Maternal Western Diet Programs Fibrosis, Loss of Antioxidant Activity, and Non-Reparative Macrophages in Juvenile Non-human Primate Livers.

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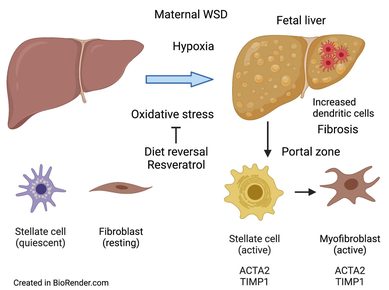
INTRODUCTION

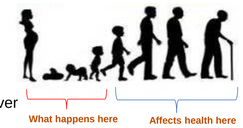
- Maternal overweight/obesity affects up to 60% of pregnancies and predisposes offspring to chronic diseases including non-alcoholic fatty liver disease (NAFLD), but the mechanisms are poorly understood (1).
- The progression and severity of NAFLD is marked by oxidative stress, inflammation and increased collagen synthesis (fibrosis) (2).
- Non-reparative macrophage are a novel subtype that do not resolve inflammation but are not classically activated, and do not facilitate tissue healing or repair. Their function might contribute to fibrosis.
- Fetal livers exposed to maternal western-diet (mWD) have increased oxidative stress, increased dendritic cells (DCs), and periportal fibrosis with hepatic stellate cell (HSC) activation (3).



References:
1. Mandala A et al. Nutrients. 16 Oct. 2020.
2. Wesolowski SR et al. Nat. Reviews Gastroenterol Hepatol. 16 Oct. 2016
3. Nash MJ et al. J Clin. Investig. Insight. 22 Dec. 2021 (in press)

HYPOTHESIS

Hypothesis: Exposure to mWD drives persistent oxidative stress and non-reparative macrophage, promoting fibrosis.



Objectives:
- Determine the effect of mWD on liver fibrosis
- Test whether mWD and pwWD drive oxidative stress, and whether mitochondria, a major ROS producer, contribute to this
- Test whether liver macrophage are programmed towards non-repair long-term due to mWD, and whether there are increased DCs in mWD-exposed livers

METHODS

Second Harmonic Generation (SHG) imaging: For each animal (**Figure 2A**), eight to twelve images (containing one to two portal triads or central veins) of 5 mm thick liver paraffin sections were were imaged using a Zeiss 780 LSM laser-scanning confocal/multiphoton-excitation fluorescence microscope. SHG signal was quantified with FIJI software.

RNA-scope and liver macrophage staining: Probes directed to ACTA2 or TIMP (**Figure 2B**), or an antibody targeted to CD68 (**Figure 4**), were applied to 5 mm thick liver paraffin sections and the number of cells positive for ACTA2, TIMP1, both, or CD68 were quantified per portal triad with ImageScope software or FIJI software, respectively.

Protein analysis: Liver whole cell extracts were analyzed with western blot via the Jess (**Figure 4**) or LiCor system (**Figure 5B**). Proteins were normalized to total protein content or vinculin.

Single cell RNA-sequencing: Non-parenchymal mononuclear cells were isolated from livers via histodenz gradient, then cryopreserved. Cells were thawed and flow sorted for 50,000 live cells, for n=3 CD/CD and n=3 WD/CD, then sequenced. Differential gene expression by diet and per cell cluster was identified and analyzed by custom R packages (**Figure 6, 7**).

Graphical summary figures were created with BioRender.

ACKNOWLEDGEMENTS

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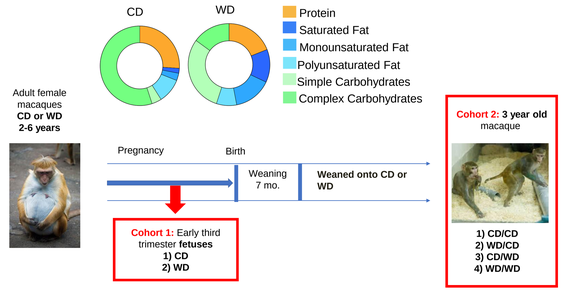
MODEL

Non-human primate (NHP) adult females were fed WD (western-style diet, 37% fat calories) or CD diet (15% fat calories) prior to pregnancy and during subsequent pregnancies.

Offspring were studied in two cohorts:
1. Early 3rd trimester fetuses (n = 31 CD, 53 WD)
2. 3 year old (3YO) juveniles weaned to a CD or WD diet yielding four groups (CD/CD, WD/CD, CD/WD, WD/WD).



