Maternal Nutrient Restriction Followed by Re-alimentation Alters Distinct Metabolic Pathways in Sheep Offspring

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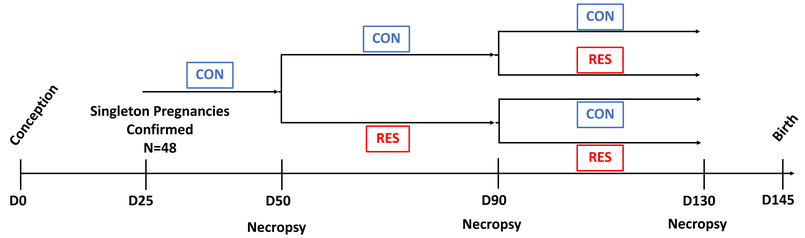
Introduction

- Maternal undernutrition can have detrimental effects on growth, development, and metabolism of fetal muscle and liver, which can have lasting consequences into adulthood (Wu et al. 2006).
- Re-alimentation is a management practice that involves providing full nutrition following nutrient restriction, which can alleviate the negative impacts of nutrient restriction on growth. This practice can lead to increased body weight (maternal and fetal), increased organ weights, and increased fat deposition (Meyer et al., 2010; Keomanivong et al., 2016). However, the effects of re-alimentation on liver, muscle, and blood metabolism are poorly understood.
- Hypothesis: Maternal nutrient restriction followed by re-alimentation will alter fetal metabolism in liver, muscle, and blood.

Materials and Methods



Diet:
CON: 100% NRC
RES: 60% NRC

Figure 1: Experimental Design

Animals: Pregnant ewes were fed 100% (CON) of NRC requirements until day 50 of gestation, then fed either 100% or 60% (RES) until day 90 of gestation. At day 90 of gestation, ewes were either maintained on their respective diet or switched to the alternative diet until day 130 of gestation. Ewes were euthanized at days 50, 90, and 130 of gestation (n=6 to 7 per treatment per time point). Fetuses were necropsied, and liver, muscle (LM, TB, and STN), and blood samples were collected, snap frozen in liquid nitrogen and stored at -80°C.

Metabolomic Analysis: Metabolomic analysis was carried out by Metabolon Inc. (Durham, NC). Briefly, metabolites were extracted from liver, muscle, and blood tissues and analyzed via UPLC-MS/MS. Bioinformatics software was used to identify metabolites. Statistical analysis was performed using two-way and one-way ANOVA, with contrast statements. Differences in metabolite fold change were considered significant at $P \leq 0.05$. Pathway enrichment analysis was performed using Metaboanalyst 4.0.

Results

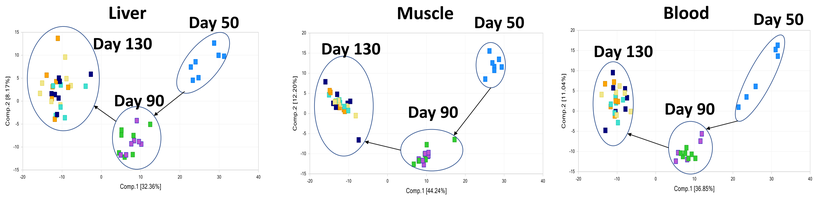


Figure 2. Principle component analysis of liver, muscle and blood metabolites. Principle components clustered by day of gestation; however differences in metabolite concentrations were observed between treatments.

