HHS Public Access

Author manuscript

Obesity (Silver Spring). Author manuscript; available in PMC 2018 June 27.

Published in final edited form as:

Obesity (Silver Spring). 2018 February ; 26(2): 310–317. doi:10.1002/oby.22090.

Tissue sodium content is elevated in the skin and subcutaneous adipose tissue in women with lipedema

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Abstract

Objective—To test the hypothesis that tissue sodium and adipose content are elevated in patients with lipedema; if confirmed, this could establish precedence for tissue sodium and adipose content representing a discriminatory biomarker for lipedema.

Methods—Participants with lipedema (n=10) and control (n=11) volunteers matched for biological sex, age, body-mass-index, and calf circumference were scanned noninvasively with 3.0T sodium and conventional proton MRI. Standardized tissue sodium content was quantified in skin, subcutaneous adipose tissue (SAT), and calf muscle. Dixon MRI was employed to quantify tissue fat and water volumes of the calf. Nonparametric statistical tests were applied to compare regional sodium content and fat-to-water volume ratio between groups (significance: two-sided $p \leq 0.05$).

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Disclosure: The authors have no conflicts of interest to disclose.

Author Contributions:

RC conceived and carried out the experiments and analyzed data. AM and PMCD conceived and carried out the experiments. PW carried out the experiments and analyzed data. HBM and SKL carried out experiments. JAB, MJD, and JT conceived the experiments and analyzed data. All authors were involved in writing the manuscript and gave approval of the submitted and published versions.

Results—Skin (p=0.01) and SAT (p=0.04) sodium content were elevated in lipedema (skin: 14.9±2.9 mmol/L; SAT: 11.9±3.1 mmol/L) relative to control (skin: 11.9±2.0 mmol/L; SAT: 9.4±1.6 mmol/L) participants. Relative fat volume in the calf was elevated in lipedema (1.2±0.48 ratio) relative to control (0.63±0.26 ratio, p<0.001) participants. Skin sodium content was directly correlated with fat-to-water volume ratio (Spearman's-rho=0.54, p=0.01).

Conclusions—Internal metrics of tissue sodium and adipose content are elevated in patients with lipedema potentially providing objective imaging-based biomarkers for differentially diagnosing the under-recognized condition of lipedema from obesity.

Keywords

obesity; diagnosis; imaging; radiology; body composition

Introduction

Lipedema is a chronic condition involving excessive and disproportionate adipose tissue deposition in the extremities of females. Importantly, adipose tissue in the legs due to lipedema does not regress in response to typical treatments for obesity such as diet, exercise, or even bariatric surgery[1]. Women with lipedema can appear similar to obese women or those with lower extremity lymphedema and misdiagnosis is common[2]. The true prevalence of lipedema is not known, likely due to a lack of awareness of the condition. Despite the availability of clinical criteria, an objective diagnostic test to distinguish lipedema from obesity represents the fundamental unmet need for the field[1, 3, 4].

Symptoms of lipo-lymphedema in advanced stages of lipedema suggest a role for compromised lymphatic function. Contrast-enhanced imaging of lymphatic vessels in several patients with lipo-lymphedema revealed dilated and permeable small vasculature throughout the legs[5]. Lymphatic vessels drain interstitial fluid and transport large lipoproteins and fatty acids[6], sodium[7], and water from peripheral tissue into the blood stream. Impaired lymphatic vessel function may reduce clearance of these components from the periphery, resulting in lipid, sodium, and water accumulation at the tissue level. Therefore, we hypothesized that adipose accumulation would be accompanied by sodium accumulation in women with lipedema.

To test this hypothesis, we employed magnetic resonance imaging (MRI) techniques to measure tissue sodium and adipose content in the legs of women with lipedema compared to that of controls tightly matched for biological sex, age, race, body mass index (BMI), and calf circumference. We show that sodium is elevated in the skin and subcutaneous adipose tissue (SAT) in patients with lipedema.

Methods

Volunteer enrollment and lipedema inclusion criteria

All volunteers provided informed consent in accordance with the Vanderbilt University Institutional Review Board (IRB). Female volunteers with lipedema (n=10, age=44.0±12.5 years, mean ± standard deviation) were recruited from the Vanderbilt Lymphedema Clinic

and an IRB-approved enrollment survey distributed through social media support groups. All recruited patients were diagnosed with lipedema by a Lymphology Association of North America (LANA)-certified lymphedema physical therapist or qualified physician. Subjects with lipedema additionally underwent a clinical examination to verify that they met all primary criteria and at least one secondary criterion for enrollment (Table 1). Criteria, lipedema stage, and type were adopted from Herbst *et al.* 2012[1]. Subjects were asked to respond to the visual analogue scale (VAS, electronic sliding scale from 0–100) regarding pain experienced in their legs on a normal day[8]. The weight and height of subjects were measured to calculate BMI. The widest girth of the subject's calf was identified and calf circumference was measured at this location.

