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Lymphatic system: a vital link between metabolic syndrome and inflammation

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Metabolic syndrome is defined by a cluster of different metabolic risk factors that include overall and central obesity, elevated fasting glucose levels, dyslipidemia, hypertension, and intimal atherogenesis. Metabolic syndrome leads to increased risk for the development of type 2 diabetes and cardiovascular disease (e.g., heart disease and stroke). The exacerbated progression of metabolic syndrome to cardiovascular disease has lead to intense study of the physiological ramifications of metabolic syndrome on the blood vasculature. These studies have particularly focused on the signaling and architectural alterations that manifest in hypertension and atherosclerosis. However, despite the overlap of metabolic syndrome pathology with lymphatic function, tangent effects on the lymphatic system have not been extensively documented. In this review, we discuss the current status of metabolic syndrome and provide evidence for, and the remaining challenges in studying, the connections among the lymphatic system, lipid transport, obesity, insulin resistance, and general inflammation.

Keywords: metabolic syndrome; lymphatic pump; lymphatics and lipid transport; inflammation

Metabolic syndrome: the disease and its present status

Metabolic syndrome (MS) is defined by a cluster of different metabolic risk factors that includes atherogenic dyslipidemia, abdominal obesity, elevated blood pressure and elevated plasma glucose associated with insulin resistance, prothrombic state, and a pro-inflammatory state.[1] These risk factors appear to directly promote atherosclerotic cardiovascular disease (ASCVD) and patients are also at an increased risk for type 2 diabetes mellitus.[2] MS has become increasingly common in the United States and in developing countries, including China and India. It is recently estimated that about 47 million U.S. adults have MS. Worldwide, it has been shown that the emerging epidemic of obesity and type 2 diabetes in young children is due to the shifts in modern diet, which likely influence the disease processes in increasingly younger populations.[3] The most prevalent disease processes, including weight gain, insulin resistance, hypertension, and hyperlipidemia, in humans are usually due to high intake of refined carbohydrates, specifically high fructose (HF), and diets rich in saturated fat.[4–7] One of the underlying risk factors for MS, atherogenic dyslipidemia consists of an aggregation of lipoprotein abnormalities including elevated serum triglyceride and apolipoprotein B (apoB), increased small low-density lipoprotein (LDL) particles, and a reduced level of high-density lipoprotein (HDL)-cholesterol (HDL-C). Regardless of cause, this syndrome identifies individuals at a high risk for ASCVD.[2] A growing body of evidence suggests that a pro-inflammatory state and the existence of a profile of inflammatory markers denote a very high risk for MS and hence cardiovascular diseases.[8] One of the most predominant underlying risk factors for the syndrome appears to be abdominal obesity, which involves abnormalities in adipose

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tissue metabolism.[9] This causes an increased production of pro-inflammatory cytokines, while there is a decrease in the levels of the potentially protective adipokine, adiponectin, emphasizing the connection between obesity and inflammation.[2,10] Other major risks include insulin resistance that directly predisposes individuals to type 2 diabetes. Furthermore, the upper body obesity correlates strongly with insulin resistance.[11] A number of studies document the evidence that an impaired nonesterified fatty acid (NEFA) metabolism contributes to the insulin-resistant state, which is prevalent in individuals with visceral obesity. Hypertrophied intra-abdominal adipocytes are characterized by a hyperlipolytic state that is resistant to the antilipolytic effect of insulin.[12,13] The resulting NEFA flux to the liver may impair liver metabolism, leading to increased hepatic glucose production. Hepatic insulin resistance is associated with decreased apoB degradation and increased production of triacylglycerol-rich lipoproteins.[14] The lymphatic system plays an important role in the uptake and transport of lipids from the intestine to circulation as well as plays a vital role in immune cell trafficking and eliciting an immune response.[15,16] The lymphatic vessels and lymph nodes are closely associated spatially, with specialized adipose tissue as well as immune cells, all of which interact extensively.[17] Recent reports suggest that the lymphatic role in lipid transport is an active and intricate process, and that when lymphatic function is compromised, there are systemic consequences to lipid metabolism and transport.[18] In spite of the vital roles played by the lymphatic system in both lipid transport and immune surveillance, the pathophysiology of this system has not been studied widely in the context of MS and a wide gap remains in our understanding. This review aims to bring together what we know about the various facets of MS and the roles played by the lymphatic system in modulating lipid transport/metabolism and inflammation, which are central to the onset and progression of this disease.

