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Lymphatic function and anatomy in early stages of lipedema

John C. Rasmussen[1] | Melissa B. Aldrich[1] | Caroline E. Fife[2,3] | Karen L. Herbst[4] | Eva M. Sevick-Muraca[1]

[1]Center for Molecular Imaging, Brown Foundation Institute of Molecular Medicine, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, Texas, USA

[2]Department of Geriatrics, Baylor College of Medicine, Houston, Texas, USA

[3]CHI St. Luke's Hospital, The Woodlands, Texas, USA

[4]Department of Medicine, University of Arizona, Tucson, Arizona, USA

Correspondence
Karen L. Herbst, Total Lipedema Care, 240 South La Cienega Blvd., Suite 200, Beverly Hills, CA 90211, USA.
Email: kaherbst@gmail.com

John C. Rasmussen, Brown Foundation Institute of Molecular Medicine, McGovern Medical School, The University of Texas Health Science Center at Houston, 1825 Pressler St., Houston, TX 77030, USA.
Email: john.rasmussen@uth.tmc.edu

Present address
Karen L. Herbst, Total Lipedema Care, Beverly Hills, California, USA and Total Lipedema Care, Tucson, Arizona, USA

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Abstract

Objective: Lipedema is an inflammatory subcutaneous adipose tissue disease that develops in women and may progress to lipolymphedema, a condition similar to lymphedema, in which lymphatic dysfunction results in irresolvable edema. Because it has been shown that dilated lymphatic vessels, impaired pumping, and dermal backflow are associated with presymptomatic, cancer-acquired lymphedema, this study sought to understand whether these abnormal lymphatic characteristics also characterize early stages of lipedema prior to lipolymphedema development.

Methods: In a pilot study of 20 individuals with Stage I or II lipedema who had not progressed to lipolymphedema, lymphatic vessel anatomy and function in upper and lower extremities were assessed by near-infrared fluorescence lymphatic imaging and compared with that of a control population of similar age and BMI.

Results: These studies showed that, although lower extremity lymphatic vessels were dilated and showed intravascular pooling, the propulsion rates significantly exceeded those of control individuals. Upper extremity lymphatics of individuals with lipedema were unremarkable. In contrast to individuals with lymphedema, individuals with Stage I and II lipedema did not exhibit dermal backflow.

Conclusions: These results suggest that, despite the confusion in the diagnoses between lymphedema and lipedema, their etiologies differ, with lipedema associated with lymphatic vessel dilation but not lymphatic dysfunction.

INTRODUCTION

First reported by Allen and Hines (1), lipedema, a disease marked by abnormal subcutaneous adipose tissue accumulation from the buttocks to the ankles and occasionally the arms, primarily develops in women and is characterized by three progressive stages. Stage I lipedema presents with sometimes painful, pearl-sized nodules in a hypertrophic subcutaneous adipose tissue layer; in Stage II, there are skin indentations with pearl- to apple-sized masses in the skin and adipose tissue; and in Stage III, there are lobules of skin and fat on the arms, hips, and thighs and around the knees. During all stages, lipolymphedema, a condition similar to lymphedema, can occur. Awareness of lipedema in medical society has been limited, and the disease is often misdiagnosed as obesity, leg swelling with venous disease, or lymphedema.

In lymphedema, genetic or acquired, lymphatic vasculature abnormalities cause impaired clearance of capillary filtrate, subsequent irresolvable edema, and subcutaneous adipose tissue accumulation. In a study of cancer-acquired lymphedema (ClinicalTrials.gov identifier NCT02949726), we used longitudinal near-infrared fluorescence lymphatic imaging (NIRF-LI) to show that chronic lymphatic dysfunction precedes edema and volumetric tissue changes and that NIRF-LI may provide early objective criteria for the future onset of clinical symptoms. However, in lipolymphedema, subcutaneous adipose deposition precedes clinical symptoms of edema. Because impaired lymphatic dysfunction has been reported to cause, as well as result from, adipose tissue expansion (2,3), lymphatic dysfunction may play a role in the etiology of lipedema. Whether subclinical lymphatic dysfunction can contribute to the inflammatory tissue environment responsible for adiposity in lipedema, can be predictive for lipolymphedema, and/or can provide an objective measure for lipedema remain open questions.

Objective criteria for a diagnosis of lipedema remain elusive. As in people with obesity, adipocytes in people without obesity but with lipedema exhibit hyperplasia and hypertrophy, with increased numbers of clustering $CD68^{+}$ macrophages found in crown-like structures surrounding dead or dying adipocytes (4). In obesity, activation of NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasomes of M1-polarized macrophages associated with crown-like structures results in elevated levels of proinflammatory cytokines (predominantly interleukin-1β [IL-1β]) that may be responsible for chronic adipose inflammation (5) and may be expected to dilate lymphatic vessels and impair lymphatic pumping activity. In acute murine studies deploying subcutaneous administration of IL-1β or lipopolysaccharide that activates the NLRP3 inflammasome, Aldrich and Sevick (6) have shown that conducting lymphatic vessels become dilated and exhibit reflux or impaired pumping from the draining lymphatic watershed. Clinically, dilated conducting vessels and impaired propulsion of lymph may precede dermal backflow into initial lymphatic capillaries, tissue edema, and the onset of lymphedema in patients with breast cancer. Whereas vessel densities and effective diameters of dermal lymphatic capillaries appear similar in patients without obesity and with lipedema and BMI-matched control patients (4,7), lymphatic dysfunction of deeper conducting lymphatic vessels could be impacted by adipose inflammation induced by M1-polarized macrophages. Although M1 macrophages are found in the adipose tissue of individuals with obesity, lipedema fat is characterized by increased numbers of anti-inflammatory, M2-polarized macrophages, which may explain, in part, the lack of insulin resistance and the comparatively low risk of diabetes in individuals with lipedema (7).

In this work, we sought to uncover whether dilation and impairment of lymphatic anatomy and function occur in individuals with Stage I to III lipedema without lipolymphedema, as assessed using NIRF-LI.

Study Importance

What is already known?

- Lipedema occasionally progresses to lipolymphedema, a condition that is similar to lymphedema, suggesting that lymphatics may contribute to the etiology of lipedema.

What does this study add?

- Unlike lymphedema, individuals with lipedema but without lipolymphedema do not exhibit dermal backflow and, in contrast, have significantly higher pumping rates and dilated vessels compared with control individuals of similar age and BMI.
- This research suggests that lymphatic failure is not involved in the etiology of early-stage lipedema, although it likely plays a role in lipolymphedema.
- The etiology of lymphatic failure in progressive lipedema and in cancer-acquired lymphedema appears to be different.

