Energy metabolism genes in the duckling liver are altered by maternal dietary methionine limitation.

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Abstract

Background: Nutritional programming is a phenomena that occurs in animals during embryonic development and changes critical metabolic pathways and influences future health and phenotype. The dam's diet, both in terms of quantity and quality, may influence the offspring's phenotypic in farmed birds as well. While mule duckling weight was lowered by 38 percent when 30 female ducks were fed a diet low in the methyl donor methionine, the weight of 180 mule ducklings from the same 30 control females was not affected by this drop. The hepatic energy metabolism of 30 of their ducklings was likewise changed by the maternal dietary methionine limitation. In comparison to the control group of 30 ducklings, their glucose and triglyceride levels were greater, but their plasma free fatty acid level was lower. Specifically, the researchers wanted to find out how maternal methionine restriction affected the livers of newly hatched male and female ducklings by examining the expression levels of 100 genes that primarily deal with energy metabolism, amino acid transport, oxidative stress, apoptotic activity and liver injury susceptibility in the livers.

Results: Sixteen of the genes were found to be expressed differently in the two groups of ducklings. mRNA levels of genes involved in several processes of energy metabolism such as glycolysis, lipogenesis and electron transport were altered by maternal dietary methionine limitation. PPARGC1B, PPARG, and RXRA nuclear receptor mRNA levels were similarly affected.

Conclusions:

In this study, we found that a 38% decrease in the amount of methionine consumed by female ducks throughout their growth and egg-laying phases had an effect on the liver transcriptome of their offspring, which might explain the differences in their liver energy metabolism previously documented. Together with the reported phenotypic data, these alterations in mRNA levels imply an early regulation of metabolic pathways.

Keywords: Nutritional programming for duck, a Methyl donor Ge expressed in a variety of ways.

Background:

As in humans and rats [1–4] and farmed animals [5–7], the effects of maternal nutrition on the phenotypes of offspring have been extensively established and reviewed in recent years. An individual's overall health and phenotypes are determined by the nutrition they get throughout their early years of life, termed as "nutritional programming," according to researchers. Direct influence on offspring phenotypes may be achieved in birds by in ovo modification of nutrition, such as injection of nutrients or elimination of components [10, 11]. A portion of the albumen was removed by Willems and colleagues to examine the long-term effects of protein malnutrition in layer hens. Chicks born from albumen-deprived eggs had lower hatching weights and hepatic proteome alterations [12], while adult chickens from albumen-deprived eggs had different gene expression in their hepatic transcriptomes [13]. The early protein deficiency in these adult chickens resulted in smaller eggs, reduced laying rates, and a larger number of secondgrade eggs [14]. Using layer hens, this team showed that nutritional programming caused by early protein undernutrition has long-lasting effects on productivity. Hepatic methyl donor availability has been shown to have an important impact in both glucose and lipid metabolism in the liver. While betaine has been demonstrated to affect cholesterol metabolism in newly born chicks and protect against corticosterone-induced steatosis in the liver, it has also been shown to have no effect on cholesterol levels in the blood. In addition, the amount and quality of the diet given to the female birds might influence the performance of their offspring. Even if hens were fed betaine, it changed the expression of genes in their chicks' livers. As part of a recent

research, we looked at the effects on female common duck Anas platyrhynchos and their newly born mule duckling morphologies of low dietary methionine (Met) laying performance [18]. The male mule duck is, in fact, the offspring of a female common duck and a Muscovy drake, an intergeneric hybrid (Cairinamoschata). Foie gras production in France is facilitated by heterosis effects that boost the formation of fatty liver (hepatic steatosis) generated by overfeeding. Met-restricted diets (R group) containing 0.25 percent Met were given to the restricted women in our previous research [18], while the control women were given meals with 0.40 percent Met, which met Met requirements throughout the growing and laying phases from 10 to 51 weeks of age (C group). Because of this, embryos hatched from mothers in the R group had less nutrients available to them throughout development because their eggs were lighter and contained less albumen. The ducklings born to mothers from the R and C groups were then allocated to their respective groups. Ducklings born to mothers in the R group had lower body weights and a higher liver-to-body weight ratio than those born to mothers in the C group. There was also a reduction in plasma alkaline phosphatase (ALP), as well as an increase in alanine transamination (ALT) activity. glucose and triglycerides (TG) concentrations increased, but FFA levels fell in their bloodstreams. Male and female newly born ducklings from Met-restricted mothers were shown to have abnormal hepatic energy metabolism. According to published research, this change might be the result of altered nutritional programming as a result of a diminished supply of Met - a methyl donor - in the mother's diet and a reduced supply of nutrients in the embryonic stage. By comparing 100 genes in the liver of newly born ducklings from male and female ducklings from Met-Restricted and Control mothers, the goal of this research was to better understand how a mother's diet affects her offspring's health. Energy metabolism, amino acid transport, oxidative stress, apoptosis, and liver damage susceptibility were among the genes targeted by these studies.

Results

Male and female ducklings from Met-restricted mothers were examined to see how maternal methionine restriction affected the hepatic energy metabolism by comparing the transcript levels of 100 target genes in their livers. After a qqnorm trans formation, the normalized relative expression of 100 tar get genes was examined in the livers of 38 ducklings from both groups (R group vs C group). Of the 100 genes and 38 liver cDNA samples analyzed, 13 exhibited more than 25% missing data and were thus excluded from the research (see Method section). One more DNA sample was also eliminated from the data set due to significant differences in data points from other samples. The remaining 87 genes and 35 liver cDNA samples from nine males and eight females from the C group and ten males and eight females from the R group have now been reported.

