Title: Ultrastructural changes are highly prevalent in pediatric non-alcoholic fatty liver disease and not limited to presence of steatohepatitis.

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Non-alcoholic fatty liver disease (NAFLD) is common in obese patients, but steatohepatitis (NASH) develops in a minority. NASH pathogenesis includes mitochondrial dysfunction. Higher prevalence of ultrastructural mitochondrial abnormalities was reported in adult cohorts with NASH, but data are lacking in children. We determined the frequency of ultrastructural changes in liver biopsies from obese adolescents. We hypothesized that NASH by light microscopy (LM) would be associated with greater mitochondrial abnormalities than fatty liver or histologically normal appearing liver in obese children. **Methods:** We retrospectively analyzed 62 adolescents with liver biopsies and electron microscopy (EM) done to evaluate for NAFLD between 2003 -2012 at CCHMC. H&E and Masson trichrome stained slides were reviewed using established NASH CRN criteria to grade steatosis, lobular/portal inflammation, hepatocellular ballooning and to stage fibrosis. 4 groups were created using LM criteria: definite NASH, borderline NASH, non-NASH fatty liver, and normal liver. EM images were scored semi-quantitatively for pleomorphism, frequency of granules and crystalline inclusions; presence or absence of megamitochondria; frequency of autophagic vacuoles; frequency and size of lipid droplets; dilation of smooth and rough endoplasmic reticulum; prevalence of sinusoidal Ito cells and inflammatory foci. Distribution of EM variables across NAFLD (LM) categories was evaluated by Fisher's Exact Test. **Results:** Fifty-five % had NASH, 27% non-NASH fatty liver, and 18% "normal" not fatty liver by LM. Mitochondrial number was normal, but 91% of subjects had mild-moderate mitochondrial pleomorphism. Autophagic vacuoles were present in 90% and sinusoidal inflammatory foci in 92%, with 63% having multiple foci, even in histologically normal liver. Lipid droplets were present by EM in all subjects, but were smaller in size in those without lipid by LM ($p=0.04$). **Conclusions:** Ultrastructural abnormalities were identified in the majority of subjects, including mitochondrial pleomorphism and swelling, inflammatory foci, autophagic vacuoles and lipid droplets, even in "normal" not fatty liver by LM. This suggests that these ultrastructural changes are systemic responses to obesity. We will determine whether systemic insulin resistance or lipid abnormalities are associated with the distribution and severity of the ultrastructural changes identified in this pilot study.

Table: Ultrastructural Characteristics of Cohort		
Electron Microscopy Variables		N (%) 62 (100)
Mitochondrial Changes		
Number:	*Normal*	61 ()
	Increased	1 ()
Pleomorphism:	*Normal shape*	5 (9)
	Mild	39 (68)
	Moderate	13 (23)
Crystalline inclusions:	*None*	40 (65)
	Rare	9 (14)
	Prominent	13 (21)
Swelling:	*None*	26 (42)
	Rare/isolated	26 (42)
	Prevalent/isolated	6 (10)
	Prevalent/severe	4 (6)
Autophagic vacuoles		
	None	-
	Rare	6 (10)
	Increased	56 (90)
Endoplasmic Reticulum Dilation/Increase		
Smooth	*None*	9 (15)
	Mild	43 (69)
	Moderate Severe	10 (16)
Rough	*None*	22 (36)
	Mild	7 (11)
	Moderate	3 (5)
	Severe	30 (48)
Inflammation		
Ito (Stellate) cells	*Normal*	52 (84)
	Increased	10 (16)
Inflammatory foci	*None*	5 (8)
	Rare (1)	18 (29)
	Multiple (>1)	39 (63)
Lipid Droplet		
Frequency	*Mild*	22 (36)
	Moderate	38 (61)
	Severe	2 (3)
Size	*Small*	18 (29)
	Variable (small-large)	44 (71)