Results

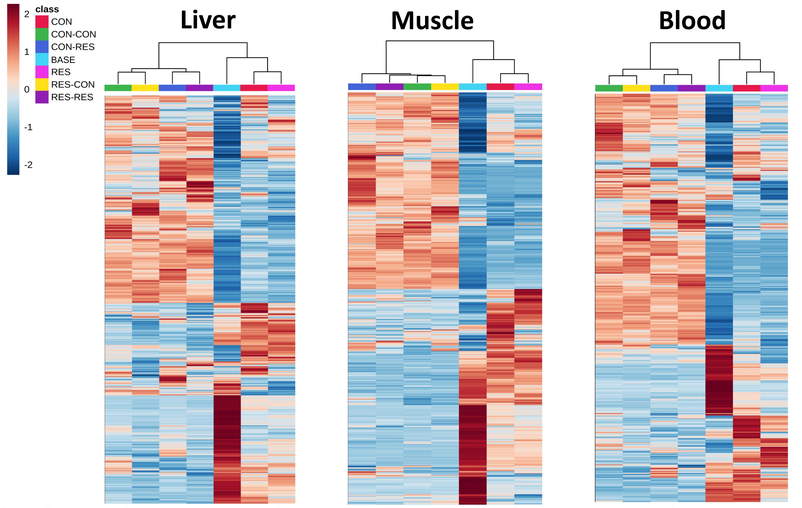


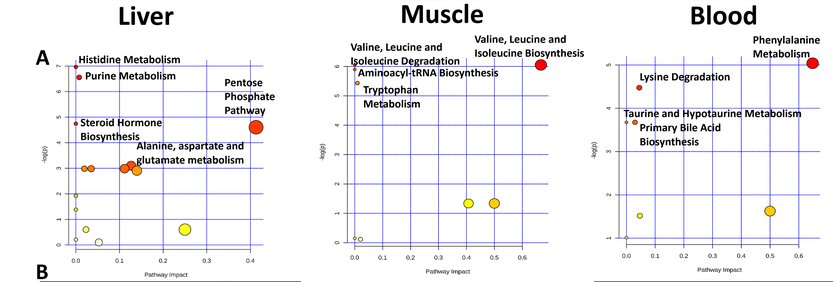
Figure 3. Hierarchical clustering analysis and heatmap of metabolite concentrations comparing treatments in the liver, muscle and blood tissues. Heatmaps highlight variations in metabolite concentrations induced by experimental treatments. Hierarchical clustering demonstrates clustering of BASE, CON, and RES treatments irrespective of tissue.

Table 1: Fetal lipidomics profile at d 50, 90, and 130 of gestation.

		Stage of Gestation								Contrast *P*-value						
		Early	Mid		Late							Day 90	Day 130			
Item[1]	Tissue	BASE	CON	RES	CON-CON	CON-RES	RES-CON	RES-RES	SEM	Day L	Day Q	Day	Trt	MG Trt	LG Trt	MG xLG Trt
CE	Liver	714.7^{A}	615.6^{A}	549.7	412.3^{B}	510.0	389.1	375.8	26.2	<0.01	0.51	0.04	0.47	0.31	0.56	0.44
	Muscle	141.1^{A}	109.6^{B}	107.5	52.8^{C}	59.1	41.5	44.7	6.0	<0.01	0.24	<0.01	0.87	0.23	0.63	0.88
	Blood	519.8^{A}	410.8^{A}	342.5	275.0^{B}	256.5	236.1	248.1	17.4	<0.01	0.71	<0.01	0.14	0.54	0.93	0.66
FFA	Liver	251.1	2315.4	2284.1	2483.3	2492.7	2233.6	2669.8	53.3	0.91	0.35	0.48	0.89	0.85	0.21	0.23
	Muscle	1886.7	1563.7	1747.0	1568.8	1581.1	1317.7	1558.2	46.4	0.08	0.27	0.98	0.28	0.34	0.35	0.40
	Blood	260.7^{A}	263.1^{A}	256.6	496.9^{B}	350.2	327.7	317.8	22.3	<0.01	0.03	<0.01	0.92	0.07	0.11	0.16
TAG	Liver	3430.7^{A}	1616.2^{B}	1235.6	948.6^{C}	843.4	618.1	688.2	142.0	<0.01	<0.01	<0.01	0.10	0.22	0.93	0.63
	Muscle	309.2	173.8	549.6	236.5^{ce}	781.6^{cf}	727.4^{de}	2375.9^{df}	886.1	0.97	0.75	0.78	0.47	0.03	0.01	0.15
	Blood	66.1^{A}	44.7^{B}	40.7	63.9^{Acg}	42.4^{ch}	31.7^{dh}	33.1^{dg}	2.7	0.77	<0.01	0.01	0.60	<0.01	0.08	0.05

