

LE&RN/FDRS Lipedema Postdoctoral Fellowship Program

2015 Award Recipients

- **Echoe Bouta, Ph.D.**

Research Fellow

Massachusetts General Hospital

Mentor: Timothy Padera, Ph.D.

Mechanisms of Impaired Lymphatic Function in Mice with Increased Adiposity

Lipedema is a chronic disorder that results in increased adipose tissue in the lower limbs and manifests as dramatic, painful swelling. Clinical studies demonstrate that patients present with at least partial lymphatic dysfunction, further demonstrated by evidence that lipedema increases in severity until lymphedema occurs (lipo-lymphedema). Anecdotal evidence showing that patients have persistent infections suggests inhibited immune function. Unfortunately, as the etiology is largely unknown, treatments are often ineffective, demonstrating a need to understand the relationship between adipose tissue, lymphatic capability, and immune function at a basic science level to catalyze more effective therapies. Studies exploring this relationship will be performed in lean mice and mice with increased adiposity (obese). Lymphatic function will be analyzed using i) near infrared fluorescence to image initial lymphatic vessels and measure lymphatic pumping pressure, ii) Doppler optical coherence tomography (DOCT) to measure lymph velocity, and iii) intravital imaging to measure contraction strength, frequency, and valve dynamics. These established methods, including the first measurement of lymph velocity without injected contrast by DOCT, will elucidate the mechanism of lymphatic dysfunction. Similarly, we will perform a detailed analysis of the ability to mount an adaptive immune response by measuring immune cell trafficking to the lymph node and the ability of T- and B-cells to establish an antigen specific response. Then, we will determine the mechanism of lymphatic dysfunction associated with increased adiposity by performing causal studies of key pathways known to be important for lymphatic function in inflammatory conditions, including tumor necrosis factor and the PGE2/EP4 signaling pathway. Finally, we will restore lymphatic contraction by targeting calcium channels important for lymphatic contraction and measure immune function, which we hypothesize will increase by enhancing cell trafficking through lymphatic vessels. This study will provide critical knowledge about the etiology of lipedema and test potential therapies that target lymphatic function.

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2015 Award Recipients

- **Rachelle Crescenzi, Ph.D.**

Postdoctoral Fellow

Vanderbilt University Medical Center

Mentor: Manus J. Donahue, Ph.D.

Functional Imaging of Sodium and Lymphatics in Patients with Lipedema

Lipedema is a chronic and incurable condition characterized by thickening of the subcutaneous adipose tissue. Diagnosis is often complicated by similar symptomatology of lymphedema and obesity. Strong evidence suggests that impaired lymphatic flow reduces the ability of lymphatic-macrophages to metabolize adipose tissue and clear sodium from the interstitium, thereby exacerbating fluid retention. The critical barrier to diagnosing lipedema is a lack of quantitative imaging modalities for characterizing the molecular and pathophysiological features of lipedema.

Hypothesis (1): Noninvasive MRI methods optimized in our laboratory for upper-extremity lymphatic physiology can be translated to the lower extremities to enable reproducible quantitation of sodium concentration, lymphatic flow velocity, and interstitial protein accumulation.

Aim (1): We will utilize multinuclear imaging to measure sodium in calf dermis in sequence with proton imaging to evaluate lymphatic velocity and interstitial protein in the popliteal nodes and surrounding tissue in healthy volunteers. Results will establish the reproducibility and normative range of these measures in healthy tissue and provide an imaging protocol that can be used for future investigations of lipedema etiology.

Hypothesis (2): Elevated dermal sodium content is associated with reduced lymphatic flow velocity and elevated interstitial protein accumulation, and this is uniquely present in patients with lipedema relative to volunteers with normal or high body mass index.

Aim (2): We will apply the MRI protocol developed in Aim (1) to patients with lipedema, healthy and obese females. Significant differences between group measures will be evaluated using a one-way ANOVA. Results will reveal to what extent the hypothesized biomarkers are individually or collectively associated with lipedema.

Findings of this study will inform the pathophysiology of lipedema, which potentially involves impaired lymphatic function and elevated dermal sodium content. This study will further provide a foundation for quickly evaluating multiple imaging biomarkers which can distinguish lipedema from other adipose disorders.

