

Jon Gonzales

Med-into-Grad: Metabolic Syndrome

Proposal: The Transport of Lipoprotein Lipase in the Metabolic Syndrome

The focus of my Med-into-Grad training consisted of various clinical aspects involved in the diagnosis and treatment of the morbidities in the Metabolic Syndrome. This included attending diabetes and lipid outpatient clinics, endocrinology and cardiology grand rounds, and diabetes and endocrinology case conferences. Through these experiences I gained a better appreciation for the complexity involved in understanding and treating the many symptoms and co-morbidities in each patient. As a PhD student, these clinical experiences have enhanced my understanding and ability to apply research to clinically relevant questions. Currently, my thesis research is focused on elucidating the mechanisms involved in the transport of lipoprotein lipase (LPL).

LPL is the critical enzyme responsible for the hydrolysis of triglyceride-rich lipoproteins in the peripheral circulation (1). Cells, such as cardiac and skeletal myocytes, macrophages, and adipocytes synthesize and secrete LPL. It then traverses the basement membrane separating these cells from the microvasculature in the tissue. The enzyme is transported across the endothelial cells to its site of action on the lumen of the endothelium.

[Impairments in the function or metabolism of LPL can lead to hypertriglyceridemia](#), a major risk factor for cardiovascular disease (1, 2). [Genetic mutations that disrupt the function of LPL have been documented in patients \(3\)](#), but [whether impairments in the transport of LPL cause disease is unknown](#). [A recent study by Bishop et al. showed the importance of collagen XVIII in LPL transport and triglyceride homeostasis in patients with mutations in collagen XVIII](#) (Bishop et al, submitted manuscript). [Conceivably, other forms of hypertriglyceridemia could result from alterations in LPL transport across the endothelium, but little is known about the transport process.](#)

The metabolic syndrome is defined by impaired metabolic conditions resulting in obesity, insulin resistance, dyslipidemia (including elevated triglyceride levels), and hypertension (4). Obesity and poorly controlled type 2 diabetes are the most common conditions associated with hypertriglyceridemia, and are often the result of increased plasma concentrations of the Very Low Density Lipoproteins (VLDL) and free fatty acids, and/or decreased LPL activity (5). This high frequency of association suggests that the metabolic stressors of obesity and diabetes may unmask subtle metabolic defects in lipid metabolism, resulting in hypertriglyceridemia (4, 5). Multiple studies have examined the gene expression and regulation of LPL in the diabetic and obese settings, but little work has been done investigating the transport of the enzyme. I hypothesize that obese and diabetic patients may have hypertriglyceridemia as a result of impaired LPL transport.

In the completion of my graduate work, I will have developed and employed in vitro and in vivo techniques to examine LPL transit. I propose that these techniques can be applied to test my hypothesis that LPL transport is impaired in the obese and/or diabetic settings. I could test this hypothesis through two specific assays developed during my thesis research:

[Assay 1: Measuring LPL transport across endothelial cells](#). A transwell assay will be employed to measure LPL transport across primary microvascular endothelial cells. A

tagged form of the enzyme will be tracked throughout its transport. Transwell assays using endothelial cells isolated from wild-type mice, db/db mice (a mouse model for diabetic dyslipidemia (6)), or ob/ob mice (a genetically obese mouse model (7)), will test LPL transport across endothelial cells in each genetic model.

