

Introduction: The broad aim of my proposal is to study the mechanisms of unbound fatty acid binding and uptake by cell membranes and the fatty acid- protein interactions in eliciting biological responses. This will help in understanding how unbound NEFAs worsen severe acute pancreatitis. To conduct this, my proposal comprised 3 specific aims and the progress on each is detailed below. We worked on Aim1 and 2 and have nearly

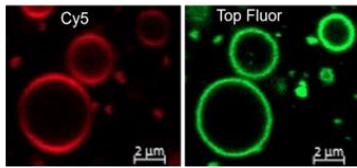
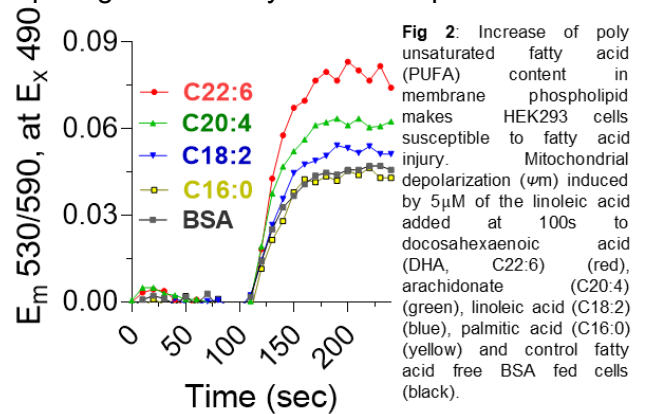


Fig 1: Fatty acid uptake by protein free membrane systems. Giant unilamellar vesicles made up of DOPC:POPG (9:1 molar ratio) with trace amount of fluorescent Cy5 DOPC lipids (red) uptake linoleic acid containing trace amounts (0.8%) of OA Top Fluor (green). Final concentration of linoleic acid used for uptake is 100μM.

accomplished Aim 2B, i.e. to understand the role of fatty acid receptors CD36 in severe acute pancreatitis.

Aim1: Determine the effect of liposomal membrane acyl chain composition on μ NEFA uptake. Giant unilamellar vesicles (GUVs) made up of DOPC, and POPG (9:1 molar ratio) also uptake unbound linoleic acid (LA) with trace amount of a fluorescent reporter (Oleic acid -Top Fluor) as (**Fig 1**). This is an extension of the unbound fatty acid uptake by POPC: POPG GUVs showed in the grant application. Ongoing work is comparing different acyl chain on uptake.

Aim2: Determine the role of membrane composition in μ NEFA uptake and Cai, Ψ m



in cells. We observed trends showing that increased PUFA content make (HEK293) cells more responsive to unbound LA (**Fig 2**). Also, we found that linolenic acid caused similar mitochondrial damage in splenocytes of CD36^{-/-} (KO) and wild type cells (**Fig 3**), as previously shown in peritoneal macrophages in the grant application.

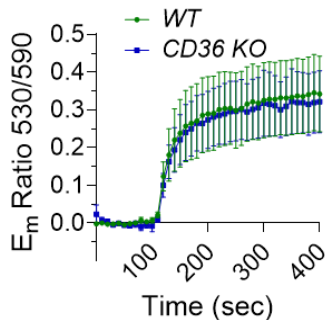


Fig 3: Deletion of CD36 does not change responsiveness towards fatty acids in splenocytes. Time course of Ψ m induced by 10μM unbound linoleic acid in splenocytes from wildtype (green) or CD36 knockout (blue) mice. uFAs

We tested whether CD36 deletion protects from lipotoxicity during severe acute pancreatitis using the IL12,18 +/- glyceryl trilinoleate (GTL).

We found that the initiation, progression, and outcomes in CD36 KO and WT were similar (**Fig 4 A, B, C, D & E**). We observed 0% survival in CD36 KO and WT mice receiving IL12/18 plus GTL while IL12/18 alone did not affect the survival in either strain. Histology analysis of pancreas showed similar acinar necrosis (5-7%) in both CD36 KO and WT mice strains (**Fig 4C**). Elevated BUN and creatinine levels supporting renal injury, was observed in both CD36 KO and WT mice receiving IL12/18 plus GTL. (**Fig 4 D & E**). Our results suggest no specific role of CD36 in fatty acid uptake

during severe acute pancreatitis when excess fatty acid release take place because of visceral fat lipolysis.

Aim3: Determine effect of membrane phospholipids on interaction of membrane proteins with μ NEFA. This aim is ongoing and is in the standardization phase. I have made progress showing the *in vitro* activity of the purified SERCA protein (ATPase activity) and its inhibition by fatty acid which needs to be repeated and confirmed.

Accomplishments:

1. Abstract titled 'The fatty acid translocase CD36 do not play a role in worsening of lipotoxic acute pancreatitis', got accepted in the upcoming Digestive Disease Week 2024 Conference.

2. Research article discussing the role of CD36 in acute severe pancreatitis will be communicated shortly.

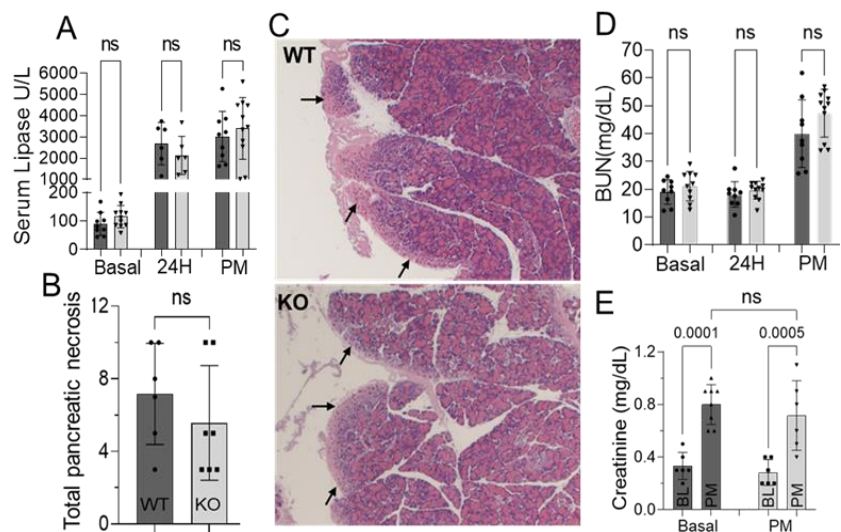


Fig 4: Initiation and progression towards severe acute pancreatitis in wild type and CD36 KO mice. (A) Serum lipase levels in mice injected with IL12 & 18 + GTL in WT (dark gray bar, n=6) and CD36 KO mouse (light gray bar, n=6) at baseline, 24 and postmortem. (B) Bar graph comparing overall pancreatic necrosis in WT (dark gray bar, n=6) and CD36 KO mouse (light gray bar, n=6). (C) H&E stained 10X magnification images of pancreas of WT and CD36 KO mouse injected with IL12 & 18 + GTL. (D & E) Bar graphs comparing serum blood urea nitrogen and creatinine at baseline and postmortem in wild type (dark gray bar, n=6) and CD36 KO (light gray bar, n=6) mouse injected with IL12 & 18 + GTL.