Introduction to the lymphatic system

The lymphatic system that runs in parallel with the blood vascular system plays an important role in maintaining body-fluid homeostasis, immune cell trafficking, and lipid transport from the intestinal lacteals.[18,19] To accomplish its normal functions, the lymphatic system, with its network of lymphatic vessels and interconnected lymph nodes, must transport lymph from the interstitial spaces, into and through the lymphatics, through the lymphatic compartment of the nodes, back into the nodal efferent lymphatics, and eventually empty them into the great veins.[19,20] This movement of lymph—which contains immune cells, antigens, lipids, macromolecules, fluid, and particulate matter within the lymphatic network, from where it is formed in the initial lymphatics to its final exit into the venous compartment—is primarily determined by the inherent pressure gradients within that network.[21,22] What is critically important is that the generation of lymph flow by intrinsic forces and the regulation of flow by both intrinsic and extrinsic forces rely on the phasic and tonic contraction of lymphatic muscle to produce a controlled net unidirectional transport of lymph.[21] The intrinsic pump relies on the spontaneous contractions of the muscle cells located within the wall of the lymphangion, a section of lymphatic between two adjacent valves,[23,24] to generate the pressure gradient required to drive lymph flow centrally. The extrinsic lymph pump depends on the cyclical compression and expansion of the lymphatic by the action of the surrounding tissues (blood vessel pulsations, gastrointestinal muscle contractions, heart contractions, skeletal muscle contractions, breathing movements, etc.) to generate lymph flow.[20,25–28] The initial lymphatics or lymphatic capillaries are blind-ended tubes containing a single-cell layer of overlapping endothelial cells without a continuous basement membrane. They are uniquely tethered to the surrounding interstitial matrix through characteristic "anchoring filaments."[29–31] The muscle cell layers invested in the outer walls of collecting and transport lymphatic vessels generate and control the movement of lymph along the lymphatic network, even against significant opposing pressure gradients. We have recently shown that lymphatic muscle contains both striated and smooth muscle contractile elements.[32] Furthermore, our data show that there are differences in the contractile machinery between blood vessels and lymphatics as well as among lymphatics from various tissue beds. However, the mechanisms of lymphatic contraction under normal condition and how they are modulated during the disease processes are not completely understood.

Lymph flow is the result of a complicated combination of lymph formation, intrinsic, and extrinsic

forces/pumps.[21] Lymphatic pump function is very sensitive to changes in mechanical load; it is likely that changes in these loads on the lymphatic system of the gut are an important regulator in lipid transport.[18] Controlled studies of the influences of imposed flow on lymphatic contractile activity were performed in isolated rat lymphatics.[33,34] Results from these studies showing a flow-dependent inhibition of the active lymph pump support the concept that the pumping activity of the lymphatics is regulated by flow/shear to adapt to the local needs to transport lymph through a continuous modulation of the extrinsic and intrinsic flows. At low levels of lymph inflow in the transporting lymphatics (i.e., low extrinsic flows), the influences of the intrinsic pump will dominate with periodic nitric oxide (NO) release owing to the phasic flow/shear patterns of the lymph pump to maintain efficient lymph transport. When the levels of lymph formation and inflow to the transporting lymphatics rise (i.e., high extrinsic flows), the influences of extrinsic forces will dominate, leading to a high NO release that inhibits the intrinsic pumping and basal tone of the transport lymphatics to optimize the conduit function of the vessel.[21] Interestingly, when lymphatic vessels isolated from various regions in the rat were compared, mesenteric lymphatics were found to be the least sensitive to this flow inhibition.[34] This is particularly important in light of the fact that the changes in load experience postprandialy by a mesenteric lymphatic are presumably substantial.[18] Failures in lymphatic transport can result in lymphedema marked by increased lipid accumulation throughout the body, resulting as an outcome of surgical procedures or acquired pathologically, resulting in significant accumulation of fluid, matrix remodeling, and adipose expansion in the affected limb.[35] However, the exact mechanisms connecting lipid pathologies (e.g., hyperlipidemia, hypercholesterolemia, obesity, and diabetes) with lymphatic physiology remain unknown.[18]