[1]LSmeans ± SEM Abbreviations: CE = Cholesterol Esters; FFA = Free Fatty Acids; TAG = Triacylglycerol. SEM = Standard Error of the Mean. Day L = BASE vs. CON-CON; Day Q = BASE and CON-CON vs CON; Day = CON vs. CON-CON; Trt = CON vs. RES; MG Trt = CON-CON and CON-RES vs. RES-CON and RES-RES; LG Trt = CON-CON and RES-CON vs. CON-RES and RES-RES; MG x LG Trt = CON-CON and RES-RES vs. CON-RES and RES-CON; Liver (nmol/g), muscle (nmol/g), and blood (μM) lipid metabolites were measured; $^{A-C}$ denote differences between stages of gestation ($P \leq 0.05$); $^{a-b}$ denote LSmeans differ between treatments within mid gestation ($P \leq 0.05$); $^{c-h}$ denote LSMeans differ between treatments within late gestation ($P \leq 0.05$). Mid-gestational restriction increased triglyceride concentrations in muscle and blood. Cholesterol ester decreased and free fatty acids increased, respectively, between days of gestations in liver, muscle, and blood tissues. Triglyceride concentrations decreased between days of gestation in liver and increased in BASE and CON-CON compared to CON in blood.

Results



B

Liver		CON-CON vs RES-CON
Pentose Phosphate Pathway	6-phosphogluconate	-1.23
	ribulose 5-phosphate	-1.82
	xylulose 5-phosphate	-1.54
	ribose 5-phosphate	-2.38
	ribose 1-phosphate	-1.64
	sedoheptulose-7-phosphate	-2.44

Muscle		CON-CON vs RES-CON
Valine, Isoleucine and Leucine Biosynthesis	leucine	-1.19
	isoleucine	-1.18
	3-methyl-2-oxovalerate	-1.18
	4-methyl-2-oxopentanoate	-1.03
	valine	-1.15
	3-methyl-2-oxobutyrate	-1.09
	isovalerylglycine	1.30

Blood		CON-CON vs RES-CON
Phenylalanine Metabolism	phenylalanine	-1.16
	N-acetylphenyl-alanine	-1.11
	1-carboxyethyl-phenylalanine	-1.28
	phenylpyruvate	-1.41
	phenyllactate (PLA)	-1.52
	phenylacetate	1.29
	2-hydroxyphenyl-acetate	-1.56

Comparison groups significantly different:
$p \leq 0.05$, group means fold of change ≥ 1.00
$p \leq 0.05$, group means fold of change < 1.00

Comparison groups trending towards significant difference:
$0.05 < p < 0.10$, group means fold of change ≥ 1.00
$0.05 < p < 0.10$, group means fold of change < 1.00

Figure 4. A) Pathway enrichment analysis of significant metabolites between CON-CON and RES-CON treatments at day 130 gestation. Significantly enriched metabolic pathways ($P \leq 0.05$) are labeled. Carbohydrate and amino acid metabolic pathways were most impacted by nutrient restriction during early gestation followed by re-alimentation. B) Fold change of metabolites involved in the metabolic pathways with the highest impact factor. Mid-gestational nutrient restriction reduced metabolites in the pentose phosphate pathway; valine isoleucine, and leucine biosynthesis; and phenylalanine metabolism pathways.

Discussion

- Nutrient restriction during mid-gestation followed by re-alimentation decreases pentose phosphate intermediates in liver and branched-chain amino and phenylalanine metabolites in muscle and blood, respectively.
- Mid-gestational nutrient restriction increases triglyceride content in muscle and decreases in blood, suggesting alterations in lipid accumulation which may predispose offspring to dyslipidemia.

References

Wu et al. (2006) *Journal of Animal Science*, *84*(9), 2316–2337
Keomanivong et al. (2017) *Physiology and Animal Nutrition*, *101*(3), 589–604.
Meyer et al. (2010) *Journal of Animal Science*, *88*(7), 2410–2424.

Acknowledgements

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