Female control volunteers (n=11, age=45.4±12.1 years, mean ± standard deviation) were recruited through the Vanderbilt University Human Imaging Core. Both subjects with lipedema and controls were in a stable condition without active infection or inflammation. Exclusion criteria included evidence of current skin infections or other signs of inflammation and past medical history of hypertension, diabetes, or arthritis in all subjects. Control volunteers were matched for biological sex, age, race, BMI, and calf circumference to participants with lipedema.

Multi-modal MRI acquisition

All volunteers were scanned at 3.0T (Philips Achieva, Philips Healthcare, Best, The Netherlands). The leg with the widest calf girth was used for imaging if asymmetry presented. The calf was then centered over four standard sodium solutions (aqueous NaCl in the physiologic range of tissue sodium content from 10 to 40 mmol/L, see Figure 1a–b) embedded in a platform on which the calf was allowed to rest for 10 minutes before acquisition of the sodium image. Sodium imaging of the calf was implemented using a quadrature knee coil tuned for sodium signal reception (Rapid Biomedical GmbH, Rimpar, Germany). A 3D gradient-echo sequence was employed: field-of-view (FOV)=192×192 mm^2, acquired matrix size=64×64; slice thickness=30 mm. An echo time (TE) of 0.99 ms was chosen for measurements of sodium content from the calf and aqueous sodium solutions using the same TE[9]. A repetition time (TR) of 130 ms was chosen to reduce error from residual T_1 signal effects; this is the shortest TR required to measure tissue sodium content with a relative error of less than 5% compared to longer acquisitions that allow full recovery[10]. The sodium image acquisition time was approximately 15 minutes.

Proton images were acquired during the same scan session without moving the subject by using the body coil for reception. The Dixon method was performed to separate the proton signal from fat and water species in the same acquisition, and provided fat-weighted ($Dixon_{FAT}$) and water-weighted ($Dixon_{WATER}$) images in the identical FOV as the sodium image (TR=200ms, TE_1=1.15ms, TE_2=2.30ms, matrix size=192×192, in-plane spatial resolution=1×1 mm^2, 6 slices each 5 mm thick). The total scan time required for proton imaging was approximately 4 minutes.

Image segmentation and analysis

Mean signal intensity was measured in each standard solution in order to calibrate the magnitude sodium signal intensity to known sodium concentrations on a per-voxel basis for each subject (Figure 1c). A quantitative sodium map was calculated and interpolated to match the matrix size of the Dixon images for each subject (Figure 1d).

Regions of interest (ROIs) were segmented on the central slice of the $Dixon_{WATER}$ image, including the outer and inner borders of the skin, and the total muscle. The skin region was further segmented as the posterior semi-perimeter of the skin because the anterior surface of the lower leg tapers along the slice dimension. The SAT region was defined as the area between the skin and muscle. Voxels in bone and blood vessels were removed from analyses. Figure 2a–c depicts example $Dixon_{FAT}$ and $Dixon_{WATER}$ images and segmented ROIs.

The circumference of the calf was measured as the perimeter of the skin in units of cm. The area of SAT (mm^2) was normalized by calf circumference (in units of mm) to provide a relative measure of the amount of SAT (units of mm) in the leg.

The total adipose tissue volume in the leg was calculated using an automated segmentation routine. A threshold was applied to the central $Dixon_{FAT}$ images based on a k-means clustering algorithm (*kmeans* function in MATLAB R2015a, MathWorks, Natick MA) assuming two compartments: fat and water. The ratio of the number of voxels in fat tissue (Figure 2d) and the number of voxels in water tissue (Figure 2e) was taken as the fat/water volume ratio (see Figure 2f). This measure accounts for the presence of adipose from all tissues in the calf and the volume of the muscle.