Samples were divided into four groups based on their mother's diet and the gender of their offspring.

The C-class. Only four ducklings were separated from their original group in each instance. 1a and 1b are the two sub-clusters that make up the first cluster. Subcluster 1a had 11 samples, 8 male ducklings from the R group, 2 male ducklings from the C group, and a female duckling from the R group. Three female and two male ducklings from the R group and one male and one female duckling from the C group comprised Te sub-cluster 1b. Subclusters 2a and 2b were created from the original cluster 2. The C group provided 6 male and 4 female samples for the sub-cluster 2a. Seven female samples were included in sub-cluster 2b, four from the R group and three from the C group. The 87 genes were classified into two primary clusters on the columns, A and B, based on their relative expression levels. The cluster A consisted of 21 genes that were overexpressed in samples from the R group of ducklings. B1 and B2 were two subclusters of the cluster B. The expression levels of 15 genes in sub-cluster B1 were identical to those in duckling clusters 1 and 2, which had previously been defined. Ducklings from the C group made up the majority of the samples in the B2 subcluster, which had 51 genes that sought to be overexpressed. The PCA (Principal Component Analysis) score plot (distribution of individuals) along the horizontal and vertical axes is shown in Fig. 1B. On the horizontal axis, the samples were divided according to the food of the mother, and on the vertical axis, according to the sex of the ducklings. Thirty-three percent of the total variability was represented by the first two main components' horizontal and vertical axes, respectively. Ducklings from the R group (MR), the C group (MC), and the F group (FR) were all divided into four groups, based on the gender of their mothers, as well as the maternal diet of their mother (FC).

Pregnant women's nutritional needs Dietary restriction affected the

expression of genes involved in energy metabolism.

The normalized and transformed relative expressions of the 87 genes examined were utilized to study the differences in gene expression between the liver samples of the offspring of the two food groups (C group vs R group)) (Additional Table 1). Analyses used a linear mixed model that included as fixed effects and as a random effect, the food of the mother and the sex of the duckling. The interaction between these variables was also included in the model. The 27 genes listed in Table 1 demonstrate a significant difference (P-value adjusted).

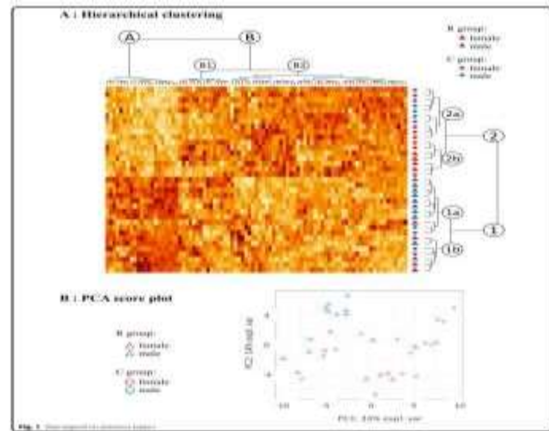
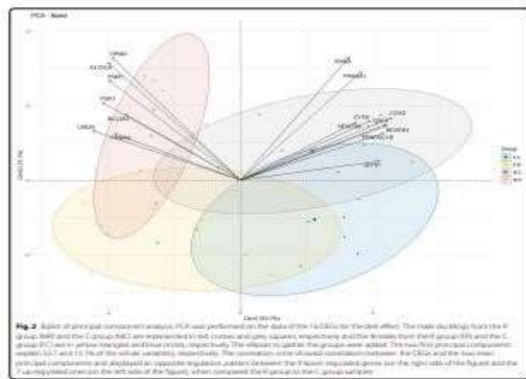


