *Arch. Biochem. Biophys.* 361, 331–339.

Smallegange, C., Hale, T. M., Bushfield, T. L., and Adams, M. A. (2004). Persistent lowering of pressure by transplanting kidneys from adult spontaneously hypertensive rats treated with brief antihypertensive therapy. *Hypertension* 44, 89–94.

Tanemura, M., Saga, A., Kawamoto, K., Machida, T., Deguchi, T., Nishida, T., Sawa, Y., Doki, Y., Mori, M., and Ito, T. (2009). Rapamycin induces autophagy in islets: relevance in islet transplantation. *Transplant. Proc.* 41, 334–338.

Van Dokkum, R. P., Jacob, H. J., and Provoost, A. P. (1998). Genetic differences define severity of renal damage after L-NAME-induced hypertension in rats. *J. Am. Soc. Nephrol.* 9, 363–371.

Van Faassen, E. E., Koeners, M. P., Joles, J. A., and Vanin, A. F. (2008). Detection of basal NO production in rat tissues using iron-dithiocarbamate complexes. *Nitric Oxide* 18, 279–286.

Wilcox, C. S. (2005). Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R913–R935.

Xie, M., Kobayashi, I., Kiyoshima, T., Nagata, K., Ookuma, Y., Fujiwara, H., and Sakai, H. (2009). In situ expression of ribosomal protein L21 in developing tooth germ of the mouse lower first molar. *J. Mol. Histol.* 40, 361–367.

Zhou, C., Zang, D., Jin, Y., Wu, H., Liu, Z., Du, J., and Zhang, J. (2011). Mutation in ribosomal protein L21 underlies hereditary hypotrichosis simplex. *Hum. Mutat.* 32, 710–714.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 19 March 2011; accepted: 02 August 2011; published online: 29 August 2011.

Citation: Wesseling S, Essers PB, Koeners MP, Pereboom TC, Braam B, van Faassen EE, MacInnes AW and Joles JA (2011) Perinatal exogenous nitric oxide in fawn-hooded hypertensive rats reduces renal ribosomal biogenesis in early life. Front. Gene. **2**:52. *doi: 10.3389/fgene.2011.00052*

This article was submitted to Frontiers in Epigenomics, a specialty of Frontiers in Genetics.

APPENDIX

METHODOLOGY MICROARRAY

After the hybridization of the samples to Illumina BeadChips RatRef-12 the raw data set containing all probes of each BeadArray was received. Each sample consisted of approximately 1 million beads, interrogating about 23K unique probes encoding genes ($n = 22523$), controls, housekeeping genes, and negative controls ($n = 825$). Each gene is probed with at least 30 beads. Before averaging the intensities of beads per probe, the outliers were removed, using the same approach to removing outliers as in BeadStudio. The removal of outliers was based on median $\pm 3\times$ median absolute deviation (MAD) and all beads with intensities outside the area of median $\pm 3\times$ MAD were removed. Outliers were frequent for each probe. In our hands close to 90% of the probes had outliers. Note that the outliers in "empty" beads, the negative controls (NC), were not removed, as they were considered to be background noise of the beads themselves. After removal of outliers (average 3 beads) the number of beads per probe averaged 39 beads.

After the test, the intensities of beads per probe are averaged for further data processing. Before the normalization procedure, the significance of a call from a gene was detected by applying a detection score that is dependent on the distribution of the intensities and the average intensity of the gene and NC (regardless of the number of NC). In this framework we performed the appropriate Student's *t*-test between the beads of a gene and the average intensity of all NC on the same array including testing (in)equality between two population variances in order to enhance reliability of the *t*-test. All probes with a significant call above the negative background were considered biologically active in the respective sample.

The software (T4Illumina) was written to process the raw data by averaging the intensities and determining the call of a gene, including determining the (un)equality of variance and many more. This software is available at request.

The significance of the presence of a gene in a group of samples (**Figure A1**) is outside the scope of the present study and will not be handled in this document. Note that all genes presented in the present study are significantly biologically active.

The averaged intensities, after removal of outliers, of the probes from all BeadArrays are Log_2-transformed and quantile normalized. Next the arrays were grouped accordingly and the average intensity per group was calculated. Finally the significance of the differences in intensities between the groups was calculated using Cyber *t*-test. Cyber-T is a statistics program designed specifically for microarray data (http://cybert.ics.uci.edu/). The normalization, averaging, and statistics procedures can all be performed by the software *FlexArray* (http://gqinnovationcenter.com/services/bioinformatics/flexarray/index.aspx?l=e).

NORTHERN BLOT

Total RNA was isolated from kidneys using Trizol using the procedure as recommended by the supplier (Invitrogen). Five microgram of RNA was run on a formaldehyde 1% agarose gel in MOPS buffer and DEPC-treated water for 4 h at 50 V. The gel was soaked in 50 mM NaOH for 15 min followed by 5 min in DEPC-treated water followed by 30 min in 10× SSC buffer. The RNA was transferred to a positively charged nylon membrane (GE Healthcare) overnight by capillary action and bound to the membrane by UV-crosslinking at 120 mJ. The blots were hybridized with DNA probes overnight in ExpressHyb Hybridization Buffer (Clontech) at 65°C. ETS and ITS1 DNA probes were made using the following primers: ETS FW: 5′-GTCTCGGTACGGGTGTGTC-3′ and REV 5′-TTTTTCCCCTTCCTCCTTTC-3′; ITS1 FW 5′-GGCCTGTGTGAGTGTTCCTC-3′ and REV 5′-TCAAGGGAAGAGCGAGAAAA-3′. Probes were labeled with ^{32}P-αCTP (Perkin-Elmer) using a random primer DNA labeling system (Invitrogen). Following hybridization, blots were washed twice for 30 min with 0.1% SDS/0.2× SSC at 65°C. Blots were exposed on storage phosphorimaging screens (Molecular Dynamics) overnight and scanned using a Typhoon Scanner (GE Healthcare).