Statistical testing

The primary objective of this study was to determine whether tissue sodium content was significantly different in each ROI in patients with lipedema compared to controls matched for biological sex, age, race, BMI, and calf circumference. To evaluate this objective, mean sodium content was measured from the skin, SAT, and muscle ROIs and standard deviations within each cohort were calculated. Group differences between tissue sodium were tested using a nonparametric Mann-Whitney test with two-sided $p \leq 0.05$ required for significance. Values are reported as group mean ± standard deviation, and the group range reflects minimum and maximum values. Group differences between age, race, BMI, and calf circumference were also tested with the same criteria to ensure participants were well-matched between groups for these parameters. As an exploratory analysis, we analyzed the subgroup of participants with lipedema that were early stage (Stages 1 or 2) and compared their imaging values to the subgroup of control volunteers who were matched for biological sex, age, race, BMI and calf circumference to this patient cohort. The assessments of these two subgroups were similar to the overall group assessments and were performed to evaluate whether the MRI protocol has potential to discriminate between early stage lipedema and non-lipedema controls.

A secondary objective was to determine whether the adipose content in the calf was significantly higher in participants with lipedema compared to controls matched for biological sex, age, race, BMI, and calf circumference. Two measures of adipose content

were evaluated including i) SAT area normalized by calf circumference and ii) total fat/water volume ratio in the calf. Group differences between normalized SAT area and fat/water volume were tested using a nonparametric Mann-Whitney test with two-sided $p \leq 0.05$ required for significance.

A third objective was to determine whether tissue sodium content was associated with normalized SAT area or fat/water volume in the calf. To evaluate this objective, a two-sided nonparametric Spearman's correlation test was applied and Spearman's-rho and significance level ($p \leq 0.05$) were determined between each study measure over all participants (n=21).

Results

Volunteer demographics

Table 2 summarizes the clinical features of patients with lipedema. Age at onset of symptoms was coincident within three years of either menarche or pregnancy in all cases. All patients confirmed that symptomatology did not respond noticeably to diet or exercise. Those participants who received liposuction or gastric bypass surgery were imaged at least one year following surgery. Those who received gastric bypass surgery reported less change in their lower body compared to upper body volume following the procedure.

Table 3 summarizes the demographic information of participants with lipedema (n=10; percent female=100%; race: 9 Caucasian, 1 Black; age=44.0±12.5 years, age range=17–61 years; BMI=33.0±7.6 kg/m^2; BMI range=21.3–48.9 kg/m^2; widest calf circumference=44.1±5.5 cm, widest calf circumference range=36.8–56.5 cm). Control volunteers (n=11; percent female=100%; race: 9 Caucasian, 2 Black; age=45.4±12.1 years, age range=33–65 years; BMI=30.6±4.5 kg/m^2, BMI range=23.8–38.6 kg/m^2; widest calf circumference=41.2±3.4 cm, widest calf circumference range=37.2–46.9 cm) were matched for biological sex (p=1.00), age (p=0.99), race (p=0.99), BMI (p=0.56), and calf circumference (p=0.32) to participants with lipedema.

Tissue sodium content and adipose composition

Calf-tissue sodium content was significantly elevated in participants with lipedema compared to controls in the skin (14.9±2.9 mmol/L vs. 11.9±2.0 mmol/L, p=0.01, Figure 3a) and SAT (11.9±3.1 mmol/L vs. 9.4±1.6 mmol/L, p=0.04). Sodium content trended higher in the muscle (17.2±3.0 mmol/L vs. 14.7±0.8 mmol/L, p=0.06), but this did not meet stated criteria for significance in our sample.

Participants with lipedema demonstrated larger normalized SAT area (13.4±4.7 mm vs. 8.7±3.8 mm, p=0.02, Figure 3b) and a greater fat/water volume ratio compared to controls (1.2±0.48 ratio vs. 0.63±0.26 ratio, p<0.01, Figure 3c).

A subgroup of patients with early stage lipedema (Stages 1 or 2, n=7, age=38.4±10.4 years, BMI=29.8±5.4 kg/m^2, calf circumference=42.2±3.2 mm, race: 7 Caucasian) and a matched subgroup of female controls (n=7, age=41.1±6.1 years, BMI=29.9±4.2 kg/m^2, calf circumference=41.1±3.1, race: 7 Caucasian) were taken from this study's volunteers. Sodium was significantly elevated in the skin (13.8±1.9 mmol/L vs. 11.4±1.3 mmol/L,

p=0.04, Figure 3d) and SAT (11.2±2.7 mmol/L vs. 8.8±1.5 mmol/L, p=0.03) of participants with early stage lipedema relative to matched controls. Normalized SAT area was not significantly different between participants with early stage lipedema and matched controls (8.8±4.7 mm vs. 11.7±3.4 mm, p=0.21, Figure 3e). Fat/water volume was significantly elevated in patients with early stage lipedema (1.1±0.32 ratio vs. 0.65±0.29 ratio, p=0.04, Figure 3f).