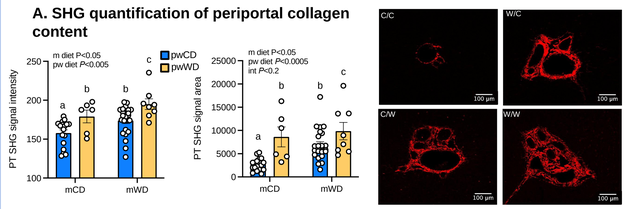
1. 3YO NHP EXPOSED TO mWD OR pwWD ARE NOT OBESE BUT HAVE SIGNS OF INSULIN RESISTANCE AND LIVER DAMAGE

MATERNAL DIET	CD	WD	CD	WD	*MATERNAL	*POSTWEAN	*INTERACTION
POSTWEAN DIET	CD	CD	WD	WD			
Body fat, %#	15.9 ± 0.3	15.5 ± 0.4	13.2 ± 1.2	12.8 ± 0.9	0.5	<0.0001	0.99
Liver weight (normalized), mg/g	22.0 ± 0.26	21.1 ± 0.42	19.3 ± 0.68	19.5 ± 0.53	0.781	<0.001	0.560
Insulin, µU/mL	4.5 ± 0.64	6.41 ± 1.15	21.8 ± 7.39	10.0 ± 1.92	0.021	<0.0001	0.002
ALT, IU/mL	44.0 ± 2.04	43.4 ± 1.80	52.6 ± 5.87	65.3 ± 4.98	0.073	<0.0001	0.049
AST, IU/mL	38.5 ± 1.52	38.3 ± 1.49	37.0 ± 1.52	44.1 ± 2.41	0.127	0.352	0.116

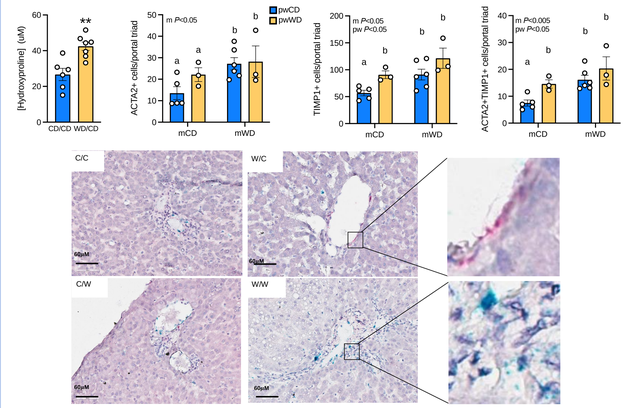
Body fat composition was measured by DEXA
* *P*-value from two-way ANOVA.

