Hepatic Lipase Deficiency (HLD) is an autosomal recessive disorder that is characterized by elevated triglyceride and cholesterol levels in the blood of affected patients [1, 2]. HLD is caused by mutations in Lipase C gene (LIPC) which encodes the enzyme hepatic lipase (HL). Hepatic lipase is predominantly involved in the conversion of intermediate-density lipoproteins into low-density lipoproteins and triglyceride-rich high-density lipoproteins (HDL) found during the well-fed state into triglyceride-poor HDL found in the fasting state [2]. There are multiple isoforms of LIPC. The full-length isoform is expressed and secreted by the liver, where it carries out the functions mentioned above. The truncated isoform of LIPC is expressed in steroidogenic tissues where it acts intracellularly and may play a role in reproductive processes. Hepatic lipase deficiency has been associated with reduced reproductive potential in mice, *though the exact mechanism for this problem has not been investigated* [3]*.* Determining how reduced LIPC activity contributes to lowered reproductive potential will uncover potential treatments for patients with hepatic lipase deficiency.

The **long-term goal** of this project is to identify how LIPC is involved in reproduction to improve infertility treatments for patients with hepatic lipase deficiency. The **goal** of this specific project is to identify how LIPC isoforms interact to promote cholesterol substrate uptake. My **hypothesis** is that the extracellular form of LIPC promotes remodeling of intermediate-density lipoproteins into low-density lipoproteins that are taken up by the granulosa cells while the intracellular truncated isoform of LIPC mobilizes cholesteryl esters from lipid droplets to free the cholesterol for the synthesis of progesterone and other reproductive hormones. *Rattus norvegicus* will be used as a model organism due to its similar progesterone synthesis mechanism to humans and the presence of a close ortholog to human LIPC [4, 5].

**AIM 1: Investigate the effects of 3 glycosylated residues in exons one and two of full-length hepatic lipase.**

**Approach:** Domain analysis has revealed the existence of three conserved glycosylated residues in exons one and two of LIPC. To investigate the purpose of these residues, I will mutagenize them using CRISPR-Cas9 to prevent their glycosylation. The effects of these changes on LIPC activity and localization can then be measured through triglyceride lipase activity assays and fluorescence microscopy.

**Rationale:** Glycosylation is important for the function of secreted proteins. The presence of these conserved residues in only the full-length LIPC isoform suggest that they are important for extracellular LIPC function.

**Hypothesis:** Mutagenesis of these conserved glycosylation residues will reduce LIPC function and potentially inhibit secretion. If these sites are the drivers for full-length LIPC function, I may see a complete switch to the truncated LIPC isoform activity.

**AIM 2: Characterize differentially expressed genes in rats that only express truncated LIPC or full-length LIPC.**

**Approach:** I will create two mutant rat lines. One will have a mutation in the exon two splice site that prevents the truncated version of LIPC from being created. In other words, this line will only express full-length LIPC. The other line will have a deletion of exons one and two, meaning all the LIPC it expresses will be in the truncated isoform. Once these lines are created, I will extract the livers and ovaries from each line for RNA-sequencing. I will then compare the results to those of a wildtype mouse line to detect changes in gene expression between wildtype mice and the mutants.

**Rationale:** RNA sequencing will determine differential gene expression of genes that are affected by the pathways involving both full-length and truncated LIPC.

**Hypothesis:** I expect to see differential expression of genes involved in lipoprotein remodeling in the mutant line that only expresses truncated LIPC. Likewise, for the line that only expresses full-length LIPC I would expect to see differential expression of genes involved in lipid droplet remodeling.

**AIM 3: Identifying protein interactions of both truncated and full-length LIPC isoforms**

**Approach:** The truncated-only and the full-length-only LIPC mutant rat lines will be used to study the protein interactions of each isoform. I will make lysates from the liver and ovaries of rats from each mutant line and perform a tandem-affinity purification (TAP) against LIPC which I will then run through mass spectrometry analysis to identify proteins interacting with truncated or full-length LIPC isoforms.

**Rationale:** Performing TAP and mass spectrometry will provide direct evidence for the proteins that interact with the full-length and truncated isoforms of LIPC.

**Hypothesis:** I expect that proteins involved with lipoprotein and chylomicron remodeling interact with the full-length LIPC isoform while proteins involved in lipid droplet remodeling interact with the truncated isoform of LIPC.

Treatment guidelines for Hepatic Lipase Deficiency (HLD) focus only on managing serum lipid levels and not on other consequences of reduced LIPC activity. By investigating the role LIPC plays in reproduction, I can further characterize the scope of HLD and improve treatment guidelines to address all complications of HLD rather than just its symptoms.

**References**

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