Lymphatics and lipid transport

The lymphatic system serves as the entry point for nearly all dietary lipids, which are taken up by enterocytes (epithelial cells on the lumen of the intestine) and packaged as large lipoproteins chylomicrons for export into the lymphatic system.[16,18,36] The mesenteric lymphatics are a special access site for ingested macromolecular lipoproteins, including the well-known propathogenic LDL, which will then be transported through the lymphatic system into the blood circulation. In the mesentery, a network of lacteals associated with the intestinal villi acts as the initial destination for chylomicrons, cholesterol, apolipoproteins, and other lipid carrier molecules that are released into the interstitium by mucosal enterocytes.[37] First, the chylomicrons enter the initial lymphatics of the small intestine, the lacteal, and are propelled through the initial vessels by the intestinal peristalsis.[38] These lymphatics are typically noted by lack of muscle cells, a discontinuous basement membrane, endothelial cells with gaps between adjacent cells, and special connections to the surrounding matrix called anchoring filaments.[29–31] This structural feature makes them ideal for the absorption of the large macromolecules of lipoproteins. Movement of this absorbed lipid through the rest of the lymphatic system occurs by the contraction of the muscular collecting lymphatics.[18] Proper functioning of the lymphatic system is essential for modulation of the rate of the chylomicron transport, and mixture of chylomicrons with lymph gives the lymphatic system its characteristic "milky" appearance and is referred to as chyle. Chylomicrons are the largest (1,000 nm) and the least dense (less than 0.95 g/ml) of the lipoproteins. They are made up of 85–88% triglycerides, approximately 8% phospholipids, 3% cholesterol esters, 1–2% proteins, and 1% cholesterol. Chylomicrons contain several types of apolipoproteins including apo-AI, II, and IV; apo-B48; apo-CI, II, and III; apo-E; and apoH.[39] Analysis of human lymph showed that the lymph concentration of HDL-C was 30% greater than that of blood.[40] Experiments on apoB knockout mice have shown that apoB is essential for the formation of chylomicron but is probably not the rate-limiting step. Hayashi *et al*.[41] demonstrated that an intraduodenal infusion of lipid causes a surge in lymphatic triglyceride output by seven- to eightfold. However, there is no distinct change in apoB output in the lymph that is indicative of the chylomicron production by the small intestine.[41] Miura *et al*.[42] have demonstrated that lipid absorption from intestinal mucosa leads to an increased flux of lymphocyte transport by almost 10-fold and this increase in lymph flow is enhanced before lipid absorption has reached its maximum. Cholesterol is transported mainly as esterified cholesterol and almost exclusively by the lymphatic system.[43] During fat absorption,