Table A1 | Collection of the top 20 strongest up- and down-regulated genes at all ages in each gender.

Entrez gene ID	Symbol	Definition	Gene expression		
			2 Days	2 Weeks	Adult
A: TOP 40 OF MALE FHH OF 2 DAYS OLD (20 INDUCED AND 20 REDUCED)					
366411	RGD1560729	Ribosomal protein S24	0.820	−0.217	−0.354
500817	RGD1563124	40S ribosomal protein S20	0.723	−0.317	−0.409
287029	RGD1562055	Ribosomal protein L31	0.709	−0.399	−0.642
501876	RGD1563431	Large subunit ribosomal protein L36a	0.680	−0.424	−0.383
499560	LOC499560	LRRG00135	0.657	0.049	0.185
289384	RGD1560186	Ribosomal protein L37	0.632	−0.192	−0.844
500451	RGD1562259	40S ribosomal protein S20	0.534	−0.114	−0.452
362896	Stat6	Signal transducer and activator of transcription 6 (predicted), transcript variant 1	0.526	−0.221	−0.058
501562	LOC501562	ORF2 consensus sequence encoding endonuclease and reverse transcriptase minus RNaseH	0.514	0.009	0.181
171069	Usmg5	Upregulated during skeletal muscle growth 5	0.508	−0.372	−0.651
367923	LOC367923	60S ribosomal protein L23a	0.503	−0.394	−0.403
362894	RGD1310066	mKIAA1002 protein	0.493	0.214	0.146
314434	RGD1559566	60S ribosomal protein L9	0.488	−0.436	−0.369
291075	Peci	Peroxisomal delta3, delta2-enoyl-coenzyme A isomerase	0.487	−0.242	0.313
680294	LOC680294	Ribosomal protein L19	0.485	−0.312	−0.510
315338	Hoxc10	Homeo box C10	0.480	−0.137	−0.223
500438	LOC500438	ORF2 consensus sequence encoding endonuclease and reverse transcriptase minus RNaseH	0.478	−0.083	0.026
500923	RGD1565798	Tumor protein, translationally controlled 1	0.478	0.030	−0.106
301434	Clk1	CDC-like kinase 1	0.474	0.081	0.282
502887	RGD1563551	Ribosomal protein L31	0.470	−0.397	−0.523
287422	Per1	Period homolog 1 (*Drosophila*)	−0.401	0.432	0.824
304063	Ets2 mapped	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	−0.415	0.104	−0.062
25458	Gss	Glutathione synthetase	−0.415	0.134	0.320
116546	Ralb	v-ral simian leukemia viral oncogene homolog B (ras related)	−0.417	−0.007	0.090
25122	Scnn1a	Sodium channel, non-voltage-gated, type I, alpha	−0.417	0.218	0.337
64017	Enpep	Glutamyl aminopeptidase	−0.422	0.102	0.787
29144	Canx	Calnexin	−0.431	0.298	0.689
24244	Calm3	Calmodulin 3	−0.443	0.037	0.067
500300	LOC500300	Hypothetical protein MGC6835	−0.446	0.090	1.036
290551	Chdh	Choline dehydrogenase	−0.456	0.256	−0.028
291081	RGD1309427	Tubulin, beta-like	−0.462	0.018	−0.344
29517	Sgk	Serum/glucocorticoid regulated kinase	−0.466	0.661	0.862
294313	Mtch1	Mitochondrial carrier homolog 1 (*C. elegans*)	−0.503	−0.072	−0.138
498290	RGD1561353	OTTHUMP00000044730	−0.506	0.040	0.267
25365	Actg2	Actin, gamma 2, smooth muscle, enteric	−0.560	0.018	0.079
117273	Rhoa	ras homolog gene family, member A	−0.572	−0.047	−0.171
501211	LOC501211	LOC501211	−0.608	0.074	−0.596
287571	Taf15	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor	−0.712	0.255	0.049
25279	Cyp24a1	Cytochrome P450, subfamily 24	−0.849	0.342	−0.634
25633	Dnase1	Deoxyribonuclease I	−0.861	0.298	0.233
B: TOP 40 OF MALE FHH OF 2 WEEKS OLD (20 INDUCED AND 20 REDUCED)					
498789	RGD1564865	20-alpha-hydroxysteroid dehydrogenase	0.056	0.685	−0.110
29517	Sgk	Serum/glucocorticoid regulated kinase	−0.466	0.661	0.862
81676	Hnmt	Histamine *N*-methyltransferase	0.028	0.625	1.057
25526	Ptgds	Prostaglandin D2 synthase (brain)	−0.089	0.586	−0.559
362188	RGD1311652	MGC52019 protein	0.149	0.584	0.291

(Continued)

Table A1 | (Continued)