Table 1 Differentially expressed genes (DEGs) in the liver of ducklings

GENE	R group LsMeans ± SD	C group LsMeans ± SD	Females LsMeans ± SD	Males LsMeans ± SD	Diet P-value (BH)	Sex P-value (BH)	Sex*Diet P-value (BH)
GPAM	0.39 ± 0.16	−0.52 ± 0.17	−0.59 ± 0.17	0.47 ± 0.15	< 0.01	**< 0.01**	0.38
[illegible]	−0.61 ± 0.19	0.64 ± 0.19	−0.02 ± 0.2	0.05 ± 0.18	< 0.01	0.06	0.09
PGM1	0.53 ± 0.15	−0.65 ± 0.15	−0.53 ± 0.16	0.41 ± 0.14	< 0.01	**< 0.01**	0.78
[illegible]	−0.55 ± 0.24	0.77 ± 0.28	0.01 ± 0.26	0.2 ± 0.25	0.01	0.91	0.85
ELOVL6	0.51 ± 0.27	−0.5 ± 0.31	−0.46 ± 0.26	0.47 ± 0.25	0.01	**< 0.01**	0.62
[illegible]	−0.54 ± 0.24	0.51 ± 0.27	−0.1 ± 0.23	0.07 ± 0.23	0.01	0.81	0.85
MTTP	−0.48 ± 0.21	0.55 ± 0.22	0.1 ± 0.23	−0.02 ± 0.2	0.01	0.87	1.00
[illegible]	0.49 ± 0.25	−0.46 ± 0.27	−0.29 ± 0.25	0.32 ± 0.23	0.01	0.07 *	0.39
PRKAA1	−0.49 ± 0.2	0.4 ± 0.2	−0.48 ± 0.21	0.39 ± 0.19	0.01	**0.02**	0.83
[illegible]	−0.78 ± 0.3	0.51 ± 0.24	−0.77 ± 0.31	0.49 ± 0.23	0.01	0.11	1.00
UGDH	0.48 ± 0.21	−0.53 ± 0.22	−0.2 ± 0.22	0.15 ± 0.2	0.01	0.42	0.83
CYTB	−0.6 ± 0.28	0.52 ± 0.33	−0.15 ± 0.29	0.07 ± 0.27	0.02	0.54	0.70
[illegible]	0.34 ± 0.21	−0.43 ± 0.21	−0.33 ± 0.22	0.25 ± 0.2	0.03	0.13	0.70
[illegible]	−0.4 ± 0.22	0.42 ± 0.22	−0.08 ± 0.23	0.1 ± 0.21	0.03	0.81	0.70
PPARG	0.47 ± 0.22	−0.5 ± 0.24	−0.1 ± 0.23	0.07 ± 0.22	0.03	0.81	0.87
PPARGC1B	−0.43 ± 0.23	0.43 ± 0.23	0 ± 0.23	0 ± 0.22	0.03	0.98	0.87
[illegible]	−0.48 ± 0.3	0.46 ± 0.34	−0.27 ± 0.3	0.25 ± 0.29	0.06 *	0.18	0.78
PPARA	−0.35 ± 0.22	0.33 ± 0.22	−0.29 ± 0.23	0.27 ± 0.21	0.16 *	0.23	0.79
ELAVL1	0.2 ± 0.24	−0.35 ± 0.28	−0.67 ± 0.24	0.53 ± 0.24	0.22	**< 0.01**	0.99
HMGCR	0.1 ± 0.33	−0.25 ± 0.37	−0.67 ± 0.32	0.51 ± 0.31	0.47	**< 0.01**	0.12
MEF2C	0 ± 0.18	−0.12 ± 0.19	−0.71 ± 0.2	0.38 ± 0.18	0.75	**< 0.01**	1.00
SCD1	0.32 ± 0.26	−0.44 ± 0.31	−0.52 ± 0.28	0.4 ± 0.26	0.18	**< 0.01**	0.70
TALDO1	0.11 ± 0.25	−0.37 ± 0.28	−0.58 ± 0.25	0.51 ± 0.24	0.18	**< 0.01**	0.70
VLDLR	−0.23 ± 0.3	0.01 ± 0.34	0.58 ± 0.29	−0.8 ± 0.28	0.66	**< 0.01**	0.00
PCK1	−0.28 ± 0.31	0.1 ± 0.35	0.36 ± 0.31	−0.54 ± 0.3	0.47	**0.01**	0.85
FASN	0.28 ± 0.27	−0.38 ± 0.31	−0.43 ± 0.27	0.33 ± 0.26	0.14	**0.02**	0.08
MAPK1	0.19 ± 0.3	−0.38 ± 0.35	−0.57 ± 0.31	0.36 ± 0.29	0.32	**0.02**	0.85
ABCA1	0.05 ± 0.29	−0.27 ± 0.32	−0.54 ± 0.29	0.32 ± 0.28	0.55	**0.04**	0.84
DGAT2	0.06 ± 0.24	−0.12 ± 0.24	−0.49 ± 0.24	0.43 ± 0.23	0.71	**0.04**	0.85
ALDOB	−0.34 ± 0.25	0.36 ± 0.28	0.36 ± 0.26	−0.35 ± 0.24	0.13	0.06 *	0.85
SDHA	−0.24 ± 0.22	0.16 ± 0.24	−0.45 ± 0.24	0.37 ± 0.22	0.35	0.06 *	0.85
DHCR24	0.11 ± 0.2	−0.15 ± 0.2	−0.37 ± 0.21	0.33 ± 0.2	0.44	0.07 *	0.08
HADH	−0.32 ± 0.23	0.21 ± 0.23	−0.44 ± 0.25	0.33 ± 0.21	0.38	0.07 *	0.83
LDHA	−0.13 ± 0.37	0.01 ± 0.43	0.26 ± 0.37	−0.38 ± 0.35	0.86	0.07 *	0.38



Correlations are found with just six genes in the R group (0.49CYTB, 0.66ENO1, 0.66ENO2, 0.66ENO3, 0.71METP, 0.56PARG1B, and

0.55PRA), but eight genes in the C group (0.55MTTP, —0,58PGK1, and —0.75UGDH) are also associated with it. No DEG was linked to the plasma activity of ALP, ALT, or AST in the C group, but only one DEG was linked to the plasma activity of each of these enzymes in the R group (ALP, 0.61 GPAM, ALT, 0.63 PPARG, and three DEGs to the plasma activity of AST, each of which

was connected to three different DEGs). In contrast, the C group showed a 0 DEG correlation. GPAM and PGM1 were negatively correlated with plasmatic FFA concentrations in both the R and C groups, however ELOVL6 was not in either group. In contrast to the C group, which showed four significant associations, the R group only had two (0.76 PGK1 and 0.49 PPARGC1B) (0.55 CYTB and 0.58 NDUFB6). The 16 hepatic DEGs had no correlation with liver content. There were only three DEGs in the C group, one in the R group (CYTB at 0.51), and one in the C group (0.50 PPARG) that were connected to the liver's lipid content. Only two C-group genes were found to influence liver weight (PGK1 at 0.57 and UGDH at 0.55). DEG correlations are higher in the C group than in the R group. UGDH and COX2 are connected to 12 and 3 DEGs, respectively, as an example of this.