How might your results change the direction of research or the focus of clinical practice?

- Lipedema appears to manifest in dilated lymphatic vessels and enhanced lymphatic pumping, potentially in response to inflammatory adipose tissue.
- Additional studies, including assays of inflammation markers, need to be performed to isolate molecular differences in the onset of lipedema and lymphedema.
- Although diagnosis of lipedema is unlikely using near-infrared fluorescence lymphatic imaging, the lack of dermal backflow in early lipedema may enable the exclusion of lymphedema.

METHODS

As part of a larger study funded by the Lipedema Foundation, individuals 18 years or older with a diagnosis of Stage I or Stage II lipedema were referred to our clinic (by KLH; Wound Care Clinic, CHI St Luke's Hospital, The Woodlands, Texas) for lymphatic evaluation using an investigational NIRF-LI technique developed in our laboratories (Center for Molecular Imaging, Brown Foundation Institute of Molecular Medicine, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, Texas). NIRF-LI has previously been used to assess lymphatics in clinical studies of healthy individuals and individuals with a variety of conditions, including lymphedema (8–12), venous disease (13,14), Dercum disease (15), and others (16–18). As part of a broader, local institutional review board- and United States Food and Drug Administration (FDA)-approved protocol ("Imaging lymphatic function in normal subjects and in persons with lymphatic disorders," ClinicalTrials.gov identifier NCT00833599), the lymphatics of participants with lipedema were imaged following the off-label, intradermal administration of indocyanine green (ICG). Individuals with an allergy to iodine were excluded from the study, and participants had to be willing and able to travel, most often via air, to

TABLE 1 Demographic information for participants with lipedema

ID	Stage	Age (y)	Sex	Race (ethnicity)	BMI (kg/m^2)	Other lymphatic-related conditions	Number of injections of ICG (total dose, μg)
L01	1	48	F	W (NHL)	34.8	Intermittent swelling	12 (300)
L02	2	37	F	W (NHL)	21.5	Venous disease; intermittent swelling	16 (400)
L03	1	41	F	W (HL)	32.8	Breast cancer survivor; intermittent swelling	15 (375)
L04	1	23	F	W (NHL)	25.8	Intermittent swelling	14 (350)
L05	2	46	F	W (NHL)	26.0	-	15 (375)
L06	2	45	F	W (NHL)	32.7	Melanoma survivor	16 (400)
L07	1	40	F	W (NHL)	24.7	-	16 (400)
L08	2	45	F	W (NHL)	24.0	-	15 (375)
L09	1	24	F	W (NHL)	27.4	Intermittent swelling[a]	14 (350)
L10	2	35	F	W (NHL)	34.8	-	16 (400)
L11	1	42	F	W (NHL)	29.6	-	14 (350)
L12	3	36	F	W (NHL)	34.8	-	14 (350)
L13	2	43	F	AA (NHL)	27.1	Venous disease	14 (350)
L14	1	43	F	W (NHL)	21.4	Venous disease; intermittent swelling	14 (350)
L15	1	33	F	W (NHL)	21.4	-	14 (350)
L16	L1/E2	37	F	W (NHL)	29.7	-	14 (350)
L17	2	44	F	W (NHL)	29.4	Liposuction of arms	14 (350)
L18	2	37	F	NR (HL)	36.0	Venous disease	14 (350)
L19	E2	45	F	W (HL)	33.3	-	14 (350)
L20	2	35	F	W (NHL)	29.1	Intermittent swelling	14 (350)

Abbreviations: AA, African American; E, early stage 2; F, female; HL, Hispanic/Latina; ICG, indocyanine green; ID, identification; L1, late stage 1; NHL, not Hispanic/Latina; NR, not reported; W, White.

[a]Individual was misdiagnosed with lymphedema prior to diagnosis with lipedema.

Houston to be imaged and to lay supine during imaging. Pregnant and breastfeeding women were excluded. Control data from previously published studies (14,19), acquired under the same FDA- and institutional review board-approved protocol, were also used herein to provide a comparative baseline for "normal" lymphatic anatomy and function. Ethical approval was provided by the Committee For the Protection of Human Subjects, The University of Texas Health Science Center at Houston.

After providing written informed consent, each participant received 12 to 16 intradermal injections of 25 μg of ICG in 0.1 mL of saline with a maximum total dose ≤ 400 μg. Most participants received 14 injections, including 2 on the dorsum of each foot, located approximately 1.5 to 2 cm above the web spaces nearest the hallux and outermost toe, 1 injection on each medial ankle, 1 on each lateral calf, and 1 on each anterior thigh. Two injections were administered below the navel, each about 2.5 cm down and to the left and right, to assess abdominal drainage to the inguinal nodal basin. Because lipedema sometimes affects arms, an injection was also administered on each medial wrist. Two additional injections, for a total of sixteen, were available if desired. Participant L01 did not receive the arm or navel injections but did receive injections on the medial calves, and L02, L03, L05-L08, and L10 received at least one additional injection (Table 1). Injection sites were covered with round bandages and black vinyl tape, as necessary, to prevent oversaturation of the NIRF-LI camera. Image exposure times were 200 milliseconds, allowing for near real-time acquisition of image sequences visualizing lymphatic pumping.

Immediately after injection, the legs, abdomen, and arms were imaged by illuminating the tissues with diffuse 785-nm excitation light and, using an intensified charge-coupled device camera, collecting the resultant 830-nm fluorescent signal emanating from the ICG-laden lymphatics. During imaging, which occurred in an examination room over 2 hours, the participant was supine. Acquired image sequences were assessed for active lymphatic pumping and to identify abnormal lymphatic anatomy, including dermal backflow, which is typified by the backward movement of lymph into the lymphatic capillaries and/or through the interstitial space across limbs of patients with lymphatic disease. Interstitial backflow is similar to dermal backflow but it remains in the immediate vicinity of the intradermal injection sites. Other anatomic abnormalities include tortuous and/or dilated vessels, superficial vessels radiating from the injection site but apparently not connected to deeper lymphatics, and vessel segmentation characterized by alternating bright and dark sections of a continuous lymphatic vessel. The percentage of limbs manifesting each abnormality was calculated for each stage of disease.