Entrez gene ID	Symbol	Definition	Gene expression		
			2 Days	2Weeks	Adult
83810	Trpv1	Transient receptor potential cation channel, subfamily V, member 1	0.022	0.583	−0.373
246234	Slc34a3	Solute carrier family 34 (sodium phosphate), member 3	−0.149	0.559	−0.108
171072	Sult1c2	Sulfotransferase family, cytosolic, 1C, member 2	0.326	0.513	0.662
57300	Aadac	Arylacetamide deacetylase (esterase)	−0.051	0.508	0.040
25256	Fmo1	Flavin containing monooxygenase 1	0.092	0.506	0.044
363227	Obfc2a	Oligonucleotide/oligosaccharide-binding fold containing 2A	0.145	0.452	−0.222
79428	Luzp1	Leucine zipper protein 1	−0.086	0.443	0.119
287422	Per1	Period homolog 1	−0.401	0.432	0.824
362216	Mrps26	Mitochondrial ribosomal protein S26	−0.154	0.427	−0.330
311826	Rexo4	REX4, RNA exonuclease 4 homolog (*S. cerevisiae*)	−0.083	0.421	0.125
299944	RGD1304605	Hypothetical LOC299944	0.126	0.406	0.477
303926	Igsf11	Immunoglobulin superfamily, member 11	0.187	0.403	0.059
293343	MGC94288	4632419K20Rik protein	−0.167	0.401	0.274
24267	Comt	Catechol-*O*-methyltransferase	−0.156	0.397	−0.180
81641	Anpep	Alanyl (membrane) aminopeptidase	0.007	0.395	0.083
364108	RGD1562073	Ribosomal protein S17	0.418	−0.517	−0.490
29258	Rps7	Ribosomal protein S7	−0.076	−0.520	−0.650
60414	Rhd	Rh blood group, D antigen	0.076	−0.521	0.066
494500	Yc2	Glutathione *S*-transferase Yc2 subunit	0.390	−0.550	−0.078
25748	Alas2	Aminolevulinic acid synthase 2, erythroid	0.021	−0.569	0.078
24615	S100a4	S100 calcium-binding protein A4	−0.090	−0.573	−0.730
300024	LOC300024	Ly6-B antigen gene	−0.202	−0.575	−0.118
500988	RGD1564560	RCK	−0.111	−0.623	0.905
500019	RGD1564980	60S ribosomal protein L29 (P23)	−0.300	−0.666	−0.375
25250	Cox8h	Cytochrome c oxidase subunit VIII-H (heart/muscle)	−0.037	−0.689	0.061
54249	Cfd	Complement factor D (adipsin)	0.001	−0.702	−0.297
361619	MGC72973	Beta-glo	−0.232	−0.747	−0.638
291541	Cidea	Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	−0.234	−0.797	0.132
25357	Thrsp	Thyroid hormone responsive protein	0.132	−0.829	0.021
293522	Eraf	Erythroid associated factor	−0.189	−0.834	−0.170
54232	Ca3	Carbonic anhydrase 3	−0.249	−0.867	−0.830
25475	Lgals5	Lectin, galactose binding, soluble 5	−0.218	−0.918	−0.225
81639	Alox15	Arachidonate 15-lipoxygenase	−0.010	−1.005	0.035
79451	Fabp4	Fatty acid binding protein 4, adipocyte	−0.189	−1.067	−0.061
24860	Ucp1	Uncoupling protein 1 (mitochondrial, proton carrier)	−0.083	−1.330	0.041
C:TOP 40 OF ADULT MALE FHH (20 INDUCED AND 20 REDUCED)					
117556	Sv2b	Synaptic vesicle glycoprotein 2b	0.046	−0.471	1.766
313089	Slc7a13	Solute carrier family 7 (cationic amino acid transporter, y + system) member 13	0.150	0.029	1.419
498211	RGD1560523	*S*-adenosylmethionine synthetase gamma form (methionine adenosyltransferase)	−0.037	0.124	1.251
50672	Ednrb	Endothelin receptor type B	0.223	−0.396	1.238
117560	Klf9	Kruppel-like factor 9	−0.138	−0.059	1.220
501085	LOC501085	Sulfotransferase K1	−0.087	0.290	1.181
497772	LOC497772	Hypothetical gene supported by NM_030827	0.183	0.259	1.178
501610	LOC501610	Gag-Pol polyprotein	0.023	0.099	1.123
316256	Tnfrsf21	Tumor necrosis factor receptor superfamily, member 21	−0.013	0.252	1.112
367201	RGD1562392	Sulfotransferase K1 (rSULT1C2)	0.007	0.136	1.109

(Continued)

Table A1 | (Continued)

