

Choline Deficient High Fat Diet for Modeling Human Liver Disease

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INTRODUCTION

- It is estimated that 5% of the US population has metabolic associated steatohepatitis (MASH).
- Choline is a precursor for phosphatidylcholine, which is required for the assembly and secretion of very-low-density lipoproteins which transport triglycerides and cholesterol out of the liver.
- Without sufficient choline, triglycerides and cholesterol accumulate in hepatocytes, leading to hepatic steatosis.

AIM

- The purpose of this study was to characterize disease progression in a CDHFD rat model and assess its translation to human disease.

METHODS

- Male Wistar Han rats were fed either a control diet (LabDiet 5CR4) or L-Amino Acid Diet With 45 kcal% Fat With 0.1% Methionine, No Added Choline and 1% Cholesterol (CDHFD; Research Diets, Cat #: A16092003) for 4, 8, 12, 16, or 20 weeks. At each time-point serum and liver samples were collected.
- Histology/Immunohistochemistry (iHC): Formalin-fixed, paraffin-embedded samples of rat CDHFD liver sectioned at 4um and placed on microscopic slides. Sections of fat CDHFD liver were stained with Picrosirius red histochemistry and ED1 iHC. For ED1 iHC, slides were deparaffinized and rehydrated through graded alcohols, followed by antigen-retrieval and quenching of endogenous peroxidases. Sections were incubated with anti-mouse ED1 antibody (1:100, BioRad, cat#MCA341R), washed, and incubated with horseradish peroxidase (HRP)-conjugated anti-mouse IgG secondary antibody, detected with 3,31-Diaminobenzidine (DAB) chromogen, counterstained with hematoxylin, dehydrated through graded alcohols, cleared in xylene and mounted on microscopic slides. All slides were scanned with an Aperio slide scanner for microscopic examination by a board-certified veterinary pathologist (MJH).

SUMMARY

- CDHFD is a model of severe liver disease with steatosis, inflammation, and progressive fibrosis that develops to cirrhosis and end stage liver disease by 20 weeks.