Active lymphatic pumping was quantified by counting the number of pumping or propulsion events in the lower leg approximately 3 to 10 cm above the medial malleolus. The total number of

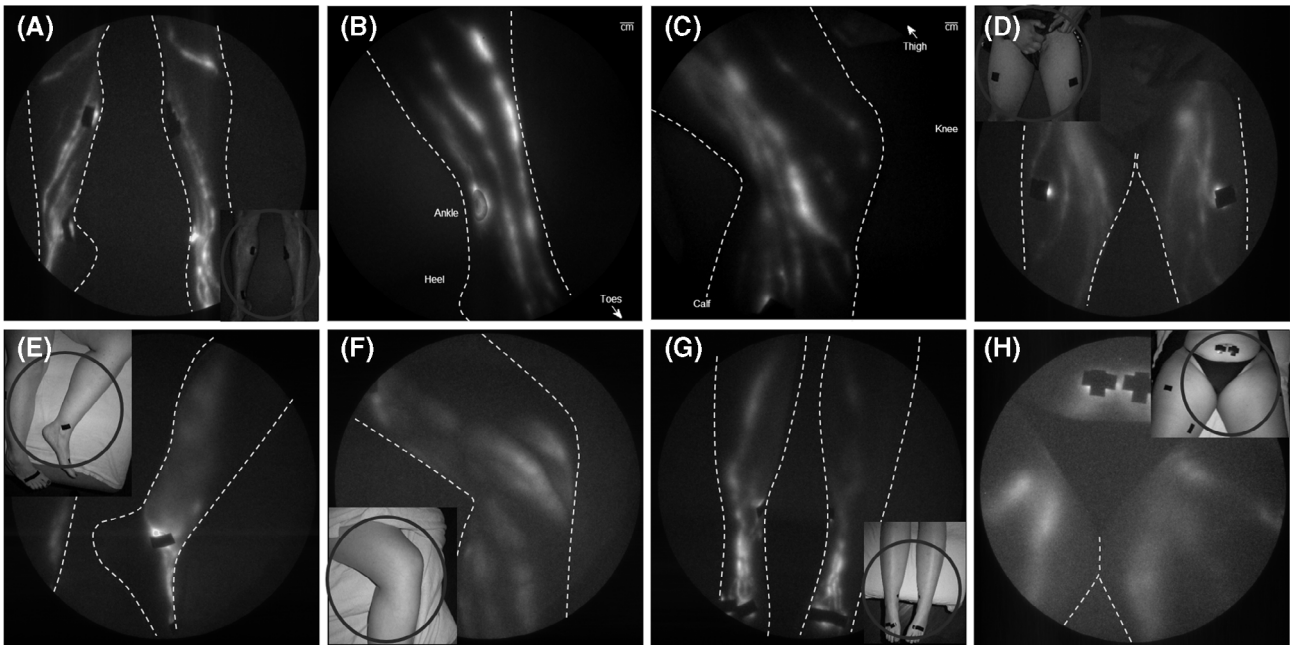

FIGURE 1 NIRF-LI images (white light image inset) illustrating typical lymphatic anatomy in (A-D) control individuals and (E-H) individuals with lipedema. In control individuals, the lymphatics in the shins were typically of a similar intensity as those in the (A) feet, and (B) the vessels in the medial ankle and (C) knee were distinct, with little dilation. As the vessels transited deeper into the tissues, they appeared more dilated, particularly in the (D) upper thigh, owing to the diffusion of fluorescent light through the tissues. In individuals with lipedema, the vessels in the (E) medial ankles and (F) knees were typically more dilated compared with control individuals. A distinct drop-off in the fluorescence intensity was often observed between the feet and the shins as shown in the left leg in panel (G), and occasionally the lymphatics were somewhat obscured in the (H) thighs, although the inguinal lymph nodes were still visible. Panel (H) also illustrates the lymphatic drainage from the injection sites near the navel toward the inguinal lymph nodes. Injection sites were covered by round bandages and/or black vinyl tape. The brightness and contrast of the NIRF-LI images have been adjusted to help visualize both the dim and bright vessels in the 16-bit images. Panels (A) and (D) are reproduced from (14), and panels (B) and (C) are reproduced from (19), with permission. NIRF-LI, near-infrared fluorescence lymphatic imaging

propulsion events was divided by the imaging time to obtain a propulsion rate for each leg. Because the published control propulsion rates used herein include only the propulsion events from the foot and ankle injections, we did not quantify the number of propulsion events originating from injection sites above the ankles in this analysis. Unpaired Student *t* tests ($\alpha = 0.05$) were used to assess statistical differences in the propulsion rates between the control, Stage I, and Stage II limbs.

RESULTS

Demographics

Over a period of about 15 months, 20 individuals with lipedema were referred to and enrolled in this observational study, including 8 Stage I, 11 Stage II, and 1 Stage III participants. After written informed consent, the participants were imaged per institutionally approved protocols. Demographic information for the individuals with lipedema is presented in Table 1. Consistent with the fact that lipedema predominately affects women, our lipedema group consisted entirely of female individuals. The median age of the lipedema group was 40.5 years (range = 23-48 years; mean = 39 years), and their median BMI was 29.3 kg/m^2 (range = 21.4-36.0; mean = 28.8). For comparison purposes, we used published data from a group of nine control individuals, six men and three women, with a median age of 43.0 years (range = 30-58 years; mean = 44.9 years) and a median BMI of 30.3 (range = 23.5-37.6; mean = 29.6) (14).

Anatomy

As illustrated in Figure 1A-D, control lymphatic vessels were generally linear in nature, with unidirectional pumping from ICG-injection sites toward the regional nodal basins. Of the 18 control limbs imaged, 4 exhibited vessels radiating from injection sites, 2 exhibited signs of vessel segmentation, 1 exhibited a tortuous vessel between the foot and ankle, and 1 exhibited interstitial backflow around an injection site. There were no apparently dilated vessels in this control group, although one individual, with BMI of 23.5, had limited lymphatic vasculature observed above the left ankle.