Skin sodium content correlated significantly with SAT sodium content (Spearmen'srho=0.66, p=0.001) and with muscle sodium content (Spearman's-rho=0.75, p<0.001). Normalized SAT area was significantly correlated with fat/water volume (Spearman's-rho=0.92, p<0.001) and with BMI (Spearman's-rho=0.54, p=0.01; Spearman's-rho=0.58, p=0.006, respectively). Only sodium content in the skin was significantly correlated with the fat/water volume (Spearman's-rho=0.54, p=0.01).

Case examples of sodium content maps and corresponding anatomical $Dixon_{FAT}$ images are presented in Figure 4 from patients with Stage 1 and Stage 4 lipedema alongside control females with similar age, race, BMI, calf circumference, and normalized SAT area.

Discussion

Due to a lack of clinical awareness of the symptomology of lipedema, and the difficulty in differentiating the source of leg swelling, patients with lipedema currently suffer from a delay in diagnosis and mismanagement for years or even decades[11]. One major unmet clinical need in lipedema management rests with the lack of a diagnostic test capable of objectively reporting internal differences in tissue composition and function in women with lipedema versus women who are obese. Such a diagnostic test would be important for understanding lipedema etiology, but also establishing precedent for lipedema as a distinct condition from obesity. In this study, we applied clinically accessible sodium MRI methodologies followed by adipose tissue composition analysis to test the hypothesis that adipose accumulation would be uniquely accompanied by sodium accumulation in women with lipedema compared to female controls. The primary findings were that tissue sodium content was significantly elevated in the skin and SAT in patients with lipedema compared to female controls matched for age, race, BMI, and calf circumference as well as in a subset of patients with early stage lipedema compared to a subset of matched controls. We additionally measured significantly elevated adipose content of the legs in women with lipedema, which correlated significantly with skin sodium content among all participants. These findings provide evidence in support of a distinct etiology of lipedema that can be measured using noninvasive MRI methods and further offer potential to diagnose lipedema at an early stage.

Skin sodium accumulation is an emerging hallmark of inflammatory diseases and cardiovascular risk factors. Tissue sodium is elevated in hypertension[12], arthritis[13], infection[14], diabetes[15], and has recently been correlated with left ventricular hypertrophy in chronic kidney disease[16]. We have shown here that skin sodium is elevated in women with lipedema who are normotensive and without diabetes or heart disease. While women with lipedema do not have an increased prevalence of diabetes compared to the population of

women in the United States[17, 18], further research is needed to investigate the impact of other vascular or metabolic disorders on the sodium levels in patients with lipedema who are already at higher risk for storing sodium in their skin and SAT. A differential diagnosis may be achieved with a combination of clinical tests or a multi-modal imaging protocol such as the methods we employed for this study. Furthermore, the emerging role of elevated peripheral sodium in many prominent diseases of the 21st century underscores the need to develop robust tools for quantifying tissue sodium content as well as treatment strategies capable of modifying sodium in specific tissues.

Elevated tissue sodium content was also measured in the SAT, including in the subset of participants with early stage lipedema. Sodium was not observed in the SAT of women with similar BMI and calf circumference. In this preliminary study we were not able to address mechanistic hypotheses, however we are intrigued by the fact that lipedema features macrophage infiltration in the SAT[17]. Inflammatory-induced macrophages are also present in abdominal adipose tissue deposits in persons with metabolic syndrome[19] that is similarly characterized by adipose volume growth at the inflamed site. In an earlier study, we showed that inflammation is associated with increased skin sodium storage[14]; here we present evidence that adipose deposition is significantly correlated with local sodium accumulation in a clinical population. Further studies should investigate the variation in sodium levels in patients at their greatest and fewest adipose deposits throughout the body, and whether local adipose deposition is regionally dependent on sodium accumulation. In particular, the function of salt-sensing macrophages in the regulation of tissue volume and metabolism[20] is likely significant to lipedema pathophysiology. MRI-based tissue sodium and adipose volume measures have potential to serve as noninvasive clinical biomarkers of inflammatory pathways in adipose tissue.

