The Link between Lymphatic Function and Adipose Biology

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Despite observations of a link between lymphatic vessels and lipids that date as far back as 300 BC, a link between lymphatic vessels and adipose tissue has only recently been recognized. This review will summarize documented evidence that supports a close relationship between lymphatic vessels and adipose tissue biology. Lymphatic vessels mediate lipid absorption and transport, share an intimate spatial association with adipose tissue, and regulate the traffic of immune cells that rely on specialized adipose tissue depots as a reservior of energy deployed to fight infection. Important links between inflammation and adipose tissue biology will also be discussed in this article, as will recent evidence connecting lymphatic vascular dysfunction with the onset of obesity. There seems little doubt that future research in this topical field will ensure that the link between lymphatic vascular function and adipose tissue is firmly established.

Key words: **lymphatic; lymphangiogenesis; obesity; adipogenesis; inflammation; lymph node; lymph**

Introduction

The first documented observation that a connection exists between lymphatic vessels and lipids dates as far back as *ca.* 300 BC, when the Ancient Greeks documented distinct mesenteric vessels filled with milk in suckling young.[1,2] In the 17th century, almost 2000 years later, Gaspar Aselli was credited with being the first to recognize the role of these vessels in lipid absorption and transport; he described the mesenteric lymphatic vessels in the gut of a dog that had consumed a lipid-rich meal as "white veins."[3] In extensive work that followed Aselli's initial recognition of the absorptive nature of these vessels, it was established that the "milky veins" he described in fact made up a vascular network distinct from the blood vasculature: the lymphatic vessels.[1] Work from many 17th century anatomists demonstrated that the lymphatic vessels of the mesentery drained their lipid-rich contents progressively via the cisterna chyli and thoracic duct to the bloodstream, at the junction of the thoracic duct with the great veins of the neck.[1] Were it not for the "illumination" of lymphatic vessels by ingested lipid-rich lymph, the discovery of these vessels would almost certainly have occurred much later in the course of history. While the role and mechanism of lymphatic vascular lipid transport have been extensively established since Gaspar Aselli's initial studies, a link between lymphatic vascular function and adipose tissue biology has only recently been recognized. This article will reflect on both historic and recent data that underpin a close relationship between lymphatic vessels and adipose tissue, and will consider the possibility that lymphatic vascular dysfunction may underlie a proportion of cases of human obesity.

Lymph Nodes, Lymphatic Vessels, and Adipose Tissue Depots

A close relationship exists between lymph nodes and adipose tissue; indeed, lymph nodes, the organizing centers of immune surveillance and response, are always found surrounded by adipose tissue. By extrapolation then, it follows that lymphatic vessels are also in close physical association with adipose tissue. Subcutaneous adipose tissue lies in close proximity to the dermal lymphatic vasculature (FIG. 1), while visceral adipose tissue surrounds the collecting lymphatic vessels of the mesentery, cisterna chyli and thoracic duct, as well as the efferent and afferent lymphatic vessels of intra-abdominal lymph nodes. The efferent and afferent lymphatic vessels of the superficial lymph nodes are also encapsulated by adipose tissue. Extensive work by Pond and colleagues has revealed that

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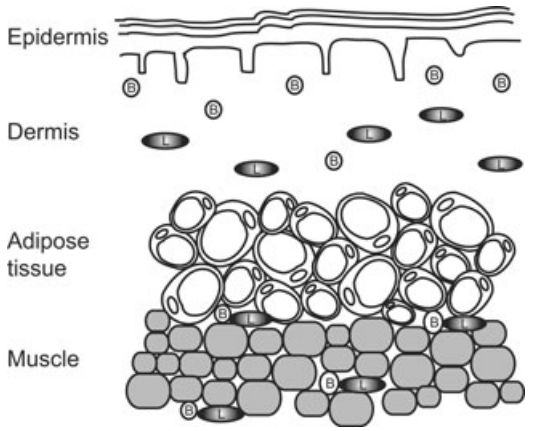

FIGURE 1. Lymphatic capillaries in the dermis are located in close proximity to the subcutaneous adipose tissue layer. L: lymphatic vessels, B: blood vessels.

the adipose tissue surrounding lymph nodes serves as a reservoir of energy that is deployed to power local immune responses.[4–7] Their work has demonstrated that the adipocytes within lymph node fat pads that are most closely apposed to the lymph node (perinodal adipocytes) respond to local immune challenge by increasing their rate of lipolysis compared to adipocytes that are more distantly located from the node.[5] Increased lipolysis liberates fatty acids and other lipid-derived mediators that fuel the ensuing immune response. Prolonged inflammation has been shown to propagate energy release by stimulating lipolysis in adipocytes closer to the periphery of the lymph node fat pad, as well as in adipose depots further afield from the site of challenge.[8] These observations suggest that the scope and magnitude of adipocyte lipolysis is progressively increased to meet the requirements of sustained immune cell activation.