Table 2 details the observations for each limb with lipedema, with sample images of observed abnormal lymphatic structure presented in Figure 1E-H and Figure 2. For identification purposes, each leg is identified by the individual's ID number followed by the side of the body (e.g., L12-L refers to the left leg of individual L12). Dilated and potentially "leaky" lymphatic vessels, particularly in the medial leg as shown in Figure 1E,F (L06-L and L05-L), were the most commonly

TABLE 2 Summary of observed lymphatic anatomic abnormalities and propulsion rates

Stage	ID-Limb	Abnormal anatomic observations	Injection-associated interstitial backflow	Proximal diffuse or unusual node-like lymphatics	Foot to shin signal attenuation	Propulsion rate, events/min	Abnormal arm observations
1	L01-L	S			Y	0.7	
1	L01-R	D, VR			Y	0.5	
1	L03-L	D		Th	Y	0.6	
1	L03-R	S, D	A	K; Th (nonfluorescent fibrotic nodules)	Y	0.8	S
1	L04-L	D			Y	1.7	S
1	L04-R	T, D	A		Y	1.9	S, IB-W
1	L07-L	SS, T, D	C	Sh	Y	2.1	
1	L07-R	SS, T, D, VR			Y	2.8	
1	L09-L	D	A		Y	1.8	Reflux
1	L09-R	D	A		Y	1.4	IB-W
1	L11-L	SS, D			Y	1.1	
1	L11-R	T, D	Th, C		Y	1.9	
1	L14-L	S, D			N	1.0	
1	L14-R	S, D, VR	Th		N	1.3	
1	L15-L	SS, D	A		Y	1.9	
1	L15-R	SS, D, VR	A		N	1.7	
2	L02-L	S, D, VR	A		Y	1.2	
2	L02-R	S, D, VR			Y	1.8	
2	L05-L	T, D			Y	0.8	
2	L05-R	T, D			N	0.5	
2	L06-L	T, D			Y	0.7	
2	L06-R	D, VR		Sh	Y	0.5	
2	L08-L	SS, D, VR	Th, A	Th	Y	0.9	D
2	L08-R	D, VR	Th, C		N	0.6	
2	L10-L	SS, T, D			Y	1.3	
2	L10-R	T, D	Th	Sh	Y	1.2	
2	L13-L	SS, D			Y	0.4	
2	L13-R	SS, T, D, VR		Sh	Y	0.7	T
L1/E2	L16-L	S, T, D	A		Y	1.9	
L1/E2	L16-R	S, D, VR			Y	3.1	
2	L17-L	D, VR			Y	1.7	T, DB-W
2	L17-R	D, VR			Y	1.8	DB-W
2	L18-L	D, VR	C		Y	1.9	
2	L18-R	D, VR			Y	2.0	
E2	L19-L	SS, D, VR	Th		N	1.7	
E2	L19-R	T, D, VR	C	Sh	N	2.1	
2	L20-L	S, D, VR			Y	1.5	
2	L20-R	S, D, VR			Y	1.6	
3	L12-L	SS, T, D	A	K	Y	1.8	
3	L12-R	SS, T, D	A		Y	1.9	

Abbreviations: A, ankle; C, calf; D, dilated vessels; DB, dermal backflow; E2, early stage 2; IB, interstitial backflow; ID, identification; K, knee; L, left; L1, late stage 1; N, no; R, right; S, vessel segmentation; Sh, shin; SS, signs of vessel segmentation, T, tortuous vessels; Th, thigh; VR, vessels radiating from injection site; W, wrist; Y, yes.

observed abnormality (98% of legs) in all individuals with lipedema regardless of disease stage. A distinct reduction of fluorescence in the shins compared with the feet, as shown in Figure 1G (L04), was also observed in 83% of the legs, possibly consistent with the sparing of the feet in lipedema. As lymphatic vessels transited the medial thighs (Figure 1H; L03), the fluorescence intensity often decreased further,

although fluorescent lymph nodes were generally observed in the inguinal regions, consistent with functional lymphatic watersheds. The lymphatic anatomy and function in arms of individuals with lipedema appeared similar to those of control individuals.

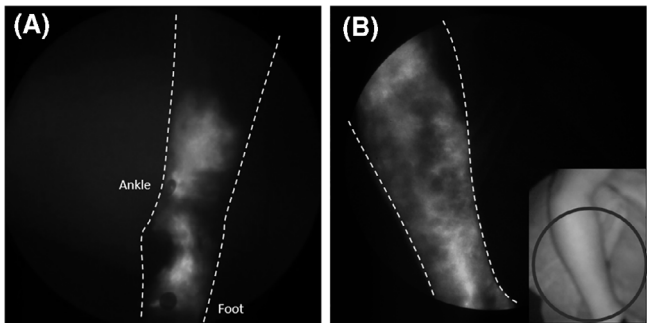

FIGURE 2 NIRF-LI images (white light image inset) illustrating the dermal backflow commonly observed in individuals with lymphedema, including (A) the leg of an individual with primary lymphedema (no lipedema) and (B) the arm of a patient with breast cancer who had extensive dermal backflow but did not yet have the extent of arm swelling needed for clinical diagnosis. Injection sites were covered by round bandages and/or black vinyl tape. The brightness and contrast of the NIRF-LI images have been adjusted to help visualize both the dim and bright vessels in the 16-bit images. NIRF-LI, near-infrared fluorescence lymphatic imaging

The most common anatomic abnormality observed in clinical and subclinical lymphedema was dermal backflow (Figure 2); however, we did not see large areas of dermal backflow in these individuals. The exception was small areas of dermal backflow in the wrists of L17 (Figure 3A), who had previously undergone liposuction of the arms. Several small areas of diffuse lymphatic structure in unusual locations (Figure 3B-3D) were observed in nine legs (L03-L, L03-R, L06-R, L07-L, L08-L, L10-R, L12-L, L13-R, and L19-L). Although one of these diffuse areas (Figure 3B; L07-L) had some similarities to early dermal backflow, it appeared to be part of the lymphatic vessel draining the foot. Whether this area will evolve into more typical dermal backflow with lipedema progression or whether it is associated with lipomas and/or fatty nodules, as seen in patients with Dercum (15), remains to be seen in longitudinal studies. The diffuse lymphatic structures observed in the other legs had bright spots similar to those originating from lymph nodes but they were not located in areas where lymph nodes are typically observed, including the mid-medial thigh (Figure 3C; L19-L) and the anterior thigh (Figure 3D; L03-R). It is possible that these structures were ectopic lymph nodes/tissues, such as those seen in rheumatoid arthritis (20), indicative of underlying inflammatory processes.