a significant and gradual increase in lymph flow has been demonstrated, which is referred to as the lymphagogic effect.[37] The lymphagogic effect generally spikes at approximately 1–2 h after feeding and then declines back to basal levels. Many studies have shown that extrinsic pump factors such as increased vascular permeability/capillary filtration, vis a tergo (formation of lymph), and peristaltic motions of the gut contribute to the increase in lymph flow.[37,44,45] However, relationships between the lipid uptake and mesenteric lymphatic function, and/or the effects of changes in the lipid or protein components of the lymph in modulating lymphatic contractility have not been established. As the mesenteric lymphatic vessels are primary routes of transport for chylomicrons and gut signaling peptides, chronic exposure to the increased transport stress load may result in functional consequences or long-term remodeling. Also, increased chylomicron loads likely alter lymph viscosity, thereby affecting force production, lymph flow, and shear stresses produced by flow. We have recently demonstrated that LDL increases phasic contraction frequency and decreases tone of the isolated rat mesenteric lymphatic vessels, resulting in a significant increase in lymph pump output.[46] LDL particles are readily taken up by both the lymphatic muscle and endothelial cell populations. Furthermore, our recent results show that mesenteric lymphatics from HF diet-fed rats exhibit an increase in contraction frequency but a decrease in phasic contraction amplitude, a significant decrease in flow-mediated contraction frequency inhibition, and a decrease in tone response to flow (Wang, W., *et al.*, unpublished observation). We have also recently demonstrated that aging affects both phasic and tonic lymphatic contractility by weakening the pressure-dependent contractile/pumping activity of the lymphatic muscle cells and by compromising the endothelium/flow/NO-dependent regulation.[47] We propose that aged-group animals showing the increasing incidence of insulin resistance, hyperlipidemia, and other cardiovascular defects could themselves serve as a MS model. The pathophysiology of lymphatics in aged-group animals correlates with our recent findings in the mesenteric lymphatic pump activity from the HF diet group animals, as we discussed earlier.

The presence of free chyle in the peritoneum or thoracic cavity, chylous ascites, and chylothorax, respectively, is indicative of a dysfunctional lymphatic transport.[48] Genetic mutations in several genes that are important for lymphatic vascular patterning and development lead to an edematous condition or formation of chylous ascites (reviewed in Tammela *et al.*[49]). Mice deficient in angiopoietin 2 exhibit lymphatic dysfunction and abnormal patterning of the lymphatic vasculature, develop chylous ascites, and edema.[50] In many individuals with lymphatic syndromes such as lymphedema and lipedema, accumulation of adipose tissue has been found in the edematous regions.[51] Subcutaneous adipose tissue accumulation has been described in the Chy mouse model of lymphedema, in which the cutaneous lymphatic vessels are dysfunctional.[52] An interesting insight into the fact that lymphatic vascular defects resulting in abnormal leakage or accumulation of lymph might promote adipogenic differentiation, adipocyte lipid accumulation, or both, and thereby trigger obesity was obtained from studies in $Prox1^{+/-}$ mice. At E14.5, all $Prox1^{+/-}$ embryos had edema, indicative of lymphatic dysfunction, with the most severe mispatterning being associated with the most pronounced edema.[53] The lymphatic vessels of the mesentery and the intestine were the most severely affected in these mice. Pups that died soon after birth showed accumulation of fat in the intestinal wall as well as chyle leakage from the mesenteric lymphatic vessels. In most $Prox1^{+/-}$ mice analyzed, the magnitude of obesity was correlated to the extent of disorganization and leakage of the lymphatic vessels, with the oldest and most obese mice having the most severely disrupted lymphatics. Further, the accumulation of fat, most obviously around lymph nodes and near mesenteric lymphatic vessels, suggested that lymphatic leakage was a key mechanism by which lymphatic defects promoted adipocyte hypertrophy or ectopic adipogenesis.[53] The authors propose that lymphatic vascular defects cause obesity in $Prox1^{+/-}$ mice and that crosstalk occurs between the lymphatic vasculature and adipose tissue.[53]