Our findings also revealed a trend for higher sodium throughout the muscle of participants with lipedema. Additionally we measured significantly higher fat/water volume in the calf of lipedema patients, indicating both elevated intramuscular adipose tissue and reduced muscle size. While elevated sodium has also been observed throughout the muscle in the case of anaerobic exercise[21], local muscle injuries[22], or essential hypertension with increasing age[12, 23], lipedema is the first condition in which we have observed both increased muscle sodium and adipose content. This suggests that myopathy may be a clinical feature of lipedema that is consistent with the frequently reported symptom of chronic fatigue. Our findings are in line with a recent report of significantly reduced muscle strength bilaterally in the legs of women with lipedema compared to women who are obese[24]. Additional experimental methods to measure energetic potential would provide insight into the involvement of the muscle in the pathophysiology of lipedema.

Mechanisms related to lymphatic dysfunction may underlie tissue sodium and adipose accumulation in patients with lipedema. Accumulation of sodium in the interstitium is consistent with impaired lymphatic capillary clearance function[7, 25]. Reduced lymphatic processing capacity is also consistent with clinical symptoms of lymphedema that occur in advanced stages of lipedema. The relationship between lymphatic function and tissue sodium storage remains to be evaluated in vivo, although these studies may be possible with the emergence of lymphatic imaging modalities including indocyanine green near-infrared

imaging[26, 27] and magnetic resonance lymphangiography[28–30]. Noninvasive lymphangiography approaches[28, 29] which exploit contrast derived from the long magnetic resonance relaxation times of lymphatic fluid relative to other tissues, may have particular relevance in this field as they can be performed in ten minutes or less and without exogenous contrast agents, thereby making them ideal candidates for surveillance imaging or evaluating therapy responses. Therefore, one logical extension of the current study is to evaluate the relationship between tissue sodium content and lymphatic function in the lower extremities of patients affected by secondary lymphedema.

Limitations

One practical limitation is the restriction of waist circumference and limb circumference by the MRI gantry bore and coil size, respectively. Participants in this study were limited to a BMI<40 kg/m^2 and a calf circumference<60 cm. This limitation likely limits patients with more advanced stages of lipedema given size constraints of a 3.0T MRI. However, patients with less severe stages of lipedema also demonstrated elevated sodium in the skin and SAT as exemplified in the case examples and subgroup analyses. Tissue sodium content may therefore be useful to distinguish lipedema from obesity prior to clinically observable features, such as SAT volume or leg girth. The order of pathological events cannot be determined from this initial cross-sectional study. Note that participants with lipedema and the control group were not identically matched for the number of participants in each group, and rather group size was determined after matching for five demographic parameters: biological sex, age, race, BMI, and calf circumference.

While our lipedema cohort was representative of a lipedema cohort in the general population, this study included patients with lipedema who have undergone a variety of prior-treatments including complete decongestive therapy (CDT), liposuction, and gastric bypass surgery. All surgical procedures had occurred more than one year prior to enrollment in this study. In a meta-analysis, we evaluated whether the trends for tissue sodium content were different in these subjects, however we found no trend for a difference in tissue sodium content in these participants compared to the other participants. The effect of CDT therapy or surgical intervention on tissue sodium or adipose content was beyond the scope of this study, and future imaging studies are needed to investigate the impact of conservative or surgical interventions on tissue composition in patients with lipedema definitively.

The acquisition of sodium MRI requires specialized multinuclear scanner capabilities and specialized coils tuned to the sodium resonance. While not as common as proton water hardware, this hardware is commercially available and actively used at many medical centers in the United States. The imaging approach we have employed is both standardized and noninvasive, and has the potential to become more widely utilized due to a growing need for clinical methods that can evaluate sodium at the tissue level[31].

Conclusion

In this study we quantified tissue sodium and adipose content using a multi-modal clinical MRI protocol in women with lipedema. We measured significantly elevated sodium in the skin and SAT, and significantly higher relative fat volume in patients with lipedema.

Accumulation of tissue sodium and adipose indicates reduced vascular clearance or increased deposition, and inflammation in the pathophysiology of lipedema. Furthermore, tissue sodium accumulation may provide a functional link between the disrupted microenvironment of the tissue and pro-inflammatory immune cell polarization in patients with lipedema. These findings underscore the potential relevance of tissue sodium as a molecular biomarker of tissues affected by lipedema. Along with MRI-based adipose volume quantitation in the leg, sodium MRI has the potential to provide an important noninvasive differential diagnosis of lipedema from obesity.