Figure 3E shows the interstitial accumulation of fluorescence around the injection site in the calf of L08-R. Figure 3F shows a small lymphatic vessel radiating from the injection site in the calf of L17-L. Figure 3G provides an image of possible lymphatic varicosity of the

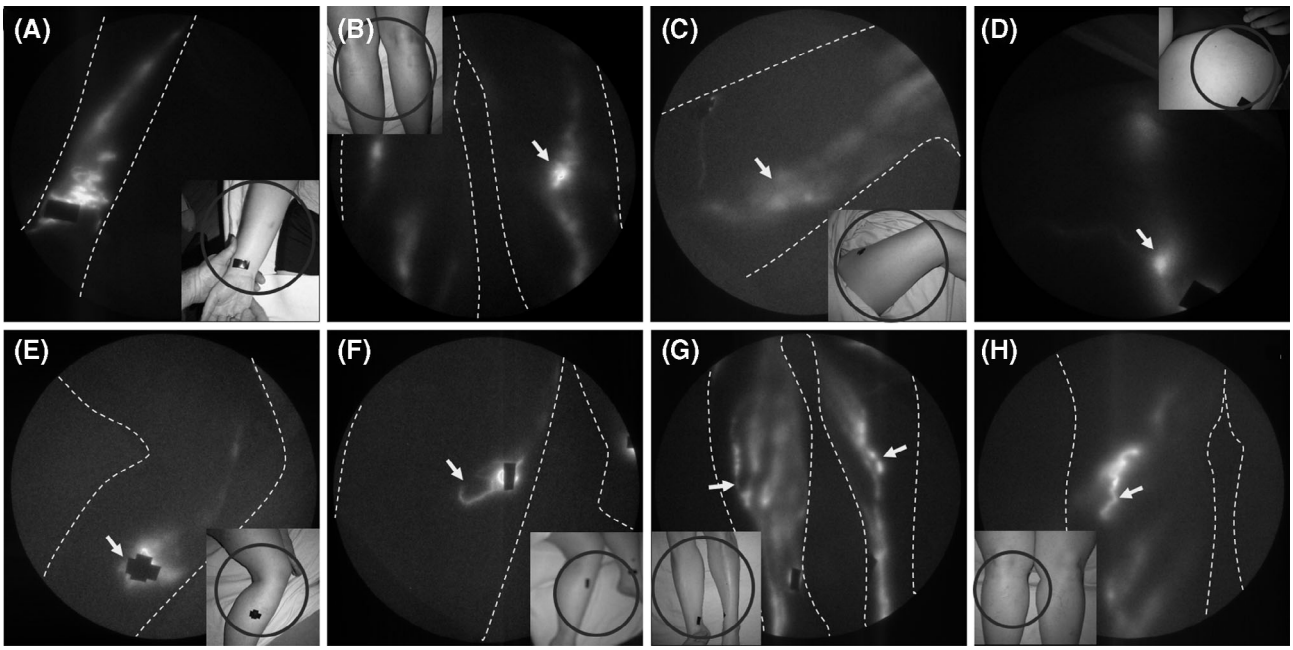

FIGURE 3 NIRF-LI images (white light images inset) of anatomic features of interest. (A) Image of dermal lymphatic backflow in the wrist of one individual (L17-R) who had previously undergone liposuction in the arms. Other notable anatomic features include (B-D, arrows) areas of diffuse lymphatic structures that did not appear to be dermal backflow and often appeared node-like but were not located in areas where nodes were typically observed in control individuals (L07-L, L19-L, and L03-R, respectively), (E) interstitial backflow (arrow) around injection sites (L08-L), (F) vessels radiating (arrow) from the injection sites (L17-R), (G) signs of segmentation (arrows; and, atypically, this patient had no signal drop-off between the feet and shins; L14), and (H) a tortuous vessel (arrow) in the shin of L05-R. Injection sites were covered by round bandages and/or black vinyl tape. The brightness and contrast of the NIRF-LI images have been adjusted to help visualize both the dim and bright vessels in the 16-bit images. NIRF-LI, near-infrared fluorescence imaging

lower legs of L14, particularly in the left limb, in which bright and dark segments were observed along the lymphatic vessels, whereas the right limb showed less distinct signs of segmentation. Prior work, using contrast enhanced magnetic resonance imaging (MRI), confirmed that these "segmented" lymphatic vessels may correspond to "corkscrew" geometry, or lymphatic varicosity, of vessels (19). Interestingly, in this same individual (L14; Figure 3G), we did not see the distinct drop-off in the fluorescent intensity between the feet and shins observed in the other participants. Figure 3H shows a tortuous lymphatic vessel observed in L05-R.

Overall, of the 16 legs with Stage I disease, 9 (56%) presented with evidence of segmentation/varicosity (L01-L, L03-R, L07-L, L07-R, L11-L, L14-L, L14-R, L15-L, and L15-R), 4 (25%) with tortuous vessels (L04-R, L07-L, L07-R, and L11-R), 15 (94%) with dilated vessels (all except L01-L), 4 (25%) with radiating vessels (L01-R, L07-R, L14-R, and L15-R), 9 (56%) with interstitial backflow around at least one injection site (L03-R, L04-R, L07-L, L09-L, L09-R, L11-R, L14-R, L15-L, and L15-R), and 3 (19%) with diffuse lymphatic structures that transported propelled lymph (L03-L, L03-R, and L07-L). In addition, 13 of 16 Stage I limbs (81%) exhibited distinct fluorescent signal attenuation between the feet and lower legs (all except L14-L, L14-R, and L15-R).

Of the 22 legs with Stage II disease, 11 (50%) presented with evidence of segmentation (L02-L, L02-R, L08-L, L10-L, L13-L, L13-R, L16-L, L16-R, L19-L, L20-L, and L20-R), 8 (36%) with tortuous vessels (L05-L, L05-R, L06-L, L10-L, L10-R, L13-R, L16-L, and L19-R), 22 (100%) with dilated vessels (all), 15 (68%) with radiating vessels (L02-L, L02-R, L06-R, L08-L, L08-R, L13-R, L16-R, L17-L, L17-R, L18-L, L18-R, L19-L, L19-R, L20-L, and L20-R), 8 (36%) with interstitial accumulation around at least one injection site (L02-L, L08-L, L08-R, L10-R, L16-L, L18-L, L19-L, and L19-R), and 5 (23%) with diffuse or unusual lymphatic structures (L06-R, L08-L, L10-R, L13-R, and L19-R). In addition, 18 of 22 Stage II limbs (82%) exhibited distinct fluorescent signal attenuation between the feet and lower legs (all except L05-R, L08-R, L19-L, and L19-R). In the single individual (L12) with Stage III disease, all of these abnormalities were observed in both legs, with the exception of radiative lymphatic vessels (L12-L and L12-R) and diffuse/unusual lymphatics in L12-R.