Table 2: Duckling characteristics may be affected by a mother's restricted dietary Met intake (from Bodin et al., 2019 [18])

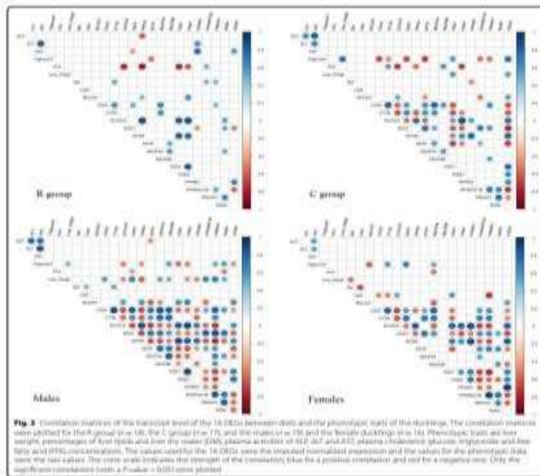
	Treatment						
	R group		C group				
	n	Mean ± SD	n	Mean ± SD	P_{Diet}	P_{sex}	$P_{interaction}$
Body weight (g)	180	33.8 ± 0.3	180	35.2 ± 0.3	<0.001	NS	NS
Liver weight (g)	28	1.51 ± 0.08	21	1.40 ± 0.11	NS	0.001	NS
Liver BW (%)	28	4.38 ± 0.17	21	3.92 ± 0.28	0.07	0.06	NS
Liver lipids (%)	28	17.23 ± 1.43	19	17.67 ± 1.44	NS	NS	NS
Liver dry matter (%)	28	41.16 ± 8.73	20	41.21 ± 1.16	NS	NS	NS
Plasma Glucose (mMol/L)	23	16.36 ± 1.88	26	10.63 ± 2.38	0.03	NS	NS
Plasma FFA (mMol/L)	28	0.27 ± 0.05	27	0.55 ± 0.05	0.01	0.07	NS
Log Plasma TG	27	0.55 ± 0.19	27	-0.09 ± 0.21	0.01	0.01	NS
Log Plasma ALP	28	5.36 ± 0.09	24	5.62 ± 0.18	0.07	<0.001	NS
Log Plasma ALT	28	2.98 ± 0.09	23	3.31 ± 0.09	0.002	0.01	NS
Log Plasma AST	27	4.42 ± 0.19	27	4.69 ± 0.21	NS	0.006	NS

Numbers, means, and standard errors of the measured traits as well as the significance of the differences between means are given. P-values <0.05 were considered significant

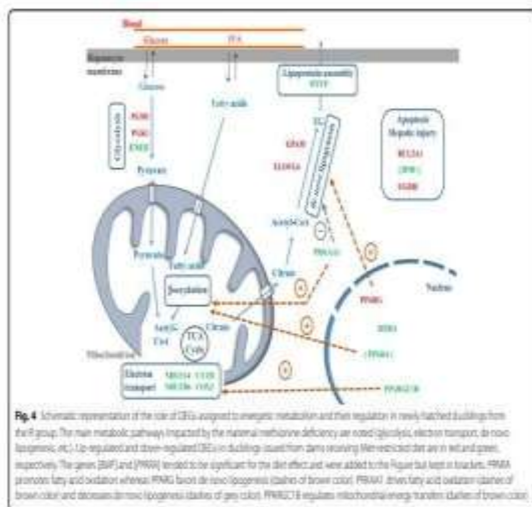
Each of these groups had CYTB at 9, ELOVL6 at 10, and ENO1 at 10, for example. Using male and female samples, it was shown that the phenotypes of males are more closely linked to the DEGs than those of women. 8 DEGs in the males (5 negative correlations: ELOVL6, GPAM, PGM1, and UGDH; 3 positive correlations: 0.54 NDUFB6, 0.51 PRKAA1 and 0.56 RXRA) and 4 DEGs in the females (0.61 BCL2A1, 0.59 CYTB, 0.68 NDUFA4 and 0.52 NDUFA4) and the liver weight is correlated to 11 DEGs in the males (7 positive correlations: 0.5 COX2, 0.6 ENO1, 0.54 NDUFA4, 0.48 NDUFA6, 0.66 PPARGC1B, 0.70 PRKAA1; and a negative correlation: -0.53 BCL2 On the other hand, men have no DEG and females have a correlation between hepatic lipid content and 5 DEGs (0.50 COX2 and CYTB and 0.73 PRKAA1 and + 0.53 ELOVL6). BCL2A1 and NDUFB6 have a larger association with other DEGs in men than in females, but the relationships with the other DEGs are stronger for both genders. It was possible to derive identical correlation matrices for each of the four subpopulations (MRs), as shown in Additional Figure 1 (previewed here). As can be seen, guys from the C group (MC) had higher DEG correlations than males from the R group, which isn't a huge surprise (MR). According to our exploratory data analysis (Fig. 1) and Principal Component Analysis, the cor relation matrixes varied not only across groups, but also by gender, with the findings agreeing with the exploratory data analysis (Fig. 1) and the Principal Component Analysis (Fig. 2).