Acknowledgments

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RESULTS

1. Physiological

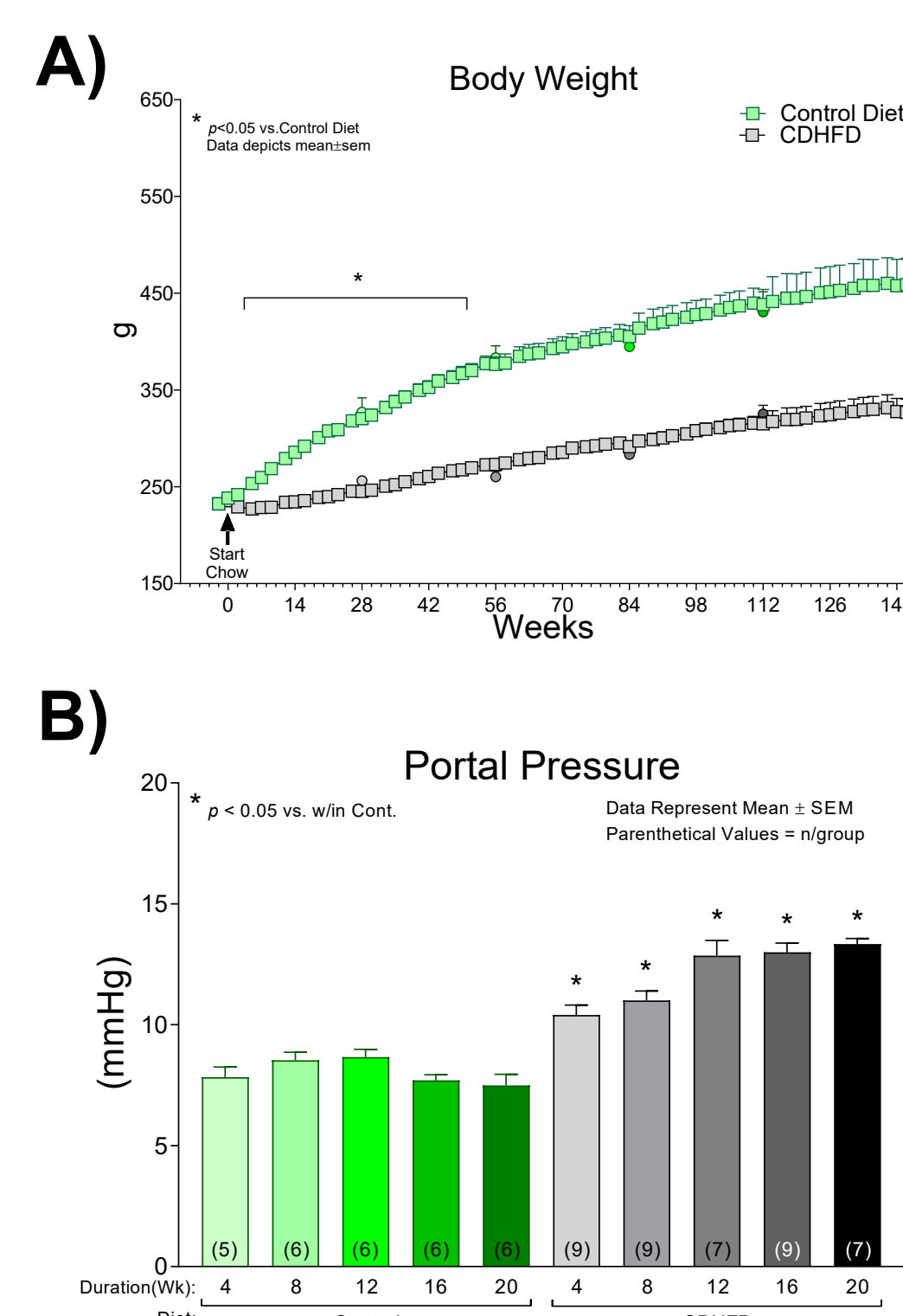


Figure 1. Physiological characteristics for animals receiving CDHFD or control chow. **(A)** Body weight, **(B)** Portal vein pressure, following up to 20 weeks of CDHFD. Animals receiving CDHFD had decreased body weight, and increased portal vein pressure at all timepoints. * = $p < 0.05$ vs control diet.