Although the emphasis of this study was on the legs, where lipedema changes are most prevalent, we also imaged the arms and abdomen. The arm lymphatics were largely normal in appearance and, of the 40 arms imaged, only 3 presented with evidence of segmentation (L03-R, L04-L, and L04-R), 2 with tortuous vessels (L13-R and L17-L), 1 with dilated vessels (L08-L), and 2 with interstitial backflow (L04-R and L09-R). L17, who had previously undergone liposuction of the arms, presented with dermal backflow in both wrists (Figure 3A). In L16, the lymphatic vessels in the upper arms were notably less bright than the lower arms, similar to the signal drop-off observed between the feet and lower legs. In L09-L, lymphatic reflux was observed. The abdominal lymphatics generally drained to the inguinal lymph nodes (Figure 1H); however, crossover drainage between the left and right injection sites was common and, in several individuals, the abdominal injections drained to both the inguinal and the axillary nodal basins.

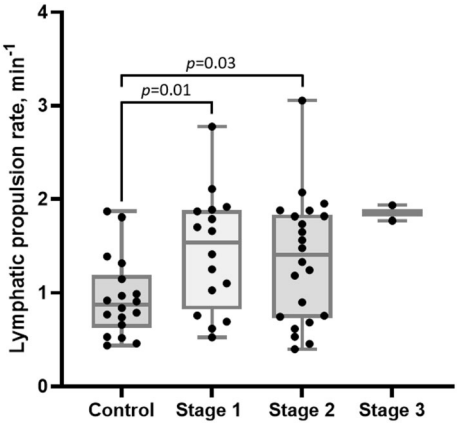

FIGURE 4 Whisker plot of the propulsion rates observed in the control individuals and individuals with lipedema

Function

The average control propulsion rate was 0.9 (0.4) events/min (range = 0.44-1.87 events/min) (14). In individuals with lipedema, the average propulsion rates were 1.4 (0.6), 1.4 (0.7), and 1.8 (0.1) events/min in Stage I, II, and III disease, respectively. Figure 4 shows a whisker plot of the propulsion rates, as well as the data point spread. Student *t* tests indicate that the differences between the control rates and the Stage I and Stage II rates are significant ($P = 0.0102$ and $P = 0.0258$, respectively), but not between Stage I and Stage II rates ($P = 0.6692$). Because only one individual had Stage III disease (L12), this stage was not included in the statistical analysis. Lymphatic pumping was observed in all arms but was not quantified for this report, as control data were available only for the lower legs.

DISCUSSION

The results of this study, in individuals with Stage I to II lipedema without lipolymphedema, demonstrate abnormal lower extremity lymphatic anatomy compared with control participants, with increased incidence of segmented, tortuous, and/or dilated vessels, as well as increased incidence of interstitial accumulation. Despite seven individuals self-reporting intermittent swelling with their disease (Table 1), not one individual presented with dermal backflow in the legs. Although present in increased numbers, the reported anatomic abnormalities are not distinguishing characteristics for diagnosis of lipedema.

Our past studies of patients with cancer showed that, once present, dermal backflow persisted over months and years (21) but that it could be reduced in extent by physiotherapies (12). In recent longitudinal studies of patients with breast cancer at increased risk for arm

lymphedema, dermal backflow predicted the future onset of irreversible edema by as much as 23 months. In a study of patients with early venous disease and lower extremity reversible edema, dermal backflow was noted (14), but whether dermal backflow occurs with lower extremity reversible edema in early lipedema or otherwise normal healthy individuals remains to be studied in individuals with edema. From our observations of patients with early-stage lipedema who self-reported past reversible edema, we did not observe dermal backflow, but rather enhanced lymphatic propulsion in their legs. Using lymphoscintigraphy, others, while reporting impaired lymphatic function, have also reported mild anatomic abnormalities, including the lack of dermal backflow in early lipedema, and have used the technique to exclude lymphedema in the diagnosis of lipedema. (22–26)

It remains to be seen whether individuals with lipolymphedema present with decreasing lymphatic propulsion and subsequent dermal backflow, as commonly observed in acquired lymphedema using NIRF-LI. It is noteworthy that L09 had been misdiagnosed with lymphedema prior to her diagnosis with lipedema, and the lack of dermal backflow indicates that her intermittent swelling, which prompted the initial misdiagnosis, likely results from transiently impaired lymphatic uptake and/or lymphatic propulsion and not catastrophic lymphatic failure. Longitudinal, observational studies may elucidate the dynamic relationships between lymphatic propulsion, dermal backflow, and reversible edema and the onset of irreversible edema (or lipolymphedema) in these individuals.

It is important to note that lymphatic dysfunction arises because of lack of uptake into initial lymphatics, reduced lymphatic propulsion, and/or increased lymphatic load associated with inflamed tissues and leaky blood vasculature. In the event of impaired lymphatic pumping, but intact lymphatic uptake, dermal backflow occurs as an early sign of lymphatic failure. Our work showed normal lymphatic uptake, increased lymphatic propulsion, and no dermal backflow. In contrast, Busco et al. (27) recently used ICG lymphography to assess proximal lymphatic transport in the legs of individuals with early-stage lipedema. Their results, using 0.8-mL subcutaneous injections, also showed no dermal backflow but negatively correlated the proximal lymph transport with BMI and duration of disease, therefore associating early lipedema with lymphatic dysfunction. However, these results may be negatively influenced by fluorescence attenuation of intervening tissue layers that proximally increase in thickness and by delayed and variable lymphatic uptake from subcutaneous injection sites. Because we measured active lymphatic propulsion near the ankle, made possible by the enhanced sensitivity of NIRF-LI and the low-dose, low-volume intradermal injections, the results presented herein are not as susceptible to attenuation artifact or impaired subcutaneous delivery.

Of note, this study was not blinded, and some bias and subjectivity are inherently present when determining whether an anatomic lymphatic "feature" is abnormal. Segmentation was particularly susceptible to this bias, as the fluorescence intensity within a vessel can vary for a variety of reasons, including variations in the ICG concentration, vessel depth, and tissue pigmentations, as well as the presence of overlying blood vasculature. To reflect this uncertainty, in situations in which the lymphatic vessel intensity was not as uniform as a "normal" lymphatic vessel and did not yet have obviously alternating segments of light and dark segments, the variation was reported as "signs of segmentation" (Table 2). Although not addressed in this contribution, efforts are underway to develop metrics to quantify lymphatic anatomic abnormalities, as observed with NIRF-LI, to further reduce the subjectivity of future analyses.