Entrez gene ID	Symbol	Definition	Gene expression		
			2 Days	2 Weeks	Adult
500621	LOC500621	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-coenzyme A thiolase	−0.238	0.034	1.104
313729	Errfi1	ERBB receptor feedback inhibitor 1	0.002	0.084	1.091
310395	LOC310395	Nocturnin (CCR4 protein homolog)	−0.232	0.105	1.074
361272	Dhtkd1	Dehydrogenase E1 and transketolase domain-containing 1	0.066	0.126	1.060
81676	Hnmt	Histamine *N*-methyltransferase	0.028	0.625	1.057
364773	LOC364773	Liver regeneration-related protein LRRG07	−0.370	−0.192	1.043
500300	LOC500300	Hypothetical protein MGC6835	−0.446	0.090	1.036
24861	Ugt1a1	UDP glycosyltransferase 1 family, polypeptide A1	0.246	0.091	1.027
171361	Eef1a1	Eukaryotic translation elongation factor 1 alpha 1	0.101	−0.260	1.007
301276	RGD1564912	Mut protein	−0.090	0.083	0.999
500180	LOC500180	IG kappa-chain V–V region K2 precursor	0.002	0.024	−0.796
502302	LOC502302	40S ribosomal protein S19	0.002	−0.034	−0.796
29191	Tac2	Tachykinin 2	−0.008	0.014	−0.805
362506	Ccl19	Chemokine (C–C motif) ligand 19	0.043	−0.170	−0.812
363074	RGD1309779	ENSANGP00000021391	−0.061	−0.042	−0.815
499300	Ptprcap	Protein tyrosine phosphatase, receptor type, C polypeptide-associated protein	0.009	−0.007	−0.817
361537	Tyrobp	Tyro protein tyrosine kinase binding protein	0.030	−0.129	−0.824
54232	Ca3	Carbonic anhydrase 3	−0.249	−0.867	−0.830
299269	isg12(b)	Putative ISG12(b) protein	−0.072	−0.169	−0.833
292654	RGD1564549	Hypothetical protein FLJ20512	−0.173	0.064	−0.840
289384	RGD1560186	Ribosomal protein L37	0.632	−0.192	−0.844
360918	Pf4	Platelet factor 4	0.106	−0.299	−0.845
498744	RGD1561310	Ribosomal protein L37	0.151	−0.454	−0.851
367077	RGD1562835	40S ribosomal protein S26	0.170	−0.348	−0.852
24440	Hbb	Hemoglobin beta chain complex	−0.237	−0.127	−0.857
25632	Hba-a2	Hemoglobin alpha, adult chain 2	−0.105	−0.016	−0.860
299848	RGD1565117	40S ribosomal protein S26	0.029	−0.217	−0.882
499131	LOC499131	NADH:ubiquinone oxidoreductase B15 subunit	−0.075	−0.156	−0.909
140608	Atp5i	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit e	−0.139	−0.008	−0.971
692000	LOC692000	Dolichol-phosphate mannosyltransferase subunit 3	−0.190	0.095	−1.014
D:TOP 40 OF FEMALE FHH OF 2 DAYS OLD (20 INDUCED AND 20 REDUCED)					
499638	LOC499638	LRRGT00057	1.238	0.031	0.087
361117	LOC361117	LRRGT00149	1.206	−0.117	−0.136
500285	LOC500285	LRRGT00176	1.198	−0.039	0.029
498105	LOC498105	LRRGT00176	1.186	0.049	−0.044
498076	LOC498076	RIKEN cDNA 2410116I05	1.124	0.041	0.103
500586	LOC500586	LRRGT00057	1.110	−0.046	−0.062
498245	LOC498245	LRRGT00176	1.105	0.037	−0.039
313278	RGD1561090	Protein tyrosine phosphatase, receptor type, D	0.978	−0.065	0.104
362803	LOC362803	Putative RNA binding protein 1	0.888	0.045	−0.109
500380	LOC500380	LRRGT00008	0.885	0.117	−0.258
498623	LOC498623	LRRGT00176	0.844	0.044	−0.102
501156	LOC501156	LRRGT00176	0.793	0.074	−0.293
500988	RGD1564560	RCK	0.791	−0.432	−0.473
498979	LOC498979	LRRGT00194	0.763	0.035	−0.147
25365	Actg2	Actin, gamma 2, smooth muscle, enteric	0.752	−0.435	−0.126
309902	Cxxc6	CXXC finger 6	0.738	0.107	−0.008

(Continued)

Table A1 | (Continued)

Entrez gene ID	Symbol	Definition	Gene expression		
			2 Days	2 Weeks	Adult
500343	LOC500343	LRRGT00176	0.734	0.110	−0.138
315604	Ddx6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	0.597	−0.305	−0.006
498217	LOC498217	LRRG00135	0.595	0.063	−0.015
308831	Odz4	Odd Oz/ten-m homolog 4 (*Drosophila*)	0.595	−0.052	0.140
24832	Thy1	Thymus cell antigen 1, theta	−0.336	−0.161	0.319
366942	RGD1565809	Ribosomal protein L15	−0.345	−0.070	0.010
362850	Angptl4	Angiopoietin-like 4	−0.352	−0.311	−0.221
25475	Lgals5	Lectin, galactose binding, soluble 5	−0.356	−0.162	0.207
64473	Bpnt1	Bisphosphate 3′-nucleotidase 1	−0.359	−0.134	0.009
338475	Nrep	Neuronal regeneration-related protein	−0.370	−0.154	0.300
294282	Rps18	Ribosomal protein S18	−0.377	−0.016	0.021
140582	Ddit4l	DNA-damage-inducible transcript 4-like	−0.389	−0.186	−0.055
360941	LOC360941	ORF7	−0.420	0.033	0.076
117273	Rhoa	ras homolog gene family, member A	−0.423	−0.239	−0.189
29222	Ptma	Prothymosin alpha	−0.426	−0.167	0.207
315745	Fem1b	Feminization 1 homolog b (*C. elegans*)	−0.448	−0.074	0.167
124440	Rpl 41	Ribosomal protein L41	−0.455	0.034	0.067
314397	Rin3	Ras and Rab interactor 3	−0.497	−0.156	−0.188
89813	Pdk4	Pyruvate dehydrogenase kinase, isoenzyme 4	−0.498	−0.357	0.533
287571	Taf15	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor	−0.503	−0.197	0.113
308568	RGD1309326	RIKEN cDNA 2410002F23	−0.539	−0.154	−0.089
246771	Slc25a25	Solute carrier family 25 (mitochondrial carrier, phosphate carrier) member 25	−0.575	−0.393	−0.111
25633	Dnase1	Deoxyribonuclease I	−0.588	−0.250	−0.274
360739	Ranbp1	RAN binding protein 1	−0.597	−0.306	0.453
E: TOP 40 OF FEMALE FHH OF 2 WEEKS OLD (20 INDUCED AND 20 REDUCED)					
24567	Mt1a	Metallothionein 1a	−0.154	0.424	−0.075
64522	Slc5a2	Solute carrier family 5 (sodium/glucose cotransporter) member 2	−0.110	0.419	−0.362
362334	Tmem140	Transmembrane protein 140	0.205	0.359	−0.333
313430	Fam151a	Family with sequence similarity 151, member A	−0.061	0.325	−0.358
360895	Rrp15	Ribosomal RNA processing 15 homolog (*S. cerevisiae*)	0.067	0.324	−0.001
362334	Tmem140	Transmembrane protein 140	0.207	0.284	0.051
289419	Nuak2	NUAK family, SNF1-like kinase, 2	0.037	0.278	−0.257
307098	Net1	Neuroepithelial cell transforming gene 1	−0.058	0.278	−0.049
24484	Igfbp3	Insulin-like growth factor binding protein 3	0.267	0.270	−0.206
303002	RGD1308952	mKIAA0665 protein	0.052	0.263	−0.163
85385	Shc1	src homology 2 domain-containing transforming protein C1	0.094	0.261	0.123
300659	Rnf26	Ring finger protein 26	−0.166	0.253	0.085
404871	Olr557	Olfactory receptor 557 (predicted)	0.023	0.251	−0.213
360228	LOC360228	WDNM1 homolog	0.257	0.248	−0.243
116565	Lrpap1	Low density lipoprotein receptor-related protein associated protein 1	0.036	0.248	−0.180
25619	Plau	Plasminogen activator, urokinase	0.225	0.242	−0.071
361367	Amfr	Autocrine motility factor receptor	0.043	0.239	−0.135
171179	Keg1	Kidney expressed gene 1	0.014	0.238	−0.115
310810	Sdfr2	Stromal cell derived factor receptor 2	0.211	0.232	0.027
299027	Eif2s3x	Eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked	−0.021	0.228	0.096