Discussion:

The liver is the primary source of lipids in birds. Overfeeding in adult mule ducks enhances the liver's ability to retain and collect lipids, mostly triglycerides, for the development of fatty liver. Prior to their first meal, the freshly born mule ducklings from the R group had a body weight that was 6% lighter and a liver-to-body weight ratio that was 10% greater than that of the C group. Furthermore, plasmatic data revealed that male and female ducklings from Met-restricted dams had altered hepatic energy metabolism (Table 2). We thus examined the livers of newly born ducklings to see whether nutritional programming may have affected the expression level of hepatic genes in ducklings whose mothers had been given decreased Met diets. " The capacity of overfed animals to develop steatosis in the liver might be affected by such a shift in gene expression, if it is followed by a change in the production of associated proteins. Te These two genes, PPARA and BMF, were shown to have a P-value (BH) of less than 0.10 in the livers of newly born R group ducklings, as seen in Figure 4. The DEGs that have been shown to be up- or downregulated are shown in red and green, accordingly. A maternal methionine deficiency affected the mRNA levels for genes involved in lipid metabolism even though the dry matter and lipid content of R group ducklings were identical to those of C group ducklings.



There are a variety of energy-related routes, including glycolysis, lipogenesis, and the mitochondrial electron transfer. ENO1 was downregulated in the R group, whereas PGM1 and PGK1 were both up-regulated in the G group. R group ducklings also had lower levels of COX2 (MTCO2), NDUFA4, NDUFB6, and CYTB (MT-CYB) in their mitochondrial electron transport chain. This suggests a less active mitochondrial electron transport chain. The increased expression of ELOVL6 and GPAM in the livers of R group ducklings also suggests a greater concentration of TG. Glycolysis and pyruvate synthesis may be boosted in this group because of the high concentration of glucose in plasma. Ten, in the mitochondria, pyruvate may be turned into AcetylCoA, which can then be transformed into citrate through the tricarboxylic acid cycle. " (TCA cycle). After this, citrate may be exported from the mitochondrion and employed as a precursor to the de novo lipogenesis process.



Lipid export may have been reduced in the R group because of the decreased expression of MTTP, the protein that regulates the assembly of lipoproteins. The 5′-prime-AMP-activated protein kinase (PRKAA1) catalytic subunit is encoded by PRKAA1 (AMPK). Protecting against fatty liver disease, PRKAA1 promotes fatty acid oxidation and reduces lipogenesis in hepatocytes. [20] Lipogenesis may have been increased in R group ducklings because of lower PRKAA1 mRNA levels. These genes' expression levels did not, however, correlate with liver lipid percentages. This gene (PPARGC1B, or PGC1B) encodes a nuclear protein that belongs to the peroxisome proliferator-activated recep tor-g (PPARG) coactivators (PGC-1s). Additionally, they work together to influence the expression of genes involved in electron transport and fatty acid oxidation or de novo lipogenesis by coactivating transcription factors and nuclear receptors, including PPARG There is evidence that PPARG is a master regulator of lipogenesis, promoting hepatic fat storage age [21–23]. There are nuclear receptors such as Ppar that are abundantly expressed in the liver, and one of them, PPARA, controls transcription of genes involved in fatty acid catabolism via controlling PPAR transcription. Ligand-activated PPARA and PPARG are both phosphorylated. This occurs in the absence of any external stimuli, such as the presence of an exocrine or extrinsic stimulant. The RXRA/PPARA heterodimer is essential for PPARA transcriptional activity on fatty acid oxidation genes since RXRA is a member of the RXR family [24]. The PPARGC1B coactivator positively controls the expression of electron transport genes such as COX2 and mitochondrial -oxidation in the liver [25]. There was a decrease in the expression of PPARGC1B (PGC1B) and RXRA (PPARA) in the liver of ducklings from the R group, but PPARG (PPARG) was up-regulated. It is possible that the downregulation of RXRA and PPARGC1B in R group ducklings may have reduced energy dissipation via fatty acid oxidation and thermogenesis, while PPARG's up-regulation may have enhanced energy storage through increased lipogenesis. The qualities measured in the ducklings from both groups revealed no significant connection with RXRA. PPARG, on the other hand, was associated with higher levels of AST and ALT plasma activity in the R group and lower levels of liver DM in the C group. There was a positive correlation between plasma FFA and liver DM concentration in the R group, and an inverse correlation between plasma Tg and FFA concentration in the C group (Fig. 3). Additionally