Although the observed lymphatic abnormalities (i.e., dilated, tortuous, and/or segmented vessels, as well as interstitial accumulation and radiating vessels) have been reported previously in other disease, the statistically higher pumping rates in Stage I and II lipedema provide a stark contrast to the reduced propulsion rates reported in lymphedema (8) and progressive venous disease (14), as well as the "sluggish" propulsion observed in Dercum's disease (15). Although the lymphatic pumping mechanism is not completely understood, the increased pumping may be mediated by lymphatic preload (28) resulting from enhanced capillary permeability (7). It is noteworthy that hypermobile joints are common in women with lipedema (29) and that varicose or dilated veins are often associated with hypermobile joints (30) and lipedema (31). Veins and joints are generally impacted in underlying connective tissue disorders. Given the propensity for swelling in lipedema, it is possible that, although enhanced lymphatic pumping may initially maintain fluid homeostasis in the face of increased capillary permeability, with disease progression, the lymphatic preload eventually exceeds lymphatic pumping capacity. The reduced clearance of metabolic waste may result in a vicious cycle of increased inflammation, including polarization of M2 macrophages to inflammatory M1 macrophages until lymphatic function may become chronically insufficient, leading to lipolymphedema. The use of complete decongestive therapy or intermittent pneumatic compression therapy to aid lymphatic transport may mitigate the sequelae that progress to lipolymphedema (32).

Compared with the lower extremities, upper extremity lymphatics of participants with lipedema were unremarkable and comparable with control participants. Because functional lower extremity lymphatics must propel lymph over greater distances and against gravity for return to the blood vasculature, and because venous hypertension is greater in the lower extremities, abnormal lymphatics associated with lipedema may be more prevalent in the lower extremities. Dermal backflow was observed in the wrists of L17; however, as no other arms presented with dermal backflow, the presence of this abnormality can most likely be attributed to the liposuction procedure performed approximately 1 year prior to imaging.

Nearly all legs presented with apparent dilated lymphatics, particularly in the medial ankles and legs. Because the lymphatics underlie subcutaneous adipose tissue, it is possible that light scattering accounts for the broadening of vessels. However, dilated vessels have also been reported with non-contrast MRI (33) and, although not a measure of subcutaneous fat distribution, the mean BMI of the control group (30.3) is actually higher than that of the lipedema group (29.3). In contrast, histological examination of paraffin-embedded lipedema adipose tissue revealed apparently nondilated lymphatic vessels (7). It is possible that actively pumping lymphatic vessels, visualized with MRI or NIRF-LI, appear dilated, whereas histologically fixed vessels, showing similar podoplanin (a lymphatic marker)

coverage of area on a microscopic side, with increased, but not significant, mean podoplanin-positive lymphatic vessel area, represent "unstretched" vessels. Unlike preclinical studies of lipopolysaccharide- or IL-1β-induced inflammation, or longitudinal clinical studies of patients with breast cancer who ultimately succumb to clinical lymphedema, vessel dilation in these individuals with lipedema did not result in reduced lymphatic pumping. In comparison with a control population of similar age and BMI, lymphatic propulsion rates were significantly higher. The discordance between conducting lymphatic vessel dilation and pumping rates is surprising and not consistent with prior preclinical studies of acute inflammation. Future studies to evaluate the relationship between the cytokine milieu and the mediators of capillary permeability and lymphatic pumping could shed new insights in the dichotomy between lymphatic function and anatomy observed in Stage I and II lipedema. Of note, lipedema and lymphedema are both characterized by increased levels of platelet factor 4 (PF4/CXCL4) in blood plasma exosomes, and this biomarker was suggested to be indicative of defective lymphatic function (34). Our findings provide evidence that increased PF4 may drive related, but different, processes of inflammation in lipedema and lymphedema that result in different vessel pulsing response.

CONCLUSION

The diagnoses of lipedema and lymphedema are often confused. Although lymphatic vessel dilation, dermal backflow, and impaired lymphatic pumping are (predictive) characteristic of acquired and congenital lymphedema, the lower extremities of individuals with Stage I and Stage II lipedema without lipolymphedema possess dilated lymphatic vessels with significantly greater lymphatic pumping than control individuals, but no dermal backflow. Our studies show that the lymphatic contributions to the etiology of lymphedema and lipedema are different and that impaired lymphatic clearance is not involved in the early progression of lipedema. The relationship of the structural changes in the lymphatic vessels of patients with lipedema requires additional study.O

CONFLICT OF INTEREST

Caroline E. Fife, John C. Rasmussen, and Eva M. Sevick-Muraca are listed as inventors on patents related to near-infrared fluorescent lymphatic imaging and might receive future financial benefit from its commercialization. Caroline E. Fife, John C. Rasmussen, Eva M. Sevick-Muraca, and the University of Texas Health Science Center at Houston have research-related financial interests in Lymphatic Science, Inc. Melissa B. Aldrich and Karen L. Herbst reported no conflict of interest.

