MCBU SBED
MANİSA CELAL BAYAR ÜNİVERSİTESİ SAĞLIK BİLİMLERİ ENSTİTÜSÜ DERGİSİ
MANISA CELAL BAYAR UNIVERSITY JOURNAL OF INSTITUTE OF HEALTH SCIENCE
ISSN: 2147-9607

ARAŞTIRMA MAKALESİ
RESEARCH ARTICLE
CBU-SBED, 2022, 9(2): 296-304

Lipödemde Subkütan Yağ Dokusu Kalınlığı ile Adipokinler Arasındaki İlişki

The Relationship Between Adipokines and the Thickness of Subcutaneous Adipose Tıssue in Lipedema

Zeliha Ünlü[1] Tuğba Çavuşoğlu[2] Funda Kosova[3*] Ali Aydın

[1]Manisa Celal Bayar Üniversitesi Fiziksel Tıp ve Rehabilitasyon AD Manisa, Türkiye.
[2]Manisa Celal Bayar Üniversitesi Farmakoloji AD Manisa, Türkiye.
3*Manisa Celal Bayar Üniversitesi Biyokimya AD Manisa, Türkiye.
[4]Manisa Celal Bayar Üniversitesi Fiziksel Tıp ve Rehabilitasyon AD Manisa, Türkiye.

e-mail: zelihaunlu@yahoo.com, tugbagurpinar@gmail.com, fundakosova@gmail.com, heya.dr.ali@gmail.com
ORCID: 0000-0003-2270-0414
ORCID: 0000-0002-9479-9655
ORCİD: 0000-0001-8070-5067
ORCID:0000-0001-6964-4363
*Sorumlu Yazar / Corresponding Author: Funda Kosova

Gönderim Tarihi / Received:24.01.2022
Kabul Tarihi / Accepted: 02.03.2022
DOI: 10.34087/cbusbed.1062223

Öz

Giriş ve Amaç: Adipokinler ve disfonksiyonel yağ dokusu arasındaki ilişkide subkutan yağ dokusundan ziyade viseral yağ dokusundaki değişikliklerin rolüne dikkat çekilmiştir. Özellikle cilt altı yağ dokusunun etkilendiği lipödemde adipokinler hakkında bilgi yetersizdir. Bu çalışmada lipödemli hastalarda adiponektin, ghrelin, resistin ve visfatin düzeylerinin ve bunların yağ doku kalınlığı ile ilişkisinin araştırılması amaçlandı. Bu amaçla cilt altı yağ dokusu kalınlığı ultrasonografi ile objektif olarak değerlendirildi.

Gereç ve Yöntemler: Lipödem tanısı almış toplam 19 kadın hasta ve yaş farkı olmayan 15 sağlıklı kadın çalışmaya dahil edildi. Deri ve deri altı yağ dokusu kalınlıkları ultrasonografik olarak ölçüldü. Tüm deneklerin serum adiponektin, ghrein, resistin ve visfatin seviyeleri sandviç ELISA protokolü kullanılarak ölçüldü.

Bulgular: Lipödemli hastalarda, uyluk ve baldırda subkutan subkutan doku kalınlığı ve toplam deri-subkutan kalınlığı kontrollere kıyasla, uyluktaki deri kalınlığı dışında önemli ölçüde arttı ($P<0.000$). Lipödemli hastalar ve kontroller arasında adiponektin, ghrelin, resistin ve visfatin serum seviyelerinde anlamlı fark yoktu ($P>0.05$). Lipödemli hastalarda ve kontrollerde ultrason ile adiponektin, ghrelin, resistin ve visfatin ile deri, deri altı ve toplam kalınlık ölçümleri arasında anlamlı bir ilişki bulunmadı ($P>0.05$). İstatistiksel olarak anlamlı olmasa da detaylı incelendiğinde adipokinler ve ultrason ölçümleri arasındaki ilişkide gruplar arasında pozitif veya negatif korelasyonlar gözlendi.