Chronic lymph node stimulation has also been shown to result in the expansion of lymph node–associated adipose tissue in a rat model.[9] In this model, chronic lymph node stimulation resulted in increased adipose tissue mass due to an increase in both the size and number of adipocytes within the stimulated fat pad. Adipose tissue expansion is presumably an immune system insurance strategy that ensures that sufficient energy is always in reserve to power the fight against infectious agents. Taken together, these data link inflammation with adipose tissue metabolism and may help to explain the changes in adipose tissue biology that accompany chronic human inflammatory disorders, including HIV-associated adipose redistribution syndrome,[10] Crohn's disease, and obesity.

An intriguing observation that provides further direct evidence of an intimate lymph node–adipose tissue relationship is that, in at least one mouse model devoid of lymph nodes, the absence of nodes results in a failure of the associated lymph node fat pads to develop.[11] This observation suggests that the establishment of adipose tissue around lymph nodes is directly dependent on as yet unidentified lymph node– or immune cell–derived signals that are liberated during lymph node growth and maturation.

The Link between Lymphatic Vessels and Adipose Tissue in Human Disease

Lymphatic vessels are a critical conduit of interstitial fluid transport and immune cell traffic. Lymphatic vascular insufficiency due to developmental lymphatic vascular abnormalities, injury, obstruction or infection results in the accumulation of interstitial fluid and protein in affected tissues—a situation known as lymphedema. There are a number of characterized primary, or inherited, lymphedema syndromes,[12,13] several of which can be ascribed to inherited mutations in genes important for the growth and development of the lymphatic vasculature. Thus far, inactivating mutations have been described in the vascular endothelial growth factor receptor-3 (*VEGFR-3*) signaling pathway in patients suffering from Milroy's disease,[14,15] and in the transcription factors *FOXC2* [16–18] and *SOX18*[19] in lymphedema–distichiasis and hypotrichosis–lymphedema–telangiectasia syndromes, respectively. By far the most prevalent form of lymphedema is secondary, or acquired lymphedema, which arises as a result of lymphatic vascular injury or infection. Secondary lymphedema is estimated to occur in up to 20% of breast cancer patients after the surgical resection of axillary lymph nodes.[20] These patients experience painful and disabling edema of the affected arm for which very little effective treatment is currently available. Secondary lymphedema of the lower limbs has also been documented to occur in 10–25% of patients after surgery and radiotherapy for the treatment of gynecologic cancer.[21] The most predominant incidence of secondary lymphedema, however, is in the tropical world, where it is estimated that more than 120 million people suffer lymphedema as a result of lymphatic filariasis.[22] Whether primary or secondary in origin, if lymphedema is not resolved, the affected tissue manifests changes that include chronic inflammation, fibrosis and, most pertinent to this review, adipose tissue accumulation.[12,13] In fact, as early as the 19th century, German dermatologist Paul Unna proposed that the stagnation of tissue because of lymphatic or venous interruption resulted in fat accumulation.[23]

One report of a cutaneous lymphatic malformation that resulted in secondary late-onset adipose tissue hypertrophy exists in the literature.[24]

Lipedema is a syndrome found predominantly in postpubertal women and is characterized by bilateral, symmetrical enlargement of the legs as a result of adipose tissue accumulation, with sparing of the feet.[12] While the term lipedema suggests the existence of edematous tissue due to lymphatic vascular insufficiency, this disease is being progressively considered to be primarily a lipodystrophy syndrome. This conclusion is being drawn because a number of groups of patients with lipedema have shown normal, or only slightly reduced lymphatic function.[25,26] The diminished lymphatic function recorded in patients with lipedema could perhaps occur secondarily to adipose tissue accumulation in the legs by virtue of an obstructive effect on lymphatic flow. A role for lymphatic vessels in the etiology of this disease cannot yet be ruled out, however, as some investigators have shown functional alterations in lymph flow in lipedema patients,[27] and microlymphatic aneurysms of the lymphatic capillaries have been described in the skin of lipedema patients.[28] Further investigations should reveal whether lipedema is primarily a lymphatic vascular disease, or a lipodystrophy syndrome that interferes with normal lymphatic vascular function.