2. Serum Chemistry and Plasma Inflammatory Markers

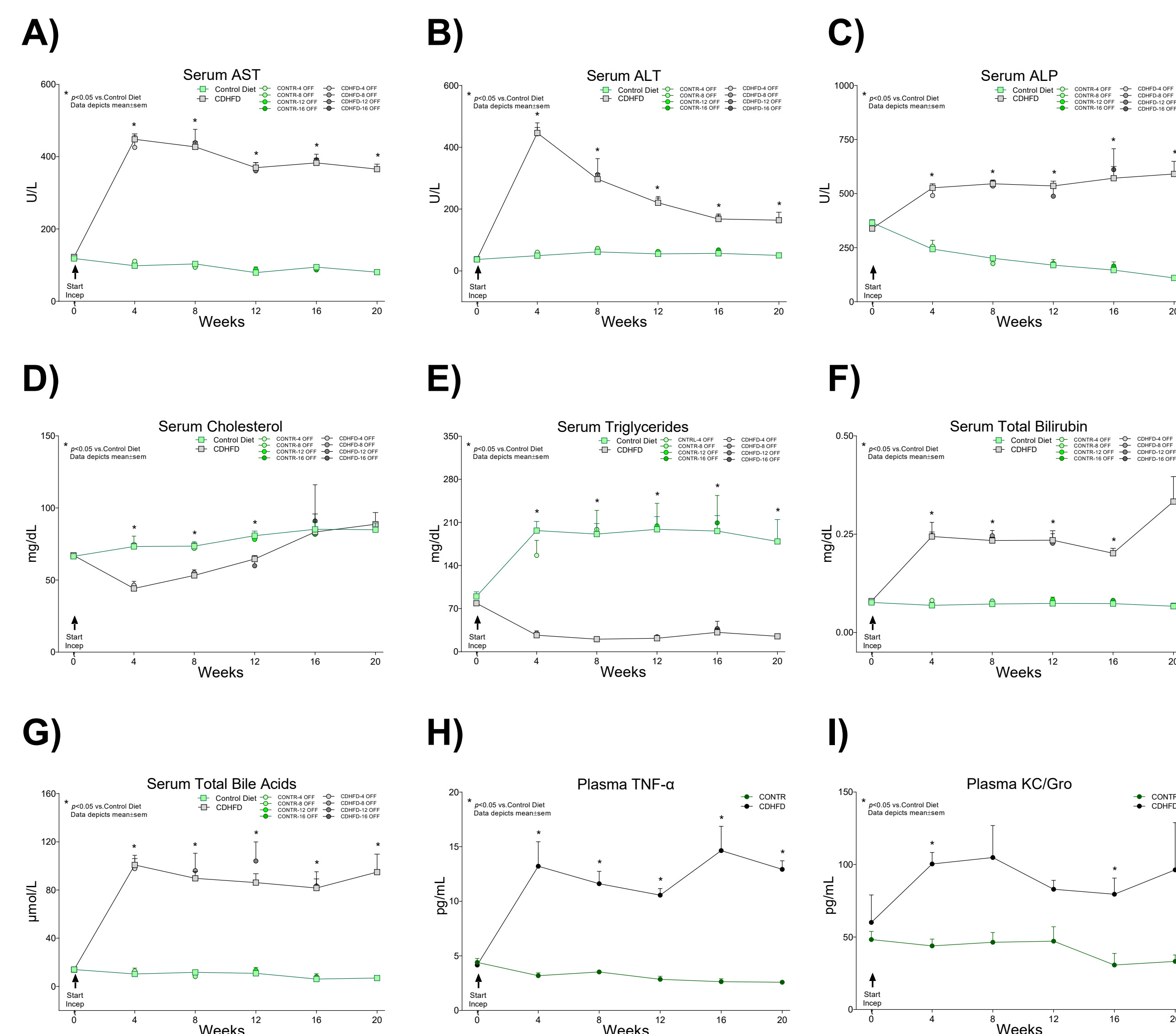


Figure 2. Serial serum clinical chemistry for assessment of liver function and plasma MSD for circulating inflammation. **(A)** AST, **(B)** ALT, **(C)** ALP, **(D)** Total cholesterol, **(E)** Triglycerides, **(F)** Total bilirubin, **(G)** Total bile acids, **(H)** TNF α , and **(I)** KC/Gro, following up to 20 weeks of CDHFD. Animals receiving CDHFD had elevated circulating liver enzymes, decreased serum lipid content, elevated bilirubin, elevated bile acids, and increased plasma inflammatory markers. These changes taken together indicate compromised liver function in the setting of CDHFD. * = $p < 0.05$ vs control diet.