Sonuç: Bulgularımıza göre, serum adipokin düzeyleri ile deri altı yağ dokusu kalınlığı arasında anlamlı bir ilişki bulunmamakla birlikte, tamamen ilgisiz oldukları tartışmalıdır. Daha geniş serilerde yapılacak çalışmalar adipokinlerin cilt altı doku kalınlığı ile ilişkisine ve ultrasonografinin önemine ışık tutacaktır.

Anahtar Kelimeler: Adipositler, Ghrelin, Lipödem.

Abstract

Objective: Attention has been drawn to the role of visceral adipose tissue changes rather than subcutaneous adipose tissue the relationship between adipokines and dysfunctional adipose tissue. Especially in lipedema in which subcutaneous adipose tissue is affected, the information on adipokines is insufficient. In this study, it aimed to investigate the levels of adiponectin, ghrelin, resistin and visfatin and their relationship with adipose tissue thickness in patients with lipedema. For this purpose, the thickness of the subcutaneous adipose tissue was objectively evaluated by ultrasound.

Materials and Methods: A total of 19 female patients diagnosed with lipedema and 15 healthy women with no age difference were included in the study. The thickness of the skin and subcutaneous adipose tissue was measured by ultrasound. The serum levels of adiponectin, ghrein, resistin and visfatin of all subjects were measured using the sandwich ELISA protocol.
Results: In patients with lipedema, the thickness subcutaneous tissue and thickness of total skin-subcutaneous were significantly increased at skin the thigh and calf, excluding skin thickness in the thigh, compared to controls ($P<0.000$). There was no significant difference in serum levels of adiponectin, ghrelin, resistin and visfatin between patients with lipedema and controls ($P>0.05$). No significant correlation was found between adiponectin, ghrelin, resistin and visfatin and subcutaneous and total thickness measurements by ultrasound in patients with lipedema and controls ($P>0.05$). Although not statistically significant, when examined as positive or negative correlations were observed between the groups in the relationship between adipokines and ultrasound.
Conclusion: According to our findings, although no significant relationship was found between serum levels of adipokines and subcutaneous adipose tissue thickness, it is controversial that they are completely unrelated. Further studies in larger series will shed light on the relationship between adipokines and subcutaneous tissue thickness and the importance of ultrasonography.

Keywords: Adipocites, Ghrelin, Lipedema.

1.Introduction

White adipose tissue, in addition to being a fat reservoir, is the largest endocrine organ with autocrine, paracrine and endocrine functions. White adipose tissue can be grouped into subcutaneous fat and visceral adipose tissue. Excessive fat accumulation in adipose tissue alters the repertoire of molecules known as adipose tissue-specific adipokines. Adipokines have been found to play an important role in the pathophysiological link between dysfunctional adipose tissue and cardiometabolic changes [1– 4]. Adipokines can cause appetite and satiety disturbances, adipocyte tissue distribution, changes in insulin secretion, insulin sensitivity, energy expenditure, endothelial function, angiogenesis, inflammation, blood pressure, and hemostasis.

Epidemiological studies have shown that accumulation of intra-abdominal (visceral) fat surrounding the gastrointestinal organs carries a greater cardiovascular risk than accumulation of subcutaneous fat in the gluteal region [5, 6]. The enlargement of subcutaneous adipose tissue does not seem to lead to adverse systemic consequences such as insulin resistance and cardiovascular risk, as in visceral adipose tissue. Despite central-visceral or ectopic adiposity, gynoid type-peripheral-subcutaneous adiposity in the hips and thighs protects against metabolic dysfunction (insulin resistance, type 2 diabetes, dyslipidemia, etc.) [7].

Significantly, in cases with extensive and distorted fat deposition in the subcutaneous tissue (multiple symmetric lipomatosis, lipedema, and Dercum's disease), the metabolic profile is often healthy. Similarly; diabetes, lipid profile disorders, cardiovascular disease-hypertension, and aortic stiffness are also less common in lipedema, though further research is needed. The sodium concentration of subcutaneous tissue in female lipedema patients was shown to be higher in the sodium MR (magnetic resonance) investigation. The link between higher sodium content and the risk of cardiovascular illness was highlighted, and it was stressed that more research into lipedema is needed [8].