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2015 Award Recipients

- **Javier Jaldin-Fincati, Ph.D.**
Postdoctoral Research Fellow
The Hospital for Sick Children
Mentor: Amira Klip, Ph.D.

Communication Between Adipose and Lymphatic Microvascular Endothelial Cells

Objective: To investigate the communication between adipocytes and lymphatic microvascular endothelial cells (L-MEC), as preamble to future examination of how this communication is affected in Lipedema. Specifically, to test the hypothesis that adipokines, fatty acids and excess insulin affect L-MEC metabolism and viability.

Adipose tissue is a highly plastic tissue that constitutes a meta-equilibrium of adipose, immune and endothelial cells, and is a major secretor of fatty acids and adipokines that control metabolism in liver and muscle. The tight endothelia of the blood microcirculation of adipose tissue cannot remove macromolecules from the interstitial space, hence contents leaving the tissue must exit through the open lymphatic microcirculation. Importantly, removal of insulin from the adipose or muscle interstitia is a key function of the lymphatic microcirculation, which thus plays a role in regulating the end of insulin action to avoid over-stimulation of adipocytes.

We hypothesize that adipokines and fatty acids secreted by insulin-responding and insulin resistant adipocytes affect the metabolic and survival properties of L-MEC.

Specific Aims:

1. To separate L-MEC and B-MEC from human adipose tissue.
2. To examine how insulin and fatty acids, alone and in combination, affect metabolic, inflammatory and survival pathways in L-MEC.
3. To investigate how human adipocytes, insulin-sensitive and insulin-resistant, affect metabolic and survival pathways in L-MEC.

Significance: There is scant information on the metabolic responses of L-MEC and on the communication between adipocytes and L-MEC. Our work will establish benchmark responses, to be investigated in the future in cells from patients with Lipedema. We hope to contribute to understanding this condition and the metabolic parameters of L-MEC in general. The postdoctoral fellow will learn how to approach a biomedical question from a fundamental perspective, to establish cell culture systems to test individual tenets, and to integrate concepts of metabolism, inflammation and cell survival.

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2015 Award Recipients

- **Annalisa Zecchin, Ph.D.**

Postdoctoral Fellow

University of Leuven, VIB

Mentor: Peter Carmeliet, M.D., Ph.D.

Supported through the Lipedema Foundation

Novel Metabolite-Based Treatment Approach of Lymphedema: Possible Relevance for Lipedema?

The lymphatic system is involved in lipid transport, inflammation and fluid homeostasis. We recently described that lymphatic endothelial cells (LECs) increase their fatty acid β -oxidation (FAO) during venous endothelial cell (VEC)-to-LEC differentiation, and this increased FAO is necessary to produce acetyl-CoA to fuel epigenetic modifications (histone acetylation) at lymphatic genes such as VEGFR3 for their differentiation and function. An important observation made was that supplementation of an exogenous source of acetyl-CoA (acetate) was able to recover impairments in VEC-to-LEC differentiation and lymphangiogenesis induced by inhibition of FAO.

In this research proposal, I hypothesize that LECs also utilize FAO to maintain lymphatic barrier function and the regulation of fluid, solute and lipid transport across the lymphatic endothelium, and aim to demonstrate that the VEGF-C/VEGFR3 signaling axis regulates lymphatic barrier function through the modulation of FAO, and that enhancement of this pathway with exogenous supplementation with acetate (or an alternative source of acetyl-CoA) can improve lymphatic function in surgical and genetic models of lymphedema

My preliminary results in this direction thus far suggest that inhibition of FAO in LECs impairs lymphatic barrier function, inducing increased extravasation of fluids and lymphedema during development. Furthermore, LEC-specific impairment in FAO in the adult leads to increased free fatty acid (FFA) levels in the lymph and decreased FFA levels in the blood.

My overall goal is to determine whether nutrient supplementation (acetate) to increase acetyl-CoA levels in LECs may improve lymphatic barrier function, either after surgery-induced lymphatic dysfunction, or genetic models which result in lymphedema (CPT1a conditional knock-out; VEGFR3 heterozygous-inactivating mutation).