3. Liver Biochemical

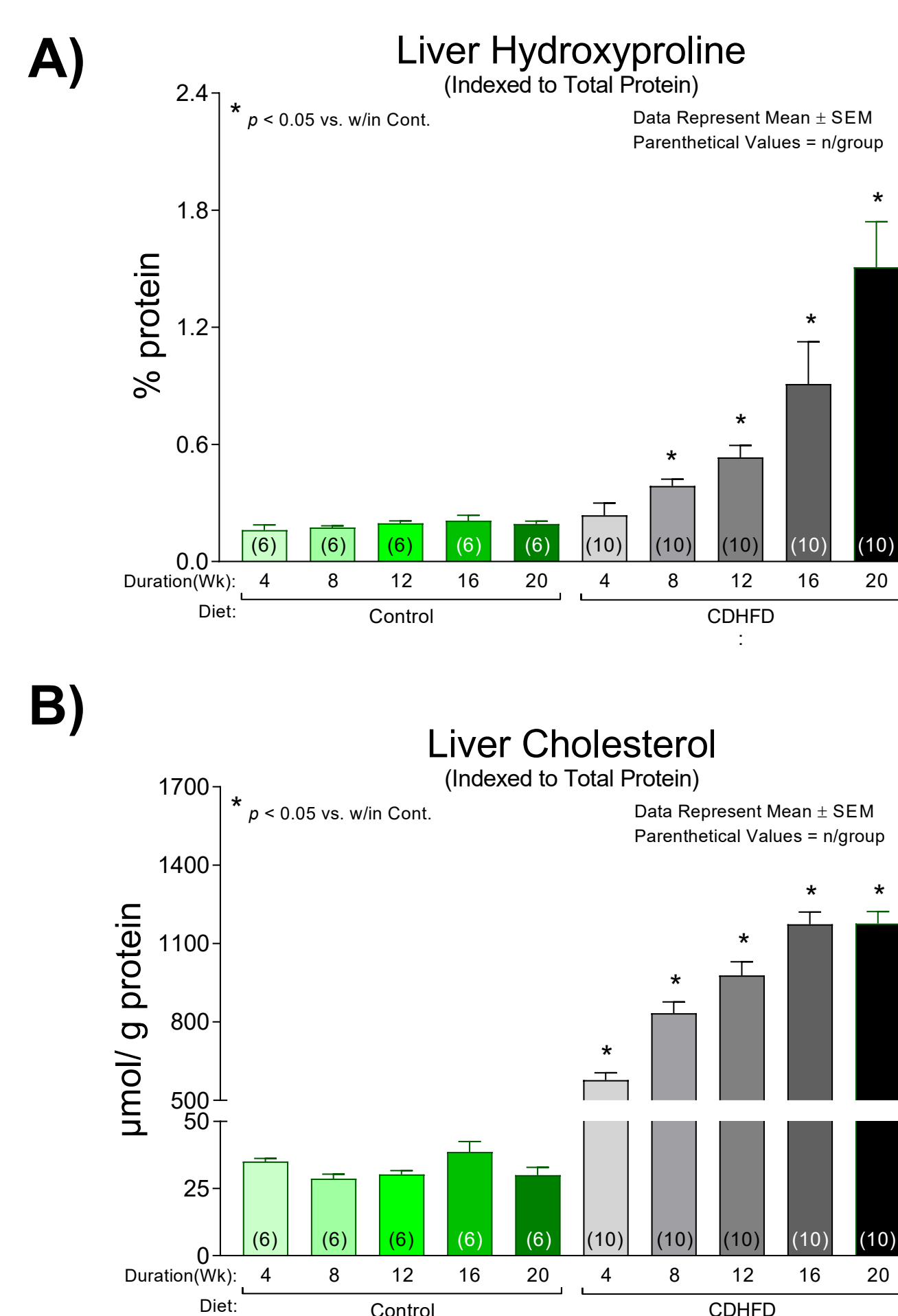


Figure 3. Biochemical assessment of liver tissue fibrosis and lipid content. **(A)** Hydroxyproline, **(B)** Total cholesterol following up to 20 weeks of CDHFD. Animals receiving CDHFD had increases in liver hydroxyproline (fibrosis) and cholesterol over the duration of diet. * = $p < 0.05$ vs control diet.