It was aimed to investigate the relationship between adipokines and subcutaneous adipose tissue thickness in lipedema, a special clinical picture in which subcutaneous adipose tissue enlarges. The thickness of subcutaneous adipose tissue was measured objectively using ultrasonography for this purpose.

2. Materials And Methods

Female patients aged 18 years and older who applied to Manisa Celal Bayar Univesity Medical Faculty Hospital Physical Medicine and Rehabilitation Department outpatient clinic and were diagnosed with lipedema were included in the study. The control group consisted of healthy people who did not differ from the patient group in terms of age. The study was approved by Manisa Celal Bayar University Health Sciences Ethics Committee with the decision numbered 20.478.486/549. Support for the Scientific Research Project numbered 2020-106 from the Presidency of Manisa Celal Bayar University was received for the study. A detailed informed consent form was obtained from all subjects who agreed to participate in the study.

For lipedema diagnosis, criterias defined by Wold et al. [9] and modified criterias (including the formers) defined by Halk and Damstra in 2017 [10] and listed by Buso et al. [11] were taken into consideration. In addition, the criteria used in the diagnosis of lipedema by Naouri [12] were also evaluated in the diagnosis.

Criterias defined by Wold et al. [9], still presently used for lipedema diagnosis: almost exclusively in women, it consists of bilateral symmetrical adipose tissue accumulation with minimal involvement in the foot, minimal pitting edema, pain-tenderness and rapid bruising, weight loss or swelling in the lower extremities that does not go away despite elevation.

The diagnostic criteria for lipedema were defined by Halk and Damstra [10] in 2017 and were minimally modified as a list by Buso et al. [11].

Patient history - Wold criteria

A; disproportionate fat distribution, fat distribution not affected by weight loss, extremity pain and bruising, tenderness to touch or fatigue in the extremity, nonpitting edema, pain or discomfort not affected by extremity lifting

Physical examination
B (lower extremity proximal); disproportionate distribution of fat, circumferential thickening of cutaneous fat
C (lower extremity distal); thickening of the proximal subcutaneous adipose tissue, cuff sign - thinning of the upper part of the foot with thickening of the distal subcutaneous adipose tissue
D (arm proximal); significant thickening of the subcutaneous adipose tissue when compared to the surrounding area, abrupt incision at the elbow
E (arm distal); thinning on the back of the hand - thickening of the subcutaneous adipose tissue with cuff sign
Additional criteria
F; pain on palpation with both hands, distal fat tissue branches in the knee (popliteus)
Diagnosis; most likely lipedema (A+B) or C or D or E
If no more than 2 criteria A and E are present, one of the two criteria in F supports the diagnosis. [11].
Six different criterias were defined by Naouri et al. [12] for lipedema; family history of lipedema, obesity, absence of injury to the lower leg, absence of Stemmer's sign, symmetrical involvement of both lower legs, and spondent or provoked pain in the legs. Swollen legs and presence of at least 4 criteria were considered lipedema.
Ultrasonographic criteria for determining the severity of lipedema were defined by Marschall and Schwahn-Schreiber [13]. Even if there is proximal lipedema, 6-8 cm above the medial malleolus is indicated as a reliable reference point for skin and subcutaneous tissue thickness measurement. 12-15 mm: mild lipedema/lipohyperplasia; 15-20 mm: moderate; >20 mm: evident; >30 mm were classified as severe lipedema. In the ultrasonographic diagnosis of lipedema, cases with a subcutaneous tissue thickness of <1.2 6-8 cm above the medial malleolus were excluded and their severity was determined according to the Marschall and Schwahn-Schreiber criteria [13].
For exclution criteria for lipedema; lack of disproportion between upper and lower body halves, asymmetry in both arms/legs, occurrence of fat accumulation in advanced adulthood independent of hormonal change stages (pregnancy or menopause), waist-hip ratio >0.85/>1.0 in females (obesity marker), waist-to-height ratio >0.5 in those younger than 40 years; between 40-50: 0.5-0.6; >0.6 in people older than 50 years (obesity marker), in obese cases, if there is no sign of step on the ankle, there is no pain with compression in the tissue, there is no predisposition to hematoma, and subcutaneous tissue thickness <1.2 mm parameters 6-8 cm above the medial malleolus in ultrasonography were evaluated [14]. Age, presence of lipedema in the mother and first-degree relatives were questioned. From the history to hormonal-estrogen; phases of hormonal change followed by lipedema; menarche, pregnancy and menopause) and the ages of the patients at this stage were questioned. Body mass index- BMI (height/m2), waist/hip and waist/height ratio were calculated from anthropometric measurements. The presence of lipolymphedema accompanying lipedema and the severity of pain were determined with a 10 cm visual analog scale (0 no pain-10 maximum pain).
Ultrasonographically, skin and subcutaneous measurements were evaluated using Toshiba Aplio 500 ultrasound device and 14 MHz linear high frequency probe.
In the ultrasonographic examination, the skin and subcutaneous tissue thickness (mm) was measured from a total of 8 places on the thigh and calf (internal-outer and upper-lower) (Picture 1). Measurement of areas scanning were as follows;
Upper medial thigh: middle of the upper half of the thigh, immediately anterior to the great saphenous vein
Upper lateral thigh: middle of the upper half of the thigh, lateral aspect of the quadriceps femoris muscle
Lower medial thigh: middle of the lower half of the thigh, immediately anterior to the great saphenous vein
Lower lateral thigh: middle of the lower half of the thigh, lateral aspect of the quadriceps femoris muscle
Upper medial leg: middle of the upper half of the leg, immediately anterior to the great saphenous vein
Upper lateral leg: middle of the upper half of the leg, lateral aspect of the tibialis anterior muscle
Lower medial leg: middle of the lower half of the leg, immediately posterior to the great saphenous vein
Lower lateral leg: middle of the lower half of the leg, lateral aspect of the tibialis anterior muscle [15,16,17], (Picture 1).