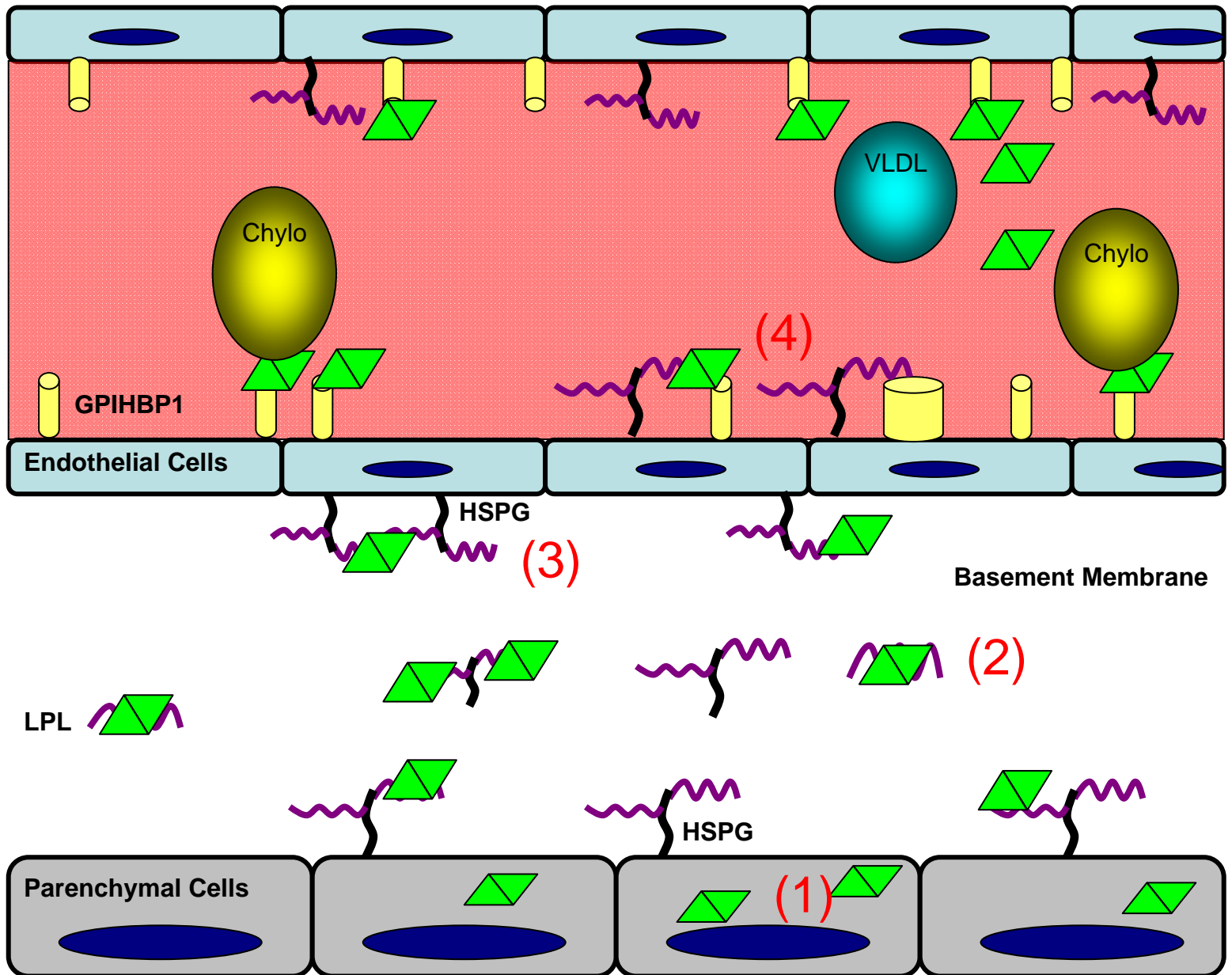
To measure transport of LPL, a tagged form of LPL will be added to the bottom chamber of the transwell, and the amount of tagged LPL appearing in the upper chamber will be assessed at various time points. To determine if the transported LPL is functional, LPL will be added to the lower chamber and the mass and lipase activity appearing in the upper chamber will be determined (17). Both the kinetics and concentration dependence of LPL transport will be measured for each cell type. This includes a time course measuring LPL in the upper chamber, whether it is bound to the apical cell surface or free, and measuring the amount of LPL transported at various enzyme concentrations. Importantly, tracking LPL mass and activity will allow us to determine if the active form of LPL is transported.

Assay 2: Detecting LPL transport in vivo. The transport of LPL in vivo will be studied using the mutant mouse models. Impairments in LPL transport would presumably result in an accumulation of tissue LPL, most likely in a subendothelial compartment. To determine the localization of LPL in the mouse models, three different studies will be performed. First, we will measure the total pool of LPL in the tissue. Next, the amount of enzyme bound to the luminal side of the endothelium can be measured by displacement of bound enzyme after an intravenous injection of a bolus of Intralipid, a fat emulsion (8). Finally, the pool of heparin-releasable LPL will be measured. This is thought to consist of LPL bound to the luminal side of endothelial cells plus LPL in sub-endothelial space (9). In this way, we can infer the location of LPL by comparing heparin-releasable and intralipid-releasable LPL in wild-type and mutant mice. In situ localization studies of tissue sections and western blotting will also be used to track the transit of LPL.

These two specific assays will test LPL transport in the genetic mouse models for obesity and diabetes. Evidence of impaired LPL transport in these models would encourage future studies of metabolic syndrome patients with hypertriglyceridemia. Indications of no difference in the transport of LPL in wild-type and obese or diabetic mice, would suggest that future studies remain focused on the expression and metabolic regulation of this enzyme.

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Figure 1. Current model for the transport of LPL



Lipoprotein Lipase (LPL) is synthesized and secreted as a dimer (1). It then diffuses across the sub-vascular basement membrane to reach the abluminal side of the endothelium. Along the way, LPL presumably comes into contact with one or more Heparan Sulfate Proteoglycans (HSPGs), such as collagen XVIII and perlecan in the basement membrane (2). LPL binds to the abluminal side of endothelial cells (3), and transcytosis across the endothelium results in its appearance on the luminal plasma membrane. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) binds LPL on the luminal side of the endothelium, where circulating lipoproteins encounter the enzyme (4). Here, the enzyme releases fatty acids for uptake and energy production.