Adipose tissue accumulation has also been described in mouse models of lymphatic vascular dysfunction. The *Chy* mouse, a naturally occurring mouse model of lymphedema due to heterozygous inactivating mutations in VEGFR-3, exhibits lymphedema as a result of hypoplastic cutaneous lymphatic vessels.[29] *Chy* mice display adipose tissue accumulation predominantly in the edematous subcutaneous adipose layer that lies in close physical proximity to the dysfunctional hypoplastic lymphatic vessels of the dermis.[29] While the mechanism of adipose tissue accumulation due to lymphatic vascular dysfunction has not yet been precisely determined in *Chy* mice, or in human lymphedema patients, insights into a potential mechanism were recently discovered in mice haploinsufficient for *Prox1*, a gene encoding a homeobox transcription factor crucial for lymphatic vascular development.[30]

A Mouse Model of Adult Onset Obesity Caused by Lymphatic Vascular Disruption

Prox1 was the first gene identified to be critical for the specification of lymphatic endothelial cell fate.[31,32] Targeted inactivation of *Prox1* in the mouse results in embryonic lethality at approximately embryonic day (E) 15, by which stage *Prox1*-nullizygous embryos display pronounced edema due to the complete absence of lymphatic vessels.[31] While many *Prox1* heterozygous mice die soon after birth, displaying phenotypes characteristic of lymphatic vascular dysfunction, such as peritoneal and/or thoracic chylous ascites, a striking feature of surviving *Prox1* heterozygous mice is adult-onset obesity.[30] Our extensive characterization of food consumption, energy expenditure, mediators of appetite and satiety control and lipid metabolism, failed to reveal any changes in any of these parameters that could account for the onset of obesity in *Prox1* heterozygous mice. A consistent correlate was, however, observed between the degree of lymphatic vascular disorganization and dysfunction and the magnitude of adipose tissue accumulation in $Prox1^{+/-}$ mice. The lymphatic vessels that were most severely affected in $Prox1^{+/-}$ animals were those of the viscera, particularly the intestine, mesentery, and thoracic duct.[30]

In addition to the abnormal patterning and dilation of lymphatic vessels in the mesentery of $Prox1^{+/-}$ mice, feeding of the mice with a fluorescent lipid illuminated regions of mesenteric lipid leakage. These were most obvious in close proximity to areas abundant in disorganized lymphatic vessels. How could lymphatic vascular disorganization and leakage be linked with adipose tissue accumulation? A search of the literature revealed early work in which both total lymph and the lipid-rich chylomicron fraction of lymph, had been shown to promote the differentiation of adipocyte precursors isolated from the stromal-vascular fraction of embryonic rabbit adipose tissue.[33] We therefore hypothesized that the lymph leaking from ruptured lymphatic vessels of $Prox1^{+/-}$ mice could potentially contain an adipogenic stimulus, and we tested this hypothesis by culturing mouse 3T3-L1 pre-adipocytes with lymph collected from newborn $Prox1^{+/-}$ pups. Our data demonstrated that lymph was a potent stimulant of 3T3-L1 adipogenic differentiation and that lymph was able to synergize with insulin to even more potently promote adipogenic differentiation in this model. No effect on 3T3-L1 differentiation was observed when safflower oil was added to culture media as a source of exogenous lipid, indicating that the lipid accumulation we observed in 3T3-L1 adipocytes cultured with lymph was indicative of true adipogenic differentiation and that this effect was mediated by an unidentified factor/factors contained in lymph. Quantitation of the size and number of adipocytes in the fat pads of $Prox1^{+/-}$ mice and their wild-type littermates revealed a two-step mechanism of increased adipose tissue mass, the first step of

which was adipocyte hypertrophy, and the second (in general restricted to the most obese *Prox1*$^{+/-}$ mice), the promotion of adipogenic differentiation to generate additional adipocytes in which to store lipid. These data provided further evidence that a true adipogenic stimulus indeed resides within lymph. We further demonstrated that increased mesenteric adipose tissue accumulation was evident in *Prox1*$^{+/-}$ mice even prior to a significant increase in total body weight, and that adipose tissue accumulation was associated with an increased number of lymphatic vascular endothelial HA receptor (LYVE-1)–positive macrophages in the mesentery.[30] Final confirmation of our hypothesis that obesity was caused by lymphatic vascular rupture and resultant lymph leakage was cemented when we inactivated *Prox1* specifically in the vasculature and demonstrated that these mice developed adult-onset obesity.[30] Generation of this mouse model proved to us that lymphatic vascular defects caused by *Prox1* haploinsufficiency were sufficient to result in obesity. What is the identity of the adipogenic factor contained in lymph? No doubt future studies will reveal the answer to this intriguing question.