Inflammation and metabolic syndrome

Chronic subclinical inflammation is an intrinsic part of MS, and several studies have shown that a pro-inflammatory state is an important component of this disease.[54] A generally enhanced adipose tissue-derived cytokine expression may be another plausible mechanism for the inflammation–MS

relationship.[55] Several chemokines, cytokines, kinases, and transcription factors have been implicated in adipose inflammation, systemic insulin resistance, and a chronic inflammatory atherogenic state that contributes to type 2 diabetes and atherosclerosis.[56] Adipose tissue not only is specialized in the storage and mobilization of lipids, but also is a remarkable endocrine organ releasing numerous cytokines and pro-inflammatory molecules such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α).[14] The discovery that TNF-α is overexpressed in the adipose tissue of obese mice provided the first clear link between obesity, diabetes, and chronic inflammation.[57] Although inflammatory markers presently do not constitute the diagnostic criteria of MS, the inflammatory markers that have been linked include C-reactive protein (CRP), TNF-α, and IL-6.[58] Macrophage infiltration of adipose tissue has been reported in obese patients that could be cause for the inflammatory profile that has been detected in abdominally obese patients. Lemieux *et al*.[59] have shown that elevated plasma level of CRP that has a predictive risk for myocardial infarction is increased in viscerally obese patients. Adiponectin is a unique adipokine whose expression from the adipose tissue is inversely correlated with the risk factors underlying MS as insulin resistance, diabetes, and atherosclerotic CVD.[56] It increases fatty acid oxidation while reducing glucose production in liver, suppresses TNF actions in nonalcoholic fatty liver disease, and inhibits nuclear factor kappa B and monocyte adhesion to endothelial cells. The link between inflammatory and metabolic signaling depends on a delicate balance.[60] Chronic inflammation may represent a triggering factor in the origin of the MS: stimuli such as over nutrition, physical inactivity, and aging may result in cytokine hypersecretion and eventually to insulin resistance and diabetes in genetically or metabolically predisposed individuals. Alternatively, resistance to the anti-inflammatory actions of insulin may result in enhanced circulating levels of pro-inflammatory cytokines, resulting in persistent low-grade inflammation.[55] For example, the neuropeptide substance P (SP) is an important mediator of inflammation and local release of SP has been shown to cause chronic inflammatory conditions. In the intestine, SP produced by several cell types may act locally in the mesenteric network in a paracrine fashion.[61] Recently, a role for SP has been shown in appetite regulation and metabolism, in addition to the already established effects of this peptide in gastric motility and digestion.[62]

Lymphatic system and modulation of immune function and inflammation

Through its function to propel lymph, the lymphatic system is strongly implicated in the adaptive immune response. It transports antigens to lymphoid tissues to allow initiation of an immune response during disease and in response to infections. Lymphoid structures such as lymph nodes are distributed along the lymphatic vessel network. They are composed of lymphatic and blood vessels spread out inside a parenchyma, subdivided into B cell follicles and a T cell area that together form the cortex and the medulla. Cellular and molecular traffic between these compartments is an essential aspect of lymph node physiology. An important feature of inflammation is infiltration of inflamed tissues by immune cells such as neutrophils, eosinophils, and macrophages.[22] Lymphatic vessels are actively involved in the inflammatory responses by transporting leukocytes from the site of inflammation to secondary lymphoid organs. Upon exposure to an inflammatory stimulus and recognition of pathogen-associated molecular patterns, dendritic cells capture antigens in peripheral tissues and migrate through afferent lymphatic vessels into lymph nodes.[63] Several studies have shown that the classical inflammatory mediators such as prostanoids,[64–69] histamine,[70–74] and nitric oxide [75] modulate the lymphatic pumping and drainage. In addition, the neuromediators that are important in immune and inflammatory responses, such as SP, calcitonin gene-related peptide, neuropeptide Y, or vasoactive intestinal polypeptide, are reported to strongly affect lymphatic vessel contractility.[76–81] Thus, there is substantial evidence that the lymphatic system is intimately involved in, and highly altered during, inflammatory diseases. Release of inflammatory mediators, in addition to increasing vascular permeability during inflammation, is thought to play a pivotal role in modulating lymphatic vessel function.[58] Recent data from our laboratory suggest that stimulation of SP, an important modulator of lymphatic contractility, activates inflammatory pathways (Chakraborty, S., *et al*., unpublished data). SP plays an important role in modulating immune responses and a local release