Acknowledgments

Grant funding:

NIH/NINR: R01 NR015079; NIH/NHLBI: R01 HL118579 Lipedema Foundation, Lymphatic Education & Research Network, and Fat Disorders Research Society Postdoctoral Research Fellowship American Heart Association 14SFRN20770008

We are grateful to Christopher Thompson, Leslie McIntosh, Clair Jones, Kristen George-Durrett, and Charles Nockowski for experimental supporting. Funding was provided by the 2015 Postdoctoral Research Fellowship award in partnership with the Lipedema Foundation (LF), Lymphatic Education & Research Network (LE&RN) and the Fat Disorders Research Society (FDRS). Additional funding was provided by NIH/NINR: R01 NR015079, NIH/NHLBI: R01 HL118579, and AHA 14SFRN20770008.

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What is already known about this subject?

- Objective diagnostic tests for distinguishing lipedema from obesity are currently not available.
- Lymphatic dysfunction is implicated in lipedema pathophysiology, distinct from obesity.
- Impaired lymphatic vessel function may reduce clearance of fat-soluble molecules and sodium from dependent tissues.

What does this study add?

- Tissue sodium content is significantly elevated in the skin and subcutaneous adipose tissue in the calf of patients with lipedema, compared to healthy females matched for age, body mass index, race, and calf circumference.
- Skin sodium content in the calf correlates with local adipose volume, and underscores the relevance of tissue sodium as a molecular biomarker of lipedema.
- Along with MRI-based adipose volume quantitation in the leg, sodium MRI has potential to provide a critical differential diagnosis of lipedema from obesity.

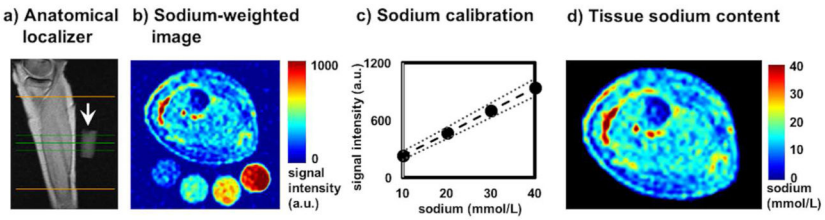

Figure 1.

Sodium MRI and analysis of tissue sodium content. **a)** Sagittal anatomical image for localization of the calf showing standard sodium solutions below the calf (white arrow). The outer green lines signify the sodium slice thickness (3 cm) and the central green line corresponds to the center of the sodium slice. **b)** Transverse sodium image (magnitude of sodium signal intensity, arbitrary units, a.u.) with standards below (concentrations from left to right are 10, 20, 30, 40 mmol/L). **c)** Sodium image signal intensity (a.u.) in the standard solutions has a linear relationship with known sodium concentrations (mmol/L). The graph represents the mean slope from all subjects' data (23.7, standard deviation=4.7) and the dotted lines represent the 95% confidence intervals of the slope (upper 25.4, lower 22.0) based on the standard error of the mean (0.88). **d)** Each subject's sodium image is calibrated individually, and applied voxel-wise to produce a quantitative map of tissue sodium content (mmol/L).

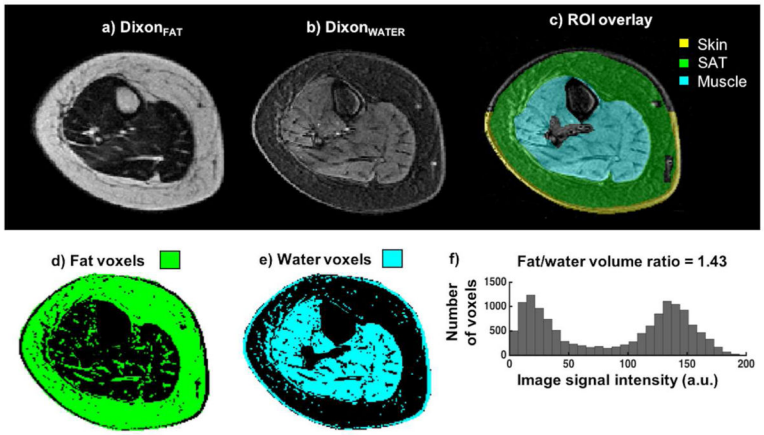

Figure 2.