(Continued)

Table A1 | (Continued)

Entrez gene ID	Symbol	Definition	Gene expression		
			2 Days	2 Weeks	Adult
24856	Ttr	Transthyretin	0.115	−0.331	0.062
309809	RGD1310495	KIAA1919 protein	−0.028	−0.336	−0.184
311676	Tmepai	Transmembrane, prostate androgen induced RNA	0.033	−0.340	0.199
89813	Pdk4	Pyruvate dehydrogenase kinase, isoenzyme 4	−0.498	−0.357	0.533
246781	Ptpn7	Protein tyrosine phosphatase, non-receptor type 7	−0.008	−0.364	0.101
501194	LOC501194	Hypothetical protein D330021B20	0.249	−0.365	−0.110
291555	Atp8b1	ATPase, class I, type 8B, member 1	0.091	−0.392	−0.097
246771	Slc25a25	Solute carrier family 25 (mitochondrial carrier, phosphate carrier) member 25	−0.575	−0.393	−0.111
303132	Aff4	AF4/FMR2 family, member 4	−0.014	−0.399	0.057
295660	RGD1564400	Eukaryotic translation initiation factor 5	−0.057	−0.407	−0.231
361296	Rnf125	Ring finger protein 125	0.270	−0.407	−0.134
64353	Pdlim5	PDZ and LIM domain 5	−0.063	−0.411	−0.297
25365	Actg2	Actin, gamma 2, smooth muscle, enteric	0.752	−0.435	−0.126
498859	LOC498859	LOC498859	−0.002	−0.449	−0.111
315190	Upk3a	Uroplakin 3A	0.317	−0.459	−0.297
501530	LOC501530	LOC501530	0.442	−0.487	0.438
290704	LOC290704	Palladin	0.371	−0.516	−0.324
94197	Rab14	RAB14, member RAS oncogene family	0.122	−0.565	−0.194
25373	Ahsg	Alpha-2-HS-glycoprotein	0.031	−0.711	0.092
498989	LOC498989	Ab2-143	0.646	−0.728	−0.269
F: TOP 40 OF ADULT FEMALE FHH (20 INDUCED AND 20 REDUCED)					
361734	Ms4a4a	Membrane-spanning 4-domains, subfamily A, member 4	−0.013	0.006	1.155
117518	Ccl17	Chemokine (C–C motif) ligand 17	−0.091	−0.034	1.052
406161	C4-2	Complement component 4, gene 2	0.048	0.197	0.885
304349	RGD1559588	Cell surface receptor FDFACT	0.010	−0.105	0.877
500172	LOC500172	Immunoglobulin kappa-chain	0.067	−0.083	0.871
29517	Sgk	Serum/glucocorticoid regulated kinase	0.142	0.027	0.861
498982	RGD1560020	Myb proto-oncogene protein	0.143	0.078	0.801
298906	Pqlc3	PQ loop repeat containing 3	−0.044	−0.012	0.795
116676	Aldh1a2	Aldehyde dehydrogenase family 1, subfamily A2	0.216	0.134	0.790
64195	Mgl1	Macrophage galactose *N*-acetyl-galactosamine specific lectin 1	−0.077	−0.030	0.769
29168	Ubd	Ubiquitin D	−0.115	0.087	0.754
313438	Dock11	Dedicator of cytokinesis 11	0.024	−0.113	0.727
681872	LOC681872	Interleukin 19	0.038	−0.033	0.726
246143	Nradd	Neurotrophin receptor associated death domain	−0.032	0.041	0.726
84032	Col3a1	Collagen, type III, alpha 1	0.057	−0.029	0.716
24366	Fgb	Fibrinogen, B beta polypeptide	0.022	0.195	0.709
502902	RGD1565140	Clecsf12 protein	−0.066	−0.009	0.692
501405	LOC501405	GTPase activating protein testicular GAP1	−0.015	−0.013	0.691
24251	Cd53	CD53 antigen	0.035	−0.049	0.681
298975	Scin	Scinderin	0.095	0.095	0.679
309527	Ch25h	Cholesterol 25−hydroxylase	0.055	−0.014	−0.713
286926	Tfpi2	Tissue factor pathway inhibitor 2	0.019	−0.005	−0.725
293538	RGD1565366	Hmx2 protein	0.256	−0.049	−0.737
24517	Junb	Jun-B oncogene	0.116	−0.039	−0.745
24451	Hmox1	Heme oxygenase (decycling) 1	0.155	0.248	−0.771
292868	Klks3	Kallikrein, submaxillary gland S3	−0.113	−0.039	−0.771