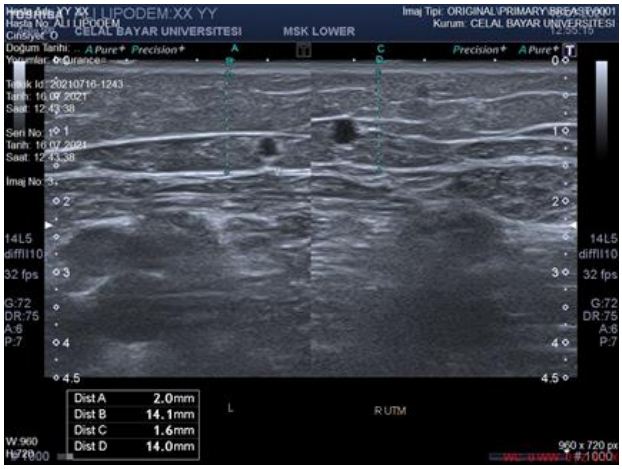

Figure 1: Skin (A, C) and subcutaneous subcutaneous tissue (B, D) thicknesses are observed in the upper thigh medial ultrasound image taken from the middle of the upper half of the thigh and in front of the great saphenous vein (V) on the right and left sides.
Right and left lower extremities were evaluated separately. The mean skin, subcutaneous subcutaneous tissue thickness and total thickness were calculated for the right and left thighs and calves. Calf/thigh ratios (%) were determined. In addition, the mean values of the right and left thighs and calves, and the calf/thigh ratio (%) were calculated.

Circumference measurements were made with a millimeter measuring tape at 3 points above the knee and 3 points below the knee with 10 cm intervals on the right and left lower extremities [17, 18]. Mean values and calf/thigh ratios (%) on the right and left were calculated.

Right and left mean values and mean calf/thigh ratio (%) were also calculated.

People who had the standards and wanted to participate in the study were included in the study by signing a consent form. For our study, 1 cc of blood was taken from the blood taken during routine practice. The collected blood was separated into serum and the materials were stored in the deep milling machine at -80 degrees until the collection of all blood was finished. Then, adiponectin (ng/mL), ghrelin (ng/mL), resistin (pg/mL) and visfatin (ng/mL) levels were measured using sandwich ELISA protocol.