to these findings, additional regulatory pathways were interestingly influenced by methionine supplementation in the ofspring liver, particularly with regard to apoptosis. Apoptosis is regulated by the BCL-2 family of proteins, which includes both BMF and BCL2A1. Mitochondrial membranes contain a large number of Bcl-2 family proteins, many of which are pro-apoptotic. In contrast to BMF, BCL2A1 is an anti-apoptotic protein that inhibits the release of cytochrome c from mitochondria and blocks caspase activation, while BMF encodes a protein that binds Bcl-2 proteins and operates as a pro-apoptotic protein. We found that BMF was down-regulated in ducklings from the R group, but BCL2A1 was up-regulated, indicating a shift in metabolism toward decreased apoptotic activity. Furthermore, the R group samples showed an increase in UGDH activity, indicating a better ability to detoxify in this group. According to the data, the UDP-Glc to UDP-Glc acid conversion enzyme UGDH is encoded by the UGDH gene (UDP GlcA). UDP-GlcA is required for the glucuronidation of harmful substances in the liver [26]. Overall, these findings imply that the maternal nutrition regulates apoptotic pathways and tissue damage. As a result of this, the plasma of ducklings from the R group had lower levels of the enzymes ALT and ALP, which suggests that they are less susceptible to liver damage. It is well known that the availability of methyl donors in the maternal diet and the effects on liver metabolism during embryonic development are well studied in mammals, where reviews highlight the interactions between onecarbon metabolism, epigenetic mechanisms, and energy metabolism [27–29]. This is also mentioned in the Background section. Betaine supplementation in birds has been researched by Ruquian Zhao's team through in ovo injection and maternal food addition. According to the findings of this research, a single in ovo injection of betaine may affect the liver cholesterol metabolism in newly born chickens [15] and shield the birds against corticosterone-induced liver disease [16]. They also found that betaine supplemented hens' offspring had decreased hepatic cholesterol level, as well as epigenetic modification of the SREBP2 and CYP7A1 genes [30], and that female chicks from betaine-supplemented hens had altered hepatic expression of the Dio1, BHMT, and DNMT1 genes [17]. In addition, when their male chicks were subjected to prolonged corticosterone exposure, the maternal supplementation had hepatoprotective effects [31]. According to our findings, maternal food availability of methyl donors has an effect on liver metabolism and hepatoprotective effects in ducks. We speculate that the lower availability of methyl donors in the mother's diet may have affected the activity of onecarbon metabolism in the liver of the duckling. As a result, in the livers of R group ducklings, methylation of gene promoters would have been changed, altering routes for energy consumption, and so affecting the availability of methyl groups. We are now investigating the influence of maternal food restriction on transcripts of one carbon metabolism genes and several epigenetic pathways in order to test this notion. The findings of this research also showed that the hepatic metabolism of mule ducklings is sex-dependent, which is interesting. Sexual dimorphism of gene expression in numerous organs, including the liver, has previously been shown [32, 33], including differences in PPAR signaling pathways in rat models [34, 35]. Nevertheless, as Aiken and Ozanne [34] and McCabe and coauthors [35] have previously examined, the application of a dietary deficiency to metabolisms that differ leads to different consequences and sex differences in response to developmental programming. We found that a number of the genes investigated were differentially expressed based on the sex of the ducklings in our research [18] (Table 2). (Table 1). In both groups of ducklings, the liver metabolism of female ducklings was different from that of male ducklings (R and C groups). Within each group, the gender correlation matrices, i.e. MR vs FR and MC versus FC, differed (Additional Fig. 1).

Conclusions:

Our work concluded by looking for transcriptome evidence of early metabolic programming in R ducklings, in which we examined the expression levels in the livers of 100 target genes in young ducks given either control or methio nine-restricted diets. Apart from that, we looked for links between gene expression differences and phenotypic findings from an earlier paper [18]. According to the present research, a 38 percent drop in methionine content in the mother's diet affects the transcriptome of her offspring and is thus modifiable. In the livers of group R ducklings, however, differences in the expression levels of the 16 DEGs may be connected to variations in the production levels of the proteins they encode. A combination of alterations in mRNA levels and observed phenotypic data suggests that early metabolic pathways are being modulated. Nutrient programming experiments were conducted with the help of Muscovy duck and female common duck heterosis in the purpose of producing fattier livers for mule ducks. For this reason, it would be

most interesting for researchers to conduct a nutritional programming experiment on the pure common duck.

Methods

Experimentation with animals and its design:

The European Council Directive 2010/63/EU was adhered to in the conduct of the experiments and animal care. INRAE, UEPFG, got accreditation number B40–037-1 for the trial at the Ducks and Goose Experimental Facility – INRAE – INRAE, (Benquet). In France, the Minister of Education, Research, and Innovation (APAFIS#1847-2015092213418825v) authorized the protocol and procedures. Detailed instructions for doing the experiment have been provided [18]. Briefly, two groups of 60 female common ducks were created and fed an acceptable amount of Met till they were 10 weeks old. From the time they were 10 weeks old until they were 51 weeks old, they were given a variety of different experimental diets. In a conventional corn-soybean diet for chicken, Met is the first limiting amino acid [36]. The recommended percentage for laying ducks is between 0.40 and 0.45 percent [37–40]. The experimental diets employed two amounts of total Met: 0.25 percent for Met restricted diets (R Group) and 0.40 percent for control diets (C Group) that met the Met needs of female laying ducks (Additional Table 2). Between the ages of 34 and 36 weeks, two artificial inseminations per week were used to produce the mule ducklings. The semen of 15 Muscovy drakes was used, and the drakes were given commercial diets instead of any nutritional treatment. Incubation of the eggs lasted 28 days at 37.6°C and 60 percent average relative humidity (incubator Sologne, La Nationale, Briaire, France). A hatcher was used to keep them at 37.3°C and 80% relative humidity for four days before they were released into the wild. Once a duckling is born, its characteristics are noted. The ducklings born to the R and C groups' females are then placed in those groups' respective nests. As stated in Bodin et al., 2019 [18], phenotypic features of ducklings were recorded at hatching on 180 and 190 ducklings from the R and C groups, respectively. There were an additional 58 ducklings that had to be destroyed because of cervical dislocation when they were only a few days old (12 females and 16 males from the C group and 15 females and 15 males from the R group). Before being sacrificed, Tese ducklings did not get any food. The weight of Teir's liver was measured. Only the livers of the C group's 8 females and 13 males and the R group's 15 females and 15 males that were successfully recovered were immediately submerged in liquid nitrogen before being transported to an 80°C freezer. This was due to gallbladder damage.