2. 3YO WD NHP LIVERS HAVE PERIPORTAL FIBROSIS

A. SHG quantification of periportal collagen content

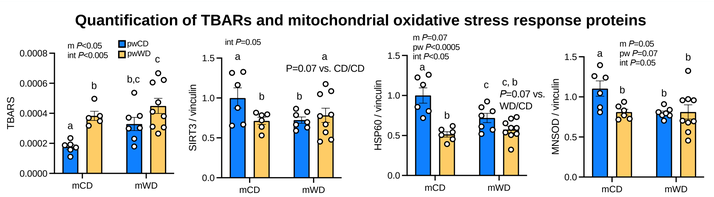


B. Quantification of periportal HSC activation via RNA-scope

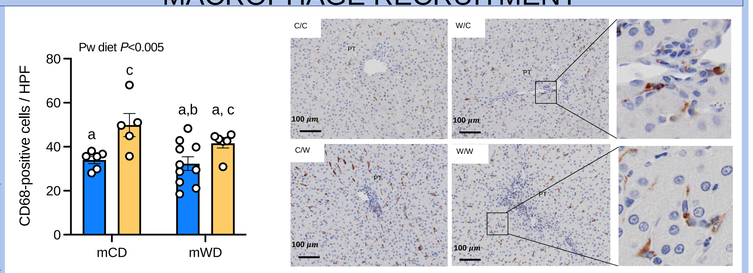


3. 3YO WD LIVERS HAVE DECREASED OXIDATIVE STRESS RESPONSE

Quantification of TBARs and mitochondrial oxidative stress response proteins

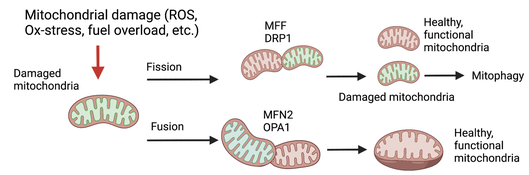


4. pwWD LIVERS HAVE INCREASED LIVER MACROPHAGE RECRUITMENT

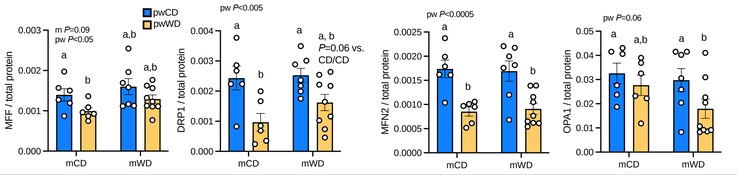


5. pwWD LIVERS HAVE DECREASED MITOCHONDRIAL QUALITY CONTROL

A. Schematic of mitochondrial fission and fusion

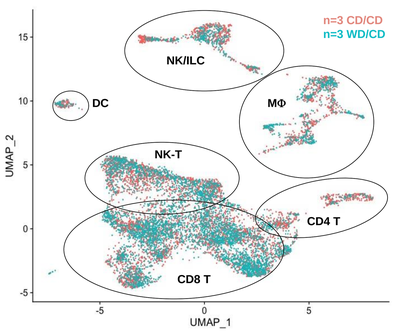


B. Quantification of mitochondrial fission (MFF, DRP1) and fusion (MFN2, OPA1) proteins in livers

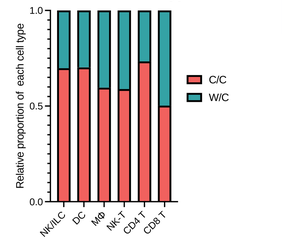


6. PROPORTIONS OF DENDRITIC CELLS AND OTHER LIVER IMMUNE CELLS ARE DECREASED BY mWD

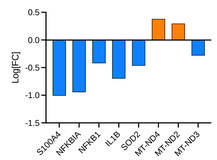
A. scRNA analysis of liver immune cell populations



B. Proportions of immune cell types in WD/CD livers

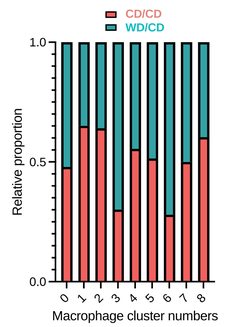


C. Differentially expressed genes in DCs by mWD

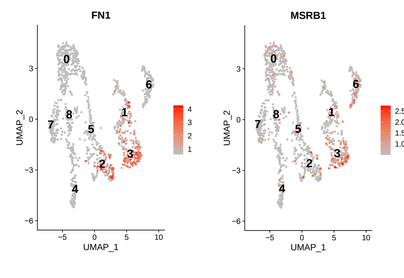


7. mWD PROMOTES NON-REPARATIVE LIVER MACROPHAGE

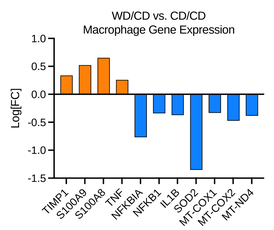
A. scRNA analysis and relative abundance of liver macrophage subsets

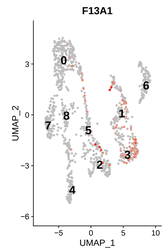


B. Clusters 3 and 6 are characterized by *FN1, MSRB1,* and *F13A1* expression, genes mediating MΦ-HSC signaling consistent with a non-reparative phenotype

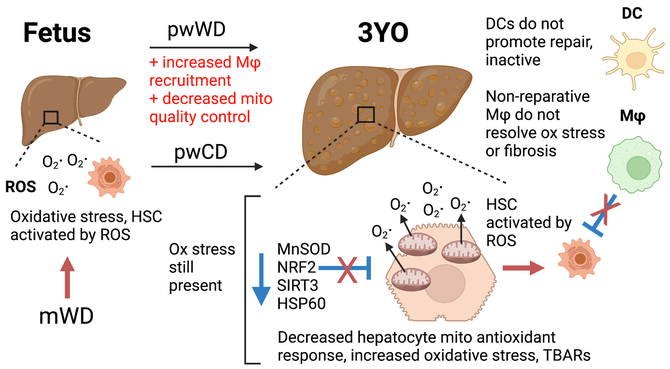


C. Top fold change genes by diet in total macrophage





SUMMARY & CONCLUSIONS



Our results indicate a paradigm for pediatric NAFLD development that begins with mWD-driven oxidative stress, loss of antioxidant activity, and a non-reparative liver macrophage phenotype, all of which promote HSC activation and fibrosis. pwWD drives mitochondrial dysfunction and macrophage recruitment, which may worsen oxidative stress and fibrosis further.

- mWD causes periportal fibrosis and oxidative stress which persists at 3YO
- mWD and pwWD decrease mitochondrial antioxidant response
- pwWD decreases mitochondrial quality control and drives liver macrophage recruitment
- mWD reduces liver DC content and activation, and decreases CD4 T-cells and NK cells
- mWD confers a non-reparative macrophage phenotype in livers

FUTURE DIRECTIONS

- Identify whether liver bile acids, which modulate oxidative stress, are increased or decreased due to mWD
- Identify whether epigenetic silencing of major antioxidant NRF2 underlies oxidative stress in WD/CD livers
- Identify whether mWD-exposed bone marrow derived immune cells share a similar non-reparative phenotype as liver macrophage
- Michael.Nash@CUAnschutz.edu for questions!