ABSTRACT

The time spanning between conception and the second year of life (first 1,000 days) is a period of human development during which the foundations for health in later life are laid. Kidneys are especially vulnerable during this perinatal period. Thus, intrauterine deficiency may predispose to decreased renal function in young adulthood, as well as to an unfavorable outcome in glomerulopathies and an elevated risk of end-stage renal diseases. Epidemiological studies have shown that experimental intrauterine growth restriction (IUGR) such as placental insufficiency increases the risk for adverse kidney outcomes in later life. Pro-inflammatory molecular signatures during early kidney development, susceptibility towards hypertension, and altered course of mesangioproliferative glomerulonephritis have been associated with experimental IUGR. On the other hand, nutritional intervention studies hint at renoprotective, anti-inflammatory, and anti-hypertensive effects of n-3 polyunsaturated fatty acids (PUFA). Therefore, the goal of this study was to investigate whether a postnatal time-limited diet intervention can ameliorate the susceptibility of IUGR-born rats towards kidney damage in later life. First, we tested the hypothesis that IUGR offspring have a higher risk for elevated blood pressure, vascular dysregulation, and an aggravated course of Thy1-nephritis. Second, we tested whether an early postnatal supplementation with n-3 PUFA and choline modifies cell membrane composition, improves blood pressure, and the severity of Thy1-nephritis.

In order to address these questions, IUGR was induced through experimental uteroplacental insufficiency by bilateral uterine artery ligation (LIG) or intrauterine stress (IUS) by sham operation of rat dams on gestational day (GD) 18. Offspring from unimpaired control dams (C) without surgery were compared to offspring after IUGR. On postnatal day (PND) 2, each foster litter was composed of six male and two female pups. From then on till PND39, rats were either fed a control diet (CONTR; 1:20 n-3/n-6 fatty acid ratio, 0.03 g docosahexaenoic acid (DHA), 1 g choline/kg food) or an intervention diet (N3PUFA; 1:1 n-3/n-6 fatty acid ratio, 5 g DHA and 5 g choline/kg food). On PND40, telemetry catheters were implanted and blood pressure and heart rate were continuously measured after recovery. On PND53, experimental glomerulonephritis was induced through Thy1.1 antibody injection in all experimental groups except group control-control (CC). For organ harvesting, rats were sacrificed on PND39 (females) and PND67 (males). Histology, proteomics, and lipidomics were performed to elucidate the molecular basis of the phenotypes induced by the different procedures and interventions longitudinally.

In both IUGR models and sexes, birth weights were significantly decreased and no significant catch-up growth was observed. On PND33, plasma parameters of males revealed differences in

triglycerides and total cholesterol in IUGR rats under diet intervention. Furthermore, the diet intervention significantly affected the phospholipid (PL) composition in kidney cortex tissue on PND39. PL containing arachidonic acid (AA, 20:4, n-6) were highly downregulated. For example, the amount of phosphatidylcholine (PC) 38:4 (20:4, 18:0) was reduced by about 75% in all diet intervention groups. In contrast, PL containing DHA (22:6, n-3) were elevated in those groups. For example, PC40:6, a composition of 22:6 and 18:0, was 2- to 3-fold increased. On PND67 and four weeks after diet intervention, we still demonstrated alterations in tissue PL content. Although there are no significant differences observed in PL containing AA anymore, the differences in PL containing DHA were still present in the diet intervention groups. Proteomics was performed in renal cortex tissue on PND39 to analyze further biological effects. By this approach, we identified 62 proteins, which were altered in LIG (LIG-CONTR vs. C-CONTR) and reversely altered by diet intervention (LIG-N3PUFA vs. LIG-CONTR). Sub-analysis revealed that LIG rats following the n-3 PUFA diet counter-regulated 11 proteins that could be assigned to the complement and coagulation cascades. Moreover, 13 proteins of the counter-regulated proteins were identified as core-matrisome and matrisome-associated. In early adulthood, LIG rats showed dysfunctional vascular tone regulation of renal interlobar arteries (RIA) including loss of myogenic tone and augmented endothelium-dependent relaxation. These findings in combination with mildly elevated blood pressure could contribute to susceptibility towards hypertensive glomerular damage. On PND67 and 14 days after Thy1-nephritis, the scores for glomerulosclerosis and tubulointerstitial (TI) lesions were not differently affected by IUGR. Kidney damage as indicated by mesangial cell transformation and proliferation was reduced in LIG offspring. In detail, α-SMA as a marker for the dedifferentiation of mesangial cells was increased in glomeruli after Thy1-nephritis (C-CONTR vs. CC-CONTR), but not in LIG-CONTR. Analyses of PDGFR-β signaling and Ki67 staining were in accordance with those findings. Interestingly, neither glomerular microaneurysms nor parietal epithelial cell involvement nor TI inflammation were present in LIG-CONTR, whereas some LIG rats that had received n-3 PUFA diet in early life were affected. In contrast, C-CONTR and IUS-CONTR rats had microaneurysms, whereas C and IUS rats that had received n-3 PUFA diet were protected. Thus, we were able to show an opposite diet effect on early life conditions. Pathway analyses of the glomerular proteome revealed that proteins regarding lipid metabolic processes, peroxisome, and branched-chain amino acid degradation were primarily altered in LIG-CONTR vs. C-CONTR. CYP4A12, which produces the AA metabolite 20-HETE was identified as a key podocyte-related enzyme in IUGR after Thy1-nephritis.

Beyond CYP4A12 and CBS, EHHADH, and EPHX2 were identified as potential targets for dietary interventions.

Taken together, the amount of AA and DHA in cell membrane PL are strongly dependent on the composition of the diet and thus, can modify the eicosanoid synthesis, by affecting inflammation and hemodynamics. During early adulthood (PND39), LIG offspring had altered proteins related to complement and coagulation cascades and matrix organization, which were counter-regulated by nutritional intervention with n-3 PUFA and choline. Furthermore, we demonstrated that experimental placental insufficiency (LIG) causes mild arterial hypertension and dysfunctional vascular tone regulation of RIA in the offspring. Contrary to expectations, we did not observe an aggravated course of Thy1-nephritis in LIG rats 14 days after Thy1-nephritis. Postnatal hyperalimentation after IUGR can lead to catch-up growth that could represent a “mismatch” that was not observed in IUGR. We speculate that the IUGR alone may not be responsible for the predisposition towards aggravated glomerulonephritis, rather the combination of both. Proteome analyses after the second hit of Thy1-nephritis indicated that proteins involved in lipid metabolic processes, peroxisomal fatty acid degradation, and branched-chain amino acid degradation are differentially regulated in LIG offspring. Furthermore, CYP4A12 was identified as a key enzyme in AA and 20-HETE metabolism. In conclusion, our study provides further evidence that adverse perinatal conditions during development affect long-term renal health. We also discovered first potential of an n-3 PUFA diet for preventing kidney damage. These results highlight the need for further studies on the effects of dietary lipid modifications on kidney health.