(Continued)

Table A1 | (Continued)

Entrez gene ID	Symbol	Definition	Gene expression		
			2 Days	2 Weeks	Adult
363483	Dmrtc1c	DMRT-like family C1c	0.096	0.076	−0.808
24498	Il6	Interleukin 6	0.055	−0.099	−0.826
78965	Csf1	Colony stimulating factor 1 (macrophage)	0.131	0.064	−0.837
24296	Cyp1a1	Cytochrome P450, family 1, subfamily a, polypeptide 1	0.102	−0.062	−0.857
24508	Irf1	Interferon regulatory factor 1	−0.214	0.033	−0.894
25542	Ccl3	Chemokine (C–C motif) ligand 3	0.102	0.064	−0.923
25464	Icam1	Intercellular adhesion molecule 1	−0.177	−0.058	−0.965
25361	Vcam1	Vascular cell adhesion molecule 1	0.133	−0.038	−1.099
362993	Rnd1	Rho family GTPase 1	0.054	−0.031	−1.161
24770	Ccl2	Chemokine (C–C motif) ligand 2	−0.007	−0.083	−1.232
116637	Ccl4	Chemokine (C–C motif) ligand 4	0.067	0.029	−1.322
114105	Cxcl2	Chemokine (C–X–C motif) ligand 2	−0.091	0.069	−1.504
25651	Selp	Selectin, platelet	−0.078	−0.032	−1.531
81503	Cxcl1	Chemokine (C–X–C motif) ligand 1	−0.075	−0.010	−2.156

The difference in gene expression between molsidomine-treated and control FHH was noted as Log_2(FM/FC), where FM and FC are the normalized intensities. Red and blue numbers indicate gene expression differentially induced and reduced by molsidomine, respectively.

Table A2 | Ribosomal protein genes in kidneys of FHH males differentially expressed by molsidomine (FM, 2 days and 2 weeks) or after perinatal molsidomine (FM, adult) vs. controls (FC).

Entrez gene ID	Ribosome	Chromosome	Gene expression		
			2 Days	2Weeks	Adult
A: 2 DAYS					
366411	S24	5	0.820	−0.217	−0.354
500817	S20	7	0.723	−0.317	−0.409
287029	L31	10	0.709	−0.399	−0.642
501876	L36a	13	0.680	−0.424	−0.383
289384	L37	13	0.632	−0.192	−0.844
500451	S20	5	0.534	−0.114	−0.452
367923	L23a	3	0.503	−0.394	−0.403
314434	L9	X	0.488	−0.436	−0.369
680294	L19	–	0.485	−0.312	−0.510
502887	L31	4	0.470	−0.397	−0.523
500322	S7	4	0.462	−0.192	−0.411
500510	L21	5	0.437	−0.044	−0.432
498078	L7a	11	0.427	−0.331	−0.354
364108	S17	14	0.418	−0.517	−0.490
294700	L21	2	0.409	−0.434	−0.293
287417	L26	10	0.399	−0.228	−0.253
295439	L21	2	0.353	−0.334	−0.175
314733	S19	7	0.329	−0.330	−0.638
289715	L37	14	0.314	−0.454	−0.554
364059	L34	13	0.299	−0.352	−0.662
365800	L36a	4	0.280	−0.014	−0.131
498828	L10	18	0.276	−0.241	−0.306
364825	L36	18	−0.199	0.117	0.126
B: 2 WEEKS					
362216	S26	3	−0.154	0.427	−0.330
308719	L27a	1	−0.068	0.243	0.044
503211	L21	8	−0.051	0.161	0.111
367030	SA	8	0.106	0.155	−0.061
287417	L26	10	0.399	−0.228	−0.253
298126	L31	5	0.018	−0.261	−0.566
313283	S12	5	0.045	−0.279	−0.548
287996	L21	11	−0.022	−0.281	0.002
296870	L34	X	−0.008	−0.295	−0.283
364828	L29	18	0.132	−0.297	−0.506
500559	S20	5	0.132	−0.302	−0.188
498360	S23	14	0.033	−0.304	−0.164
680294	L19	–	0.485	−0.312	−0.510
500817	S20	7	0.723	−0.317	−0.409
367102	S9	8	−0.068	−0.325	−0.458
299935	L31	7	0.001	−0.328	−0.461
295439	L21	2	0.353	−0.334	−0.175
364059	L34	13	0.299	−0.352	−0.662
300731	L21	8	0.061	−0.352	−0.638
499133	L27a	1	0.119	−0.363	−0.477
687298	S19	10	0.059	−0.365	−0.541
366656	L10a	6	0.188	−0.370	−0.260
502854	L31	4	0.166	−0.376	−0.624
297755	L7	5	0.252	−0.386	−0.308
294781	L21	2	0.397	−0.388	−0.627