Reverse transcription of RNA:

Samples of fresh-hatched ducklings of both sexes and of both control and restricted maternal diet groups were pulverized using a Retsch grinder at 30Hz for 45 seconds in liquid nitrogen to extract the frozen liver samples. In order to extract and purify RNA from 80 to 100mg of tissue powder, we used the TRIzol® technique (Invitrogen, California, USA) followed by a column from the Nucleospin RNA kit (Macherey Nagel, France) and followed the manufacturer's instructions. To prevent DNA contamination, we followed the manufacturer's instructions and used 20 l of rDNAse (Macherey Nagel) and 80 l of reaction buffer for 20 minutes [42, 43]. NanoDrop 8000 spectrophotometer (Termo Fisher, Illkirch, France) was used to measure total RNA and kept at 80°C. Electrophoresis and an Agilent 2100 Bioanalyzer with the RNA 6000 Nano Lab Chip Kit were used to verify its integrity (Agilent Technologies, Massy, France). All experimental samples were reverse transcribed immediately after the quality control evaluations, and the same quantity of total RNA was utilized for each sample. SuperScriptTM II (Invitrogen, California, USA), RNase H-MMLV reverse transcriptase (Promega Corporation, USA), and oligo (dT)15 were employed in the process (Sigma Aldrich, France). In RNase-free water, the cDNAs were then diluted and kept at 80°C.

Analyses based on statistics

Missing values for the other 87 genes were imputed by combining the function imputPCA with the missMDA package's three primary components [53] and applying it to each group with comparable sex and maternal diet. The data were modified to reflect a centered reduced normal distribution using the function qqnorm(Y)$x, which normalized and imputed the relative expressions. The whole dataset was then described using the converted data. Heatmaps and Principal Component Analysis (PCA) on 87 genes were done using the R program Mix Omics [54] to identify groupings of animals and genes with similar expression patterns. The first two main components of the PCA score plot were plotted for each participant. It was used to run ten ANOVAs on the transformed normalized relative expressions of 87 genes using a linear mixed model using ASReml software that included the maternal diet

and the sex of a duckling as fxed effects, along with the duckling related to its connection matrix. Dietrelated genes having a significant P-value

Abbreviations:

Albumin, Aldolase, Fructose-Bisphosphate B, and Alkaline Phosphatase are all abbreviations for the acronym ALDOB. One of the enzymes involved in the conversion of amino acids into glucose; ANCOVA: a study of differences; Components of AST, BCL2A1 and cDNA are referred to as BCL2 related proteins A1 and BMF, respectively; cDNA is the complementary deoxyribonucleic acid of these proteins. Cytochrome c oxidase subunit II; COX2 A cycle threshold (Cq) and a cycle threshold value (Ct). This is Cytochrome b, or CYTB Differentially expressed gene (DEG) DGAT2 stands for Diacyl Glycerol O-acyltransferase 2, while DHCR24 stands for 24-Dehydrocholesterol Reductase; DNAse: Deoxyribonuclease; DM: Dry mater FASN: Fatty acid synthase; ENO1: Enolase; ELAVL6: ELOVL Fatty Acid Elongase 6; ELOVL1: ELAV Like RNA Binding Protein; ELOVL6: ELOVL Fatty Acid Elongase 6 Mitochondrial GAPDH; Glycerol-3phosphate acyltransferase, mitochondrial; HMBS; HMGCR; HPRT1; LDHA; MC: Males from the C group; Met: Methionine; MR: Males from R group; mRNA: Messenger ribonucleic acid Triglyceridetransfer protein in microsomes In addition to NDUFA10 and NDUFA4, the mitochondrial complex is also known as NDUFA4 and NDUFB6, which is a subunit of NADH:Ubiquinone Oxidoreductase Subunit A10. Using Principal Component Analysis (PCA) DNA: Polymerase Chain Reaction; PCK1: Phosphoenolpyruvate carboxykinase 1 Coactivator of the Peroxisome proliferator-activated receptor-g PGM1: Phosphoglucomutase 1; PGK1: Phosphoglycerate kinase 1 Alpha 1, Catalytic Subunit of DNA Polymerase A, or POLA1. Proliferator-activated receptor alpha (PPARA) and proliferator-activated receptor gamma (PPARG) Protein Kinase AMPActivated Catalytic Subunit Alpha 1; qPCR: Quantitative Real-Time PCR; RNAse: Ribonuclease; RPS13: Ribosomal Protein S13; RXRA: Retinoid X Receptor Alpha; SCD1: Stearoyl-CoA desaturase (delta-9-desaturase); STA: Specifc target amplifation; TALDO1: Transaldolase 1; TBP: TATA box binding protein; TCA cycle: Tricarboxylic acid cycle; TE: Tris-EDTA bufer; TG: Triglycer**References**

1. Lee H-S. Impact of Maternal Diet on the Epigenome during In Utero Life and the Developmental Programming of Diseases in Childhood and Adulthood. Nutrients. 2015;7:9492–507.

2. Langley-Evans SC. Nutrition in early life and the programming of adult disease: a review. J Hum Nutr Diet. 2015;28(Suppl 1):1–14.

3. Koletzko B, et al. Nutrition During Pregnancy, Lactation and Early Childhood and its Implications for Maternal and Long-Term Child Health: The Early Nutrition Project Recommendations. Ann NutrMetab. 2019;74:93–106.