of SP in lymph nodes may contribute to chronic inflammatory conditions.[82] SP directly stimulates lymphocytes and augments mitogen- and antigen-induced lymphocyte proliferation.[83] Around 40% of human blood lymphocytes are known to express SP receptors, and SP increases lymphocyte trafficking and proliferative responses in mesenteric lymph nodes and Peyers patches.[84] SP induces immune cells to produce pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, interferon-γ, granulocyte–macrophage colony-stimulating factor, and stem cell factor.[84] Recently, we have found a significantly greater amount of adipose tissue deposition around mesenteric lymphatic vessels from rats fed the HF diet, which leads to smaller-diameter mesenteric lymphatics with a decrease in pumping activity. Furthermore, the SP-induced effect on the lymphatics is completely abolished in rats fed the HF diet, indicating that these mesenteric lymphatics are sensitized to SP (Zawieja, S., *et al*., unpublished data). We have previously shown that SP (10 nM) increased both contractile frequency and strength of mesenteric lymphatics, and improved the pump efficiency independent of the effects of preload and broadening of the working range of the lymphatic pump.[77] Karagiannides *et al*.[85] have also shown that SP increases mesenteric preadipocyte viability, reduces apoptosis, stimulates proliferation of fat tissue, creates a pro-inflammatory state, and contributes overall to the process of gut inflammation. In addition, SP has been implicated as an anti-obesity target.[85,86] It has also been shown that both lymph and the lipid-rich chylomicron fraction of lymph promote the differentiation of adipocyte precursors.[87]

Adipose tissue activation and link with lymphatics

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 reservoir of energy deployed to fight infection.[17,53] Subcutaneous adipose tissue lies in close proximity to the dermal lymphatic vasculature, whereas visceral adipose tissue surrounds the collecting lymphatic vessels of the mesentery, cisterna chili, 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.[53] Pond and Mattacks[88] have shown that sustained local activation of a single popliteal lymph node recruits additional adipocytes in the node-containing depots and these inflammatory signals spread from lymph nodes to surrounding adipocytes. Prolonged inflammation stimulated lipolysis in adipocytes closer to the periphery of the lymph node fat pad, as well as in adipose depots further from the site of application of the inflammatory lipopolysaccharide. Chronic inflammation of the peripheral lymph nodes of rats has been shown to increase the number of adipocytes that surround the node.[89] In the $Prox^{+/-}$ mouse model with the lymphatic vascular disruption causing adult-onset obesity, the authors propose two possible mechanisms of inflammation-stimulated adipose tissue accumulation, one where chronic inflammation could promote an increase in adipose tissue mass in order to fulfill the energy requirements of sustained immune cell activation by means of direct immune cell–adipocyte signaling events and a second mechanism whereby chronic inflammation could promote increased adipogenesis by stimulating neolymphangiogenesis in the mesentery of $Prox1^{+/-}$ mice, thereby exacerbating the release of lymph-derived adipogenic stimuli.[17,53]

Conclusions and remaining challenges

Despite a close association of the lymphatic system with adipose tissue and its important function in the transport of dietary lipids as well as in eliciting an inflammatory response, the lymphatic system has been grossly understudied with respect to metabolic disorders. Dysfunction of the lymphatic system can lead to altered lipid transport and fat depositions, and can lead to lymphedema along with progress of the metabolic risk factors underlying MS. A number of recent studies suggest that although these studies have provided important information relating to independent issues, such as obesity, insulin resistance, general inflammation, etc., the important challenge in this area is to identify the central locus that will provide a comprehensive understanding of the complex nature of MS. Further studies are required to delineate the axis between lymph transport, lipid metabolism, and the development of MS, which will truly be a major paradigm shift and will provide novel insights in understanding the pathogenesis of MS.

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Conflicts of interest

There are no conflicts of interest.

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