(Continued)

Table A2 | (Continued)

Entrez gene ID	Ribosome	Chromosome	Gene expression		
			2 Days	2Weeks	Adult
65139	S12	1	0.225	−0.393	−0.765
367923	L23a	3	0.503	−0.394	−0.403
287029	L31	10	0.709	−0.399	−0.642
501604	L7a	X	0.287	−0.402	−0.218
81772	S9	1	0.252	−0.406	−0.620
289401	L31	13	0.243	−0.424	−0.735
501876	L36a	13	0.680	−0.424	−0.383
299740	L31	7	−0.024	−0.426	−0.539
294700	L21	2	0.409	−0.434	−0.293
314434	L9	X	0.488	−0.436	−0.369
300278	S9	X	0.414	−0.436	−0.590
289715	L37	14	0.314	−0.454	−0.554
498744	L37	17	0.151	−0.454	−0.851
501058	S23	8	0.054	−0.467	−0.297
363418	L17	12	0.068	−0.469	−0.316
81770	L37	2	−0.136	−0.472	−0.618
500817	S20	7	0.080	−0.484	−0.347
363861	L29	12	0.212	−0.490	−0.467
366887	L31	7	0.379	−0.497	−0.497
686564	L23a	14	0.520	−0.497	−0.446
364139	L21	14	0.150	−0.497	−0.758
364108	S17	14	0.418	−0.517	−0.490
29258	S7	5	−0.076	−0.520	−0.650
500019	L29	4	−0.300	−0.666	−0.375
C: 36 WEEKS					
360710	L7a	11	0.034	0.157	0.218
365300	L7	1	−0.007	0.004	−0.213
294282	S18	20	−0.094	−0.010	−0.337
498837	S27a	18	0.196	−0.043	−0.342
315521	L32	8	0.339	0.010	−0.363
367250	L7a	9	0.021	0.060	−0.371
365560	L36a	20	0.030	−0.097	−0.384
500451	S20	5	0.534	−0.114	−0.452
367102	S9	8	−0.068	−0.325	−0.458
299935	L31	7	0.001	−0.328	−0.461
499133	L27a	1	0.119	−0.363	−0.477
500559	S20	5	0.220	−0.339	−0.490
309408	S12	1	−0.079	−0.134	−0.495
498954	S16	19	0.099	−0.171	−0.499
302497	L10a	X	0.334	−0.262	−0.499
25347	L39	X	0.014	−0.145	−0.512
302898	L1	10	0.034	0.076	−0.515
302528	L37a	X	0.006	−0.041	−0.520
502887	L31	4	0.470	−0.397	−0.523
499752	L7a	3	0.115	−0.093	−0.527
124323	S23	2	0.163	−0.260	−0.529
293754	L16	1	−0.278	0.116	−0.548
313283	S12	5	0.045	−0.279	−0.548
289715	L37	14	0.314	−0.454	−0.554
315642	L27a	8	−0.162	−0.126	−0.560
298495	L35a	5	0.296	−0.332	−0.565

(Continued)

Table A2 | (Continued)

Entrez gene ID	Ribosome	Chromosome	Gene expression		
			2 Days	2 Weeks	Adult
298126	L31	5	0.018	−0.261	−0.566
366689	L21	6	−0.128	−0.317	−0.575
81768	L22	5	0.002	−0.148	−0.580
300278	S9	X	0.414	−0.436	−0.590
292539	L17	1	0.237	−0.246	−0.592
498363	P2	14	0.481	−0.130	−0.597
299041	P1	X	0.041	−0.157	−0.601
57809	L35a	1	−0.112	−0.210	−0.614
81772	S9	1	0.252	−0.406	−0.620
502854	L31	4	0.166	−0.376	−0.624
294781	L21	2	0.397	−0.388	−0.627
314054	S10	6	−0.053	−0.170	−0.631
314733	S19	7	0.329	−0.330	−0.638
287029	L31	10	0.709	−0.399	−0.642
498555	P2	15	0.002	−0.072	−0.645
503110	S19	7	0.008	−0.047	−0.651
64360	L23	1	−0.247	0.029	−0.654
297459	S25	4	−0.247	0.160	−0.659
314248	S17	6	0.000	−0.064	−0.661
364059	L34	13	0.299	−0.352	−0.662
171061	L17	1	0.219	−0.170	−0.663
366485	–	5	−0.275	−0.376	−0.665
500714	L6	6	0.023	−0.218	−0.686
289401	L31	13	0.243	−0.424	−0.735
27139	S26	7	0.205	−0.240	−0.760
65139	S12	1	0.225	−0.393	−0.765
498523	L23a	15	−0.134	−0.253	−0.766
289932	S7	15	0.098	−0.295	−0.785
502302	S19	1	0.002	−0.034	−0.796
289384	L37	13	0.632	−0.192	−0.844
498744	L37	17	0.151	−0.454	−0.851
367077	S26	8	0.170	−0.348	−0.852
299848	S26	7	0.029	−0.217	−0.882