4. Saf-Stibler S, Gabory A. Epigenetics and the Developmental Origins of Health and Disease: Parental environment signalling to the epigenome, critical time windows and sculpting the adult phenotype. Semin Cell Dev Biol. 2020;97:172–80.

5. Chavatte-Palmer P, Tarrade A, Rousseau-Ralliard D. Diet before and during Pregnancy and Ofspring Health: The Importance of Animal Models and What Can Be Learned from Them. Int J Environ Res Public Health. 2016;13(6):586. https://doi.org/10.3390/ijerph13060586.

6. Sinclair KD, et al. Epigenetics and developmental programming of welfare and production traits in farm animals. Reprod Fertil Dev. 2016. https://doi. org/10.1071/RD16102.

7. Khanal P, Nielsen MO. Impacts of prenatal nutrition on animal production and performance: a focus on growth and metabolic and endocrine function in sheep. J Anim Sci Biotechnol. 2017;8:75. 8. Dixon LM, Sparks NHC, Rutherford KMD. Early experiences matter: a review of the efects of prenatal environment on ofspring characteristics in poultry. Poult Sci. 2016;95:489–99.

9. Morisson M, et al. Nutritional Programming and Efect of Ancestor Diet in Birds. In: Handbook of Nutrition, Diet, and Epigenetics: Springer
International Publishing; 2017. p. 1–18.

10. Jha R, Singh AK, Yadav S, Berrocoso JFD, Mishra B. Early Nutrition Programming (in ovo and Post-hatch Feeding) as a Strategy to Modulate Gut Health of Poultry. Front Vet Sci. 2019;6:82.

11. Calini F, Sirri F. Breeder Nutrition and Ofspring Performance. Braz J Poultry Sci. 2017;9:77–83.

12. Willems E, et al. Embryonic protein undernutrition by albumen removal programs the

hepatic amino acid and glucose metabolism during the perinatal period in an avian model. PLoS One. 2014;9:e94902.

13. Willems E, et al. Diferential Expression of Genes and DNA Methylation associated with Prenatal Protein Undernutrition by Albumen Removal in an avian model. Sci Rep. 2016;6:20837.

14. Willems E, et al. Partial albumen removal early during embryonic development of layer-type chickens has negative consequences on laying performance in adult life. Poult Sci. 2013;92:1905–15. 15. Hu Y, et al. In Ovo injection of betaine afects hepatic cholesterol metabolism through epigenetic gene regulation in newly hatched chicks. PLoS One. 2015;10:e0122643.

16. Hu Y, et al. In ovo injection of betaine alleviates corticosterone-induced fatty liver in chickens through epigenetic modifcations. Sci Rep. 2017;7:40251.

17. Hou Z, et al. Maternal betaine administration modulates hepatic type 1 iodothyronine deiodinase (Dio1) expression in chicken ofspring through epigenetic modifcations. Comp BiochemPhysiol B Biochem Mol Biol. 2018;218:30–6.

18. Bodin L, et al. Dietary methionine defciency reduces laying performances of female common ducks and impacts traits of interest of their mule ducklings. Poult Sci. 2019;98:5590–600.

19. Marie-Etancelin C, et al. Genetics and selection of mule ducks in France: A review. World's Poultry Sci J. 2008;64:187–207.

20. Boudaba N, et al. AMPK Re-Activation Suppresses Hepatic Steatosis but its Downregulation Does Not Promote Fatty Liver Development. EBioMedicine. 2018;28:194–209. 21. Ahmadian M, et al. PPARγsignaling and metabolism: the good, the bad and the future. Nat Med. 2013;19:557–66.

22. Steensels S, Qiao J, Ersoy BA. Transcriptional Regulation in Non-Alcoholic Fatty Liver Disease. Metabolites. 2020;10(7):283. 23. Pei K, et al. An Overview of Lipid Metabolism and Nonalcoholic Fatty Liver Disease. Biomed Res Int. 2020;2020:4020249.

24. Hardwick JP, Osei-Hyiaman D, Wiland H, Abdelmegeed MA, Song B-J. PPAR/RXR Regulation of Fatty Acid Metabolism and Fatty Acid omegaHydroxylase (CYP4) Isozymes: Implications for Prevention of Lipotoxicity in Fatty Liver Disease. PPAR Res. 2009;2009:952734.

25. Sonoda J, Mehl IR, Chong L-W, Nofsinger RR, Evans RM. PGC-1beta controls mitochondrial metabolism to modulate circadian activity, adaptive thermogenesis, and hepatic steatosis. Proc Natl Acad Sci U S A. 2007;104:5223–8.

26. Vatsyayan J, Lin C-T, Peng H-L, Chang H-Y. Identifcation of a cis-Acting Element Responsible for Negative Regulation of the Human UDPGlucose Dehydrogenase Gene Expression. BiosciBiotechnolBiochem. 2006;70:401–10.

27. Cai D, Liu H, Hu Y, Jiang Y, Zhao R. Gestational Betaine, Liver Metabolism, and Epigenetics. In: Handbook of Nutrition, Diet, and Epigenetics: Springer International Publishing AG; 2017. p. 2017.

28. Chango A, Pogribny IP. Considering maternal dietary modulators for epigenetic regulation and programming of the fetal epigenome. Nutrients. 2015;7:2748–70.

29. Clare CE, Brassington AH, Kwong WY, Sinclair KD. One-Carbon Metabolism: Linking Nutritional Biochemistry to Epigenetic Programming of LongTerm Development. Annu Rev Anim Biosci. 2019;7:263–87