Inflammation and Obesity

Low-grade inflammation is increasingly recognized as being linked with, and contributing to, obesity and obesity-associated metabolic complications such as insulin resistance, type 2 diabetes, and cardiovascular disease.[34] The adipose tissue of obese mice and humans produces proinflammatory cytokines, chemokines, and peptides including TNF-α,[35] TGF-β,[36] interleukin 6,[37] monocyte chemoattractant protein-1,[38] and leptin[39]—all of which are able to recruit and stimulate cells of the immune system. Many of these proinflammatory mediators also have documented angiogenic activity,[40,41] or are able to indirectly stimulate angiogenesis via promoting the production of angiogenic growth factors from adipocytes[42] or macrophages. Macrophages, increased in number in the adipose tissue of both obese mice and humans,[43,44] have recently been shown to produce lymphangiogenic growth factors including vascular endothelial growth factors A, C and D (VEGF-A, -C, -D) in response to inflammatory stimuli.[45–47] Macrophages have also been demonstrated to promote lymphangiogenesis in mouse models of inflammatory disease[46,47] and to stimulate angiogenesis in epididymal adipose tissue.[48] It is thereby plausible that obesity-stimulated inflammation could result in the promotion of both angiogenesis and lymphangiogenesis within, and in close vicinity to, adipose tissue. Increased lymphangiogenesis and lymphatic vascular hyperplasia have previously been associated with inflammatory conditions, including psoriasis [49] and chronic airway inflammation.[50]

Could inflammation contribute to adipose tissue accumulation in *Prox1*$^{+/-}$ mice? On the basis of the data discussed above, we reasoned that the influx of inflammatory cells that we observed in the liver and adipose tissue of obese *Prox1*$^{+/-}$ mice could indeed potentially contribute to further adipose tissue accumulation in this mouse model. Two likely mechanisms of inflammation-stimulated adipose tissue accumulation could be envisioned: (*1*) that chronic inflammation could promote an increase in adipose tissue mass in order to fulfill the energy requirements of sustained immune cell activation via direct immune cell–adipocyte signaling events; and (*2*) that chronic inflammation could promote increased adipogenesis by stimulating neo-lymphangiogenesis in the mesentery of *Prox1*$^{+/-}$ mice, thereby exacerbating the release of lymph-derived adipogenic stimuli (FIG. 2). This mechanism would likely involve the direct transmission of lymph-derived signal(s) to local adipocytes, although the involvement of cells of the immune system in this process cannot be ruled out. Multiple lines of evidence suggested to us that the initial trigger of increased adipose tissue mass in *Prox1*$^{+/-}$ mice was ruptured leaky lymphatic vessels, as defects in lymphatic vessels were obvious at early stages of embryonic lymphatic development, and an increase in mesenteric adipose tissue mass was obvious in *Prox1*$^{+/-}$ mice compared to their wild-type counterparts even prior to a noticeable increase in overall body weight. It seems entirely plausible, though, and even likely, that once established, inflammation could propagate pro-adipogenic stimuli even further in *Prox1*$^{+/-}$ mice, resulting in a cyclical exacerbation of adipose tissue accumulation.

Adipose Tissue as a Source of Lymphangiogenic Factors

As discussed above, adipose tissue has been demonstrated to liberate numerous signals that have an impact on vascular development. The requirement for macrophages in adipose tissue angiogenesis, important to sustain adipose tissue expansion, has recently been demonstrated by Cho and colleagues.[48] This work illustrated that macrophages are recruited into the hypoxic tip region of epididymal adipose tissue via signals mediated by stromal cell–derived factor 1, VEGF, and matrix metalloproteinases (MMP), and then act to promote angiogenesis dependent on MMP and VEGF