4. Histopathological Evaluation and Image Analysis

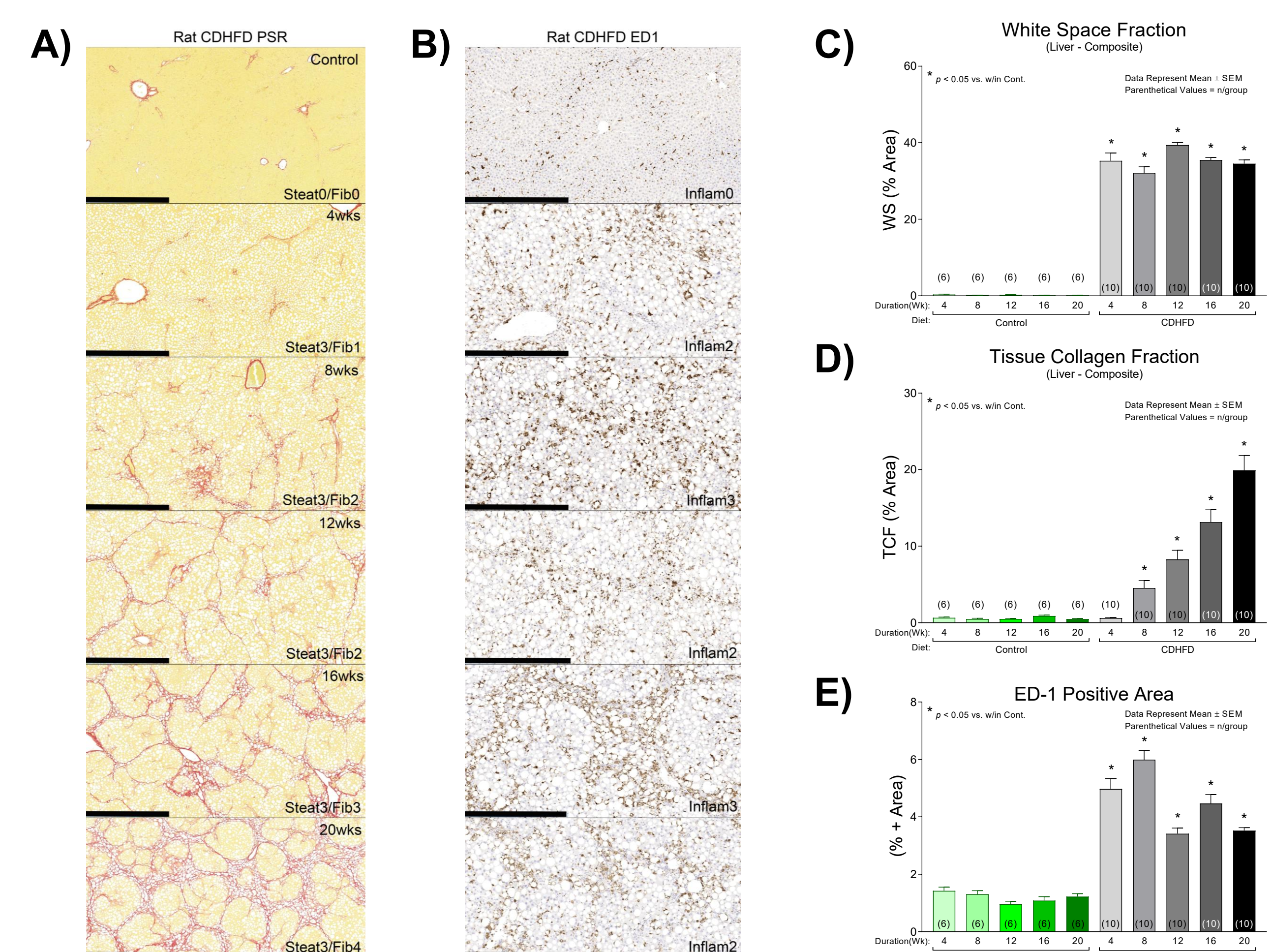


Figure 4. Histopathological evaluation and image analysis of fibrosis and inflammation in the CDHFD treated liver. **(A)** Rats treated with CDHFD have diffuse and severe steatosis/lipidosis at all time points and progressive hepatic fibrosis leading to cirrhosis by 20 weeks on diet (Picrosirius red histochemical staining, Bar = 1mm). **(B)** In rat CDHFD liver, there is hyperplasia of resident macrophages (Kupffer cells) progressing from 4 days, suggestive of cleanup during progressive hepatocellular degeneration, with overall reduction by 20 weeks as tissue remodeling and fibrosis progresses to cirrhosis. **(C-E)** Image analysis of **(C)** White space fraction (steatosis), **(D)** Tissue collagen fraction, and **(E)** ED-1 positive area.