Anatomical images used for segmentation of regions of interest and analysis of fat tissue content. **a–b)** Example fat-weighted (Dixon$_{FAT}$) and water-weighted (Dixon$_{WATER}$) images. **c)** ROIs were segmented manually from the Dixon$_{WATER}$ image, including the skin and total muscle; the subcutaneous adipose tissue (SAT) region was defined as the area between the inner border of the skin and the outer border of the muscle. The bone and large blood vessels were removed from all ROIs. **d–e)** Colored masks represent the voxels containing fat or water defined above or below a threshold of the Dixon$_{FAT}$ image. **f)** A histogram of the Dixon$_{FAT}$ image shows two distinct compartments; a k-means clustering algorithm was used to set a threshold between higher and lower signal intensity compartments corresponding to fat and water tissue, respectively. The fat/water volume ratio was calculated as a ratio of the number of voxels within each tissue.

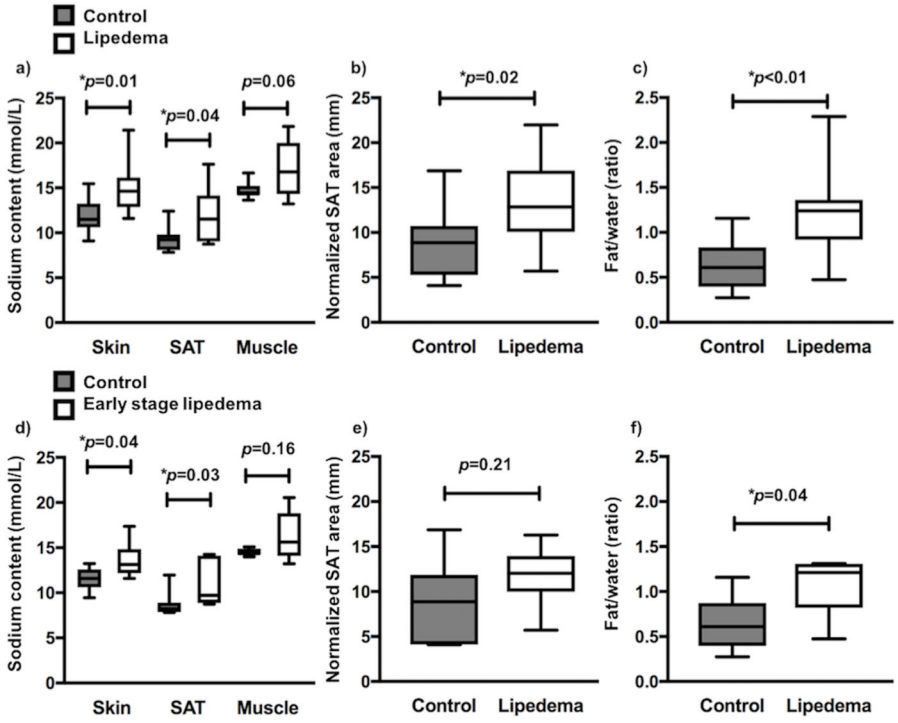

Figure 3.

Group results of **a)** tissue sodium content (mmol/L) in the skin, subcutaneous adipose tissue (SAT), and muscle regions; **b)** SAT area (mm^2) normalized by calf circumference (mm) has units of mm; and **c)** tissue composition in terms of fat/water volume ratio. **d–f)** These trends and significant differences in the skin and SAT are observed in a subset of patients with early stages of lipedema (Stages 1 or 2). Significant group differences (*$p \leq 0.05$, two-sided) were determined by a Mann-Whitney test.

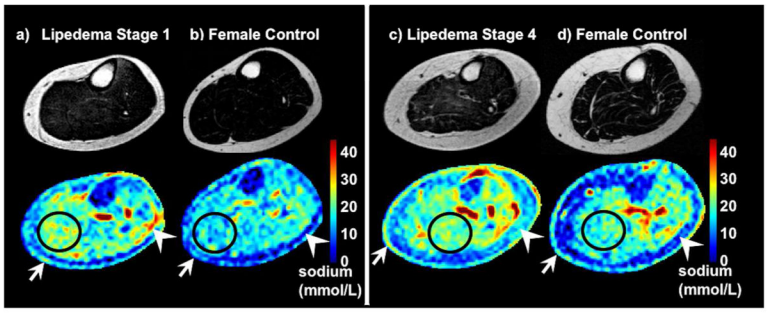

Figure 4.