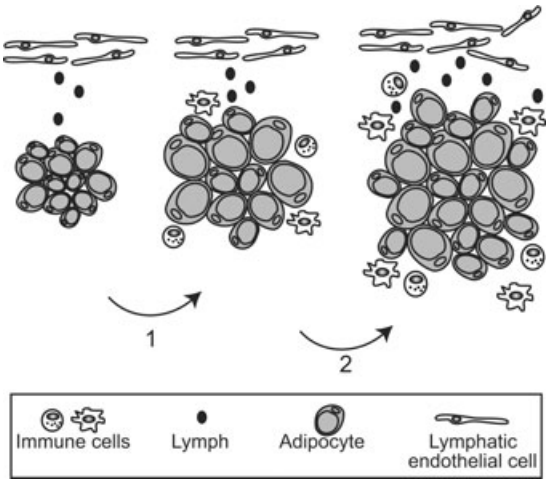

FIGURE 2. *Prox1*$^{+/-}$ mouse model for adipose tissue expansion initiated by lymphatic vascular dysfunction. In this model, it is proposed that the leakage of lymph from ruptured lymphatic vessels results in a biphasic increase in adipose tissue mass due to the exposure of adipose tissue to lymph-derived adipogenic signals. The initial phase of increased adipose tissue mass is proposed to occur via adipocyte hypertrophy (1), while the second phase is facilitated via the promotion of adipocyte differentiation from precursor adipocytes (2). The identity of the factor(s) within lymph that promote adipose tissue accumulation have not yet been identified. In addition to the direct adipogenic activity contained within lymph, it is proposed that adipose tissue accumulation is exacerbated by the inflammation that accompanies lymph leakage. Chronic inflammation has been closely linked with increased adipose tissue accumulation, obesity, neo-angiogenesis, and neo-lymphangiogenesis.

signals. While no lymphatic vessels were observed growing into the tip region of epididymal adipose tissue during the timeframe of this study, it is possible that lymphangiogenesis might secondarily follow establishment of the blood vascular network, primary construction of which is critical to sustain the metabolic demands of tissue growth and expansion. Indeed, studies from a number of laboratories have demonstrated that mouse adipose tissue can be ablated by targeted destruction of the vasculature,[51–53] confirming the importance of maintaining an adequate supply of oxygen and nutrients for adipose tissue integrity. Investigation of the ocular adipose tissue in human patients suffering the inflammatory conditions of orbital mucormyosis and panendophthalmitis suggest that lymphangiogenesis can be promoted within adipose tissue.[54] The studies of this group revealed that lymphatic vessels were present within ocular adipose granulation tissue, while absent from normal healthy controls,[54] suggesting that inflammation is able to promote lymphangiogenesis within adipose tissue.

An interesting, recently described factor produced by white adipocytes, among other tissues, is fasting-induced adipose factor (Fiaf).[55] Fiaf seems to play a role in lymphatic partitioning from the blood vasculature, as the majority of *Fiaf*-deficient mice die within 3 weeks of birth, and exhibit abnormal lymphatic–venous connections in the intestinal and mesenteric lymphatic vasculature. Analysis of embryonic and postnatal lymphatic development in *Fiaf*$^{-/-}$ mice demonstrated that the importance of Fiaf for lymphatic vascular development and function appears to be restricted to vessels of the intestine and mesentery. This effect could potentially be due to localized production of *Fiaf* in this anatomic region, or to distinct Fiaf-dependent developmental events that occur selectively in the mesenteric and intestinal lymphatics during a specified timeframe. The mechanism by which Fiaf acts to effect the separation of blood and lymphatic vascular networks in the mesentery remains, thus far, uncharacterized.

Questions to Be Answered in Order to Establish the Link between Lymphatic Function and Adipose Biology

The data summarized in this review document some of the many observations made by scientists and clinicians that link lymphatic vascular function with adipose tissue biology. Lymphatic vessels, lymph nodes, and cells of the immune system all interact extensively with adipose tissue. Many questions remain to be answered before we will fully understand the complex interplay between the cells that make up these two biological systems, both of which are of fundamental importance to human health. Some of the most topical in the current research climate include: What is the identity of the adipogenic stimulus/stimuli in lymph? Are dysfunctional lymphatics responsible for human obesity? Do other models of lymphatic dysfunction result in obesity? Are mutations/SNPs in *Prox1* associated with human obesity? Which signaling mechanisms are used in adipocyte–immune cell communications? If answers to any of the foregoing questions implicate lymphatic vascular dysfunction in the etiology of obesity in the human population, how might lymphatic vessels be targeted in order to treat obesity and obesity-associated metabolic complications? In view of the epidemic of obesity in the Western world, the answers to these questions could have a potentially large impact on the generation of new therapeutics for

the treatment of obesity-associated health complications. New-generation therapeutics targeted to ablate inflammation-stimulated adipogenesis might also provide a treatment option for patients who suffer from lymphedema that is more efficient than those currently available. The next chapter in this exciting research field will no doubt report answers to some of these questions, thereby further confirming the link between lymphatic vessels and adipose tissue biology.

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Conflicts of Interest

The author declares no conflicts of interest.

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