*All genes that are significantly differentially expressed by molsidomine are arranged per age [**(A)** is newborn males, i.e., 2 days, **(B)** is 2-week-old males, and **(C)** is adult males, i.e., 36 weeks]. The difference in gene expression between molsidomine-treated and control FHH was noted as Log_2(FM/FC), where FM and FC are the normalized intensities. Red and blue numbers indicate gene expression differentially induced and reduced by molsidomine, respectively. The symbols and the chromosomal locations are also shown. Ratios indicated in bold and red are increased differential expression and ratios indicated in bold and blue are decreased differential expression. Note that some genes in **(A)** may reappear in **(B,C)** and some genes in Table A2B may reappear in Table A2C.*

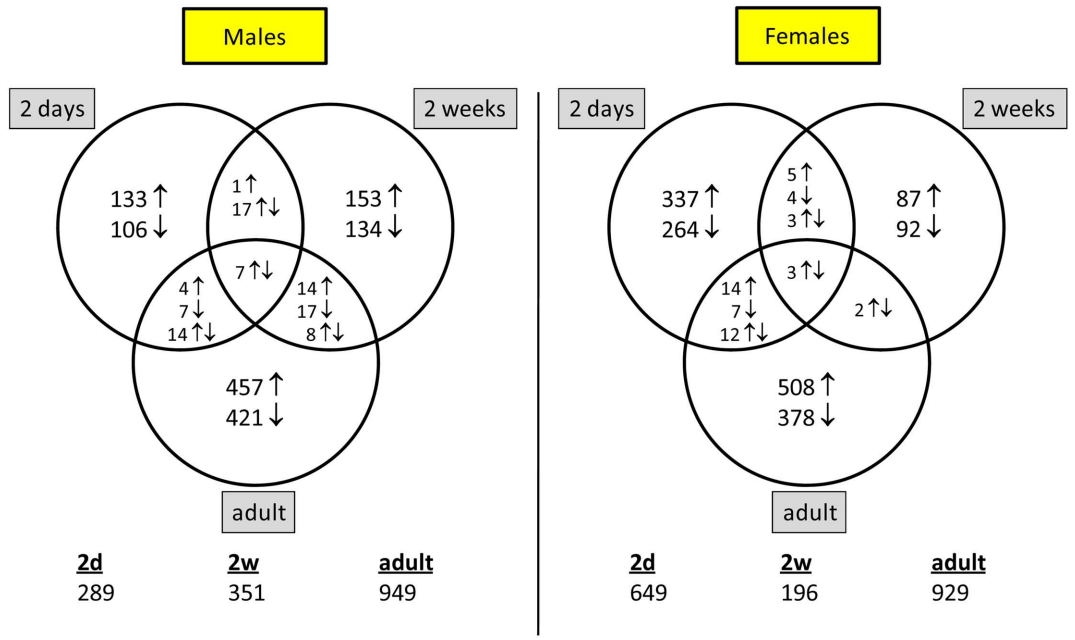


FIGURE A1 | Total number of genes differentially expressed by molsidomine at each age per gender. Hundreds of genes were significantly affected by molsidomine at each age in both genders. The distribution of the genes over all ages is shown in a Venn diagram. The up and down arrows indicate genes that are differentially induced or reduced by molsidomine vs. control, respectively. Below the diagram are the total numbers of genes that are differentially expressed by molsidomine vs. control.

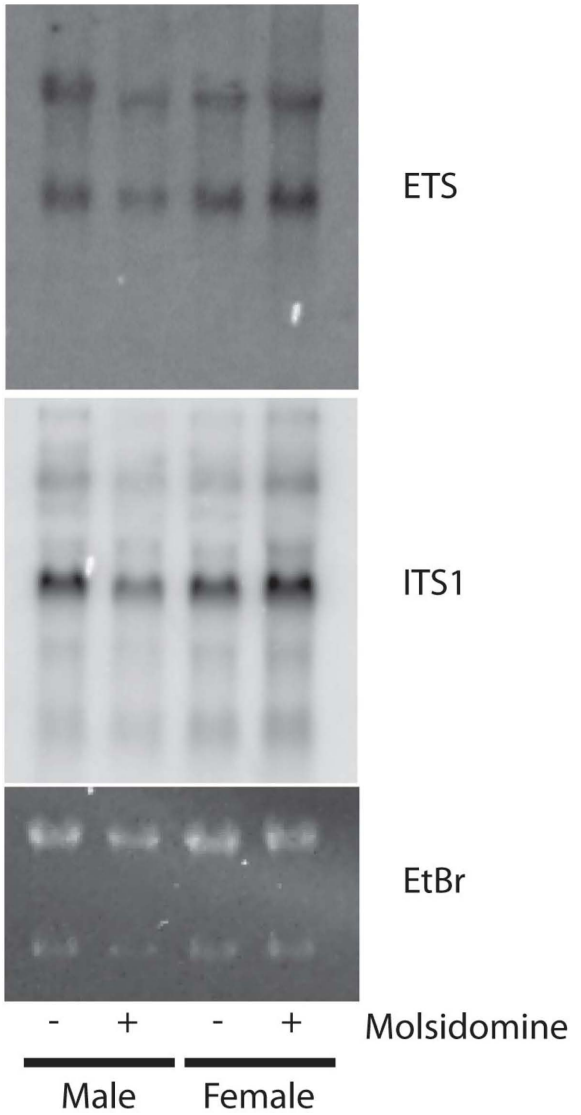


FIGURE A2 | Northern Blots. Northern blotting was performed on kidneys from 2 week FHH in order to determine rates of pre-rRNA processing. ETS: external transcribed spacer. ITS1: internal transcribed spacer 1. Ethidium bromide (EtBr) is shown as loading control. Note the slight decrease of molsidomine-treated males is due to loading (seen by EtBr) and not due to processing defects of pre-rRNA.