The $Dixon_{FAT}$ image (top row) and corresponding map of tissue sodium content (bottom row) in patients with lipedema adjacent to matched female controls. **a)** A patient with Stage 1 lipedema is 37 years old and has a BMI of 21.3 kg/m^2. She is similar in age, race, and BMI to the female control **(b)** who is 33 years old with a BMI of 23.8 kg/m^2. Structural measures of calf circumference and subcutaneous adipose tissue (SAT) area are also similar (lipedema vs. control values, calf circumference 36.8 vs. 37.2 cm, normalized SAT area 5.7 vs. 5.4 mm). However, the tissue sodium content is higher in the skin (14.4 vs. 9.1 mmol/L, arrow), SAT (14.1 vs. 9.2 mmol/L, arrowhead) and muscle (17.9 vs. 13.6 mmol/L, circle). The fat/water volume ratio in the calf is also greater (0.47 vs. 0.35 ratio). **c–d)** A patient with Stage 4 lipedema is 55 years old and has a BMI of 37.2 kg/m^2. She is similar in age, race, and BMI to the female control who is 49 years old with a BMI of 36.4 kg/m^2. Structural measures of calf circumference and SAT area are also similar (lipedema vs. control values, calf circumference 41.0 vs. 41.6 cm, normalized SAT area 11.2 vs. 11.8 mm). The tissue sodium content is higher in the patient with lipedema in all regions, including skin (15.7 vs. 12.6 mmol/L, arrow), SAT (13.3 vs. 8.2 mmol/L, arrowhead), and muscle (19.8 vs. 14.3 mmol/L, circle). The fat/water volume ratio in the calf is also higher (0.95 vs. 0.87 ratio) in the patient with lipedema compared to the matched female control.

Table 1

Enrolled participants with lipedema were required to meet all of the primary criteria and at least one secondary criterion.‡

Primary criteria:
■ Bilateral swelling of the legs
■ Negative Stemmer's sign
■ Positive for a stage and type of lipedema‡
Secondary criteria:
■ Pain in lower limbs measured using the visual analogue scale (VAS)
■ Family history of lipedema
■ Non-pitting edema in the legs
■ Easy bruising of the legs
■ Hypermobility of the joints

‡Inclusion criteria, stages, and types were adopted from Herbst *et al.* 2012[1]

Table 2

Clinically relevant features of participants in the lipedema cohort.

Study ID	Age (years)	Age at onset (years)	Stage (1–4)	Type (1–5)‡	VAS* for Pain (0–100)	Clinical features
1	48	38	2	3	40	Hypermobility; easy bruising
2	38	12	1	3	30	Stage and type reflects post-gastric bypass surgery; met all secondary criteria
3	55	37	4	3, 4	90	Hypermobility; severe pain
4	61	12	4	3, 4	65	Fibrosis present with pitting edema; met all secondary criteria
5	41	13	2	3, 4	0	Hypermobility; easy bruising; treatment with liposuction reduced pain
6	55	25	4	2, 4	52	Treatment with gastric bypass surgery did not reduce leg size; actively treats symptoms using CDT**
7	48	17	2	3, 4	21	Family history; few lipomas present in upper extremities
8	37	34	1	3	4	Easy bruising; family history; adipose deposits which reduced after CDT**
9	17	14	1	3	80	Relative of patient 10; hypermobility; easy bruising
10	40	20	1	3, 4	42	Family history; adipose deposits below knees
Mean ± std	44.0 ± 12.5	22.2 ± 10.6	2 ± 1	3 ± 0.5	47 ± 26	

*Visual analogue scale (VAS)

**Complete decongestive therapy (CDT)

‡Lipedema Type 4 indicates lipedema adipose in the arms in addition to the primary type describing leg morphology. Mean ± standard deviation (std) of the lipedema type reflects the leg morphology so the first type indicated was used if Type 4 was also present.

Table 3

Volunteer demographics

	Controls n=11	**Patients n=10**	***p*-value***
Age (years)	45.4 ± 12.1 (33, 65)	44.0 ± 12.5 (17, 61)	0.99
Race£	9 (C), 2 (B)	9 (C), 1 (B)	0.99
BMI (kg/m^2)	30.6 ± 4.5 (23.8, 38.6)	33.0 ± 7.6 (21.3, 48.9)	0.56
Calf circumference (cm)	41.2 ± 3.4 (37.2, 46.9)	44.1 ± 5.5 (36.8, 56.5)	0.32

Values represent mean ± standard deviation (minimum, maximum).

All control and patient participants are female.

£C: Caucasian, B: Black

*Mann-Whitney test with two-sided $p \leq 0.05$ required for significance.