Statistical analysis of data; data distribution in the lipedema and control groups was done by Mann Whitney U and Wilcoxon tests for non-parametric ones using Microsoft Excel and SPSS 16. Correlation was performed to analyze the relationship of continuous variables. Bivaried Spearman test was used. In terms of age, the sample was tested with the Kolmogorov Smirnov test. P: 0.2 was found in Kolmogorov Smirnov test (P>0.05) and it was determined that it showed normal distribution in terms of age. Therefore, the T test was used in the analysis. The number of pregnancies was analyzed in 2 categories as 2 or less and 3 or more pregnancies. The severity of lipedema was also categorized into two groups as mild-moderate and common-severe. Chi-square test was performed in the analysis of pregnancy and lipedema severity. Values with P<0.05 were considered statistically significant.

3. Results and Discussion

3.1. Results

19 cases diagnosed with lipedema by clinical examination (age: 51.9 ±13.2 years (minimum 19- maximum- 65)) and 15 healthy controls with no significant difference in age (age: 44.9±6.3 years (minimum 36- maximum- 64)) were included (P: 0.06).

Lipedema was accompanied by lymphedema-lipolymphedema in 5 (26.3%) of 19 patients with a diagnosis of lipedema. Nearly half of the cases defined a family history of lipedema (9 cases - 47.3%). As the initial hormonal stage of lipedema, 12 cases (63.1%) after pregnancy, 4 (21%) menopause, and 3 (15.7%) puberty were defined. The mean age of onset of lipedema was 26.7±9.7 (minimum 17- maximum 48 years). Patients with lipedema defined pain with a mean intensity of 7.0±1.5 cm (minimum 5- maximum 9 cm) according to the visual analog scale.

According to body mass index, the mean in lipedema was 34.4±7.7 height/m2 (minimum 18.7- 52.7 maximum); mean height was 25.5±2.7 height/m2 (minimum 21.0- maximum 32.0) in controls. When grouped as 18.5-24.9 normal, 25-30 overweight, 30-35 type 1 obese and 35-40 type 2 obese according to BMI; patients with lipedema close to the upper limit type 1 obese, controls were overweight close to the upper limit of normal and the difference between them was significant (P: 0.000). Waist/hip ratio was below the obesity cut-off value of >0.85 in both groups; although there is no significant difference in between two groups (P: 0.19), averages in cases with lipedema (0.77±0.1(minimum 0.36- maximum 0.85)) were lower than controls (0.82±0.0 (minimum 0.72- maximum 1.0). There was a significant difference between the groups in terms of waist/height ratio, and the values were higher in lipedema (P: 0.02). However, both lipedema (0.6±0.1 (minimum 0.32- maximum 0.88)) and control group (0.5±0.0 (minimum 0.47-maximum 0.79)) were not above the limit values determined for obesity according to age.

Ultrasonographically, the severity of lipedema; were of mostly widespread (9 cases- 75%) and moderate (7 cases- 36.8%) severity, mild (2 cases- 10.5%) and severe (one case-5.2%) were less common.

Table 1 shows the mean skin, subcutaneous and total thickness values, calf/thigh ratios and P values of the lipedema cases and controls ultrasonographically on the right and left sides. Subcutaneous and total thicknesses were significantly thicker in patients with lipedema compared to controls (P<0.000). However, in patients with lipedema, the mean skin thickness on the thigh did not differ significantly compared to the controls, unlike the calf (P>0.05). The significant difference detected in the skin and subcutaneous total value in the thigh was due to the significant thickening under the skin rather than the skin thickness. There were significant differences in the mean values of both sides between the groups in terms of skin thickness on the calf (P: 0.006). Thickening of the skin on the calf showed a significant difference with the controls. There was no significant difference in total skin and subcutaneous thickness calf/thigh ratios on the right and left sides and the mean of both sides (P>0.05). However, the rates were found to be higher in cases with lipedema.

Significant differences were found between lipedema and controls