ORCID

John C. Rasmussen https://orcid.org/0000-0002-0557-6899

REFERENCES

1. Allen EV, Hines EA. Lipedema of the legs: a syndrome characterized by fat legs and orthostatic edema. *Proc Staff Meet Mayo Clinic*. 1940; 15:184-187. https://lipedemaproject.org/wp-content/uploads/2016/02/1940_Allen_Lipedema-of-the-Legs.pdf
2. Blum KS, Karaman S, Proulx ST, et al. Chronic high-fat diet impairs collecting lymphatic vessel function in mice. *PLoS One*. 2014;9: e94713. doi:10.1371/journal.pone.0094713
3. Escobedo N, Proulx ST, Karaman S, et al. Restoration of lymphatic function rescues obesity in Prox1-haploinsufficient mice. *JCI Insight*. 2016;1:e85096. doi:10.1172/jci.insight.85096
4. Al-Ghadban S, Cromer W, Allen M, et al. Dilated blood and lymphatic microvessels, angiogenesis, increased macrophages, and adipocyte hypertrophy in lipedema thigh skin and fat tissue. *J Obes*. 2019; 2019:8747461. doi:10.1155/2019/8747461
5. Vandanmagsar B, Youm Y-H, Ravussin A, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med*. 2011;17:179-188.
6. Aldrich MB, Sevick-Muraca EM. Cytokines are systemic effectors of lymphatic function in acute inflammation. *Cytokine*. 2013;64: 362-369.
7. Felmerer G, Stylianaki A, Hollmén M, et al. Increased levels of VEGF-C and macrophage infiltration in lipedema patients without changes in lymphatic vascular morphology. *Sci Rep*. 2020;10:10947. doi:10.1038/s41598-020-67987-3
8. Rasmussen JC, Tan IC, Marshall MV, et al. Human lymphatic architecture and dynamic transport imaged using near-infrared fluorescence. *Transl Oncol*. 2010;3:362-372.
9. Aldrich M, Guilliod R, Fife CE, et al. Lymphatic abnormalities in the normal contralateral arms of subjects with breast cancer-related lymphedema as assessed by near-infrared fluorescent imaging. *Biomed Opt Express*. 2012;3:1256-1265.
10. Agollah GD, Gonzalez-Garay ML, Rasmussen JC, et al. Evidence for SH2 domain-containing 5′-inositol phosphatase-2 (SHIP2) contributing to a lymphatic dysfunction. *PLoS One*. 2014;9:e112548. doi:10.1371/journal.pone.0112548
11. Greives MR, Aldrich MB, Sevick-Muraca EM, Rasmussen JC. Near-infrared fluorescence lymphatic imaging of a toddler with congenital lymphedema. *Pediatrics*. 2017;139:e20154456. doi:10.1542/peds.2015-4456
12. Gutierrez C, Karni RJ, Naqvi S, et al. Head and neck lymphedema: treatment response to single and multiple sessions of advanced pneumatic compression therapy. *Otolaryngol Head Neck Surg*. 2019; 160:622-626.
13. Rasmussen JC, Aldrich MB, Tan I-C, et al. Lymphatic transport in patients with chronic venous insufficiency and venous leg ulcers following sequential pneumatic compression. *J Vasc Surg Venous Lymphat Disord*. 2016;4:9-17.
14. Rasmussen JC, Zhu B, Morrow JR, et al. Degradation of lymphatic anatomy and function in early venous insufficiency. *J Vasc Surg Venous Lymphat Disord*. 2021;9:720-730.e2.
15. Rasmussen JC, Herbst KL, Aldrich MB, et al. An abnormal lymphatic phenotype is associated with subcutaneous adipose tissue deposits in Dercum's disease. *Obesity (Silver Spring)*. 2014;22:2186-2192.
16. Rasmussen JC, Zvavanjanja RC, Aldrich MB, Greives MR, Sevick-Muraca EM. Near-infrared fluorescence lymphatic imaging of Klippel–Trénaunay syndrome. *J Vasc Surg Venous Lymphat Disord*. 2017;5:533-537.
17. Rasmussen JC, Fife CE, Sevick-Muraca EM. Near-infrared fluorescence lymphatic imaging in lymphangiomatosis. *Lymphat Res Biol*. 2015;13:195-201.
18. Tan I-C, Balaguru D, Rasmussen JC, et al. Investigational lymphatic imaging at the bedside in a pediatric postoperative chylothorax case. *Pediatr Cardiol*. 2014;35:1295-1300.
19. Burrows PE, Gonzalez-Garay ML, Rasmussen JC, et al. Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man. *Proc Natl Acad Sci*. 2013;110:8621-8626.

20. Noort AR, van Zoest KPM, van Baarsen LG, et al. Tertiary lymphoid structures in rheumatoid arthritis: NF-κB-inducing kinase-positive endothelial cells as central players. *Am J Pathol*. 2015;185:1935-1943.
21. Rasmussen JC, Tan I-C, Naqvi S, et al. Longitudinal monitoring of the head and neck lymphatics in response to surgery and radiation. *Head Neck*. 2017;39:1177-1188.
22. Bilancini S, Lucchi M, Tucci S, Eleuteri P. Functional lymphatic alterations in patients suffering from lipedema. *Angiology*. 1995;46:333-339.
23. Boursier V, Pecking A, Vignes S. Comparative analysis of lymphoscintigraphy between lipedema and lower limb lymphedema [article in French]. *J Mal Vasc*. 2004;29:257-261.
24. Forner-Cordero I, Oliván-Sasot P, Ruiz-Llorca C, Muñoz-Langa J. Lymphoscintigraphic findings in patients with lipedema. *Rev Esp Med Nucl Imagen Mo (Engl Ed)*. 2018;37(6):341-348. doi:10.1016/j.remn.2018.06.008
25. Tartaglione G, Visconti G, Bartoletti R, Ieria FP, Salgarello M. Rest/stress intradermal lymphoscintigraphy in diagnosis of lipedema. *World J Nucl Med*. 2020;19:376-381.
26. Gould DJ, El-Sabawi B, Goel P, Badash I, Colletti P, Patel KM. Uncovering lymphatic transport abnormalities in patients with primary lipedema. *J Reconstr Microsurg*. 2020;36:136-141.
27. Buso G, Favre L, Maufus M, et al. Indocyanine green lymphography as novel tool to assess lymphatics in patients with lipedema. *Microvasc Res*. 2022;140:104298. doi:10.1016/j.mvr.2021.104298
28. Scallan JP, Zawieja SD, Castorena-Gonzalez JA, Davis MJ. Lymphatic pumping: mechanics, mechanisms and malfunction. *J Physiol*. 2016;594:5749-5768.
29. Beltran K, Herbst KL. Differentiating lipedema and Dercum's disease. *Int J Obes (Lond)*. 2017;41:240-245.
30. el-Shahaly HA, el-Sherif AK. Is the benign joint hypermobility syndrome benign? *Clin Rheumatol*. 1991;10:302-307.
31. Wold LE, Hines EA, Allen EV. Lipedema of the legs; a syndrome characterized by fat legs and edema. *Ann Intern Med*. 1951;34:1243-1250.
32. Kruppa P, Georgiou I, Biermann N, Prantl L, Klein-Weigel P, Ghods M. Lipedema-pathogenesis, diagnosis, and treatment options. *Dtsch Arztebl Int*. 2020;117:396-403.
33. Cellina M, Gibelli D, Soresina M, et al. Non-contrast MR lymphography of lipedema of the lower extremities. *Magn Reson Imaging*. 2020;71:115-124.
34. Ma W, Gil HJ, Escobedo N, et al. Platelet factor 4 is a biomarker for lymphatic-promoted disorders. *JCI Insight*. 2020;5:e135109. doi:10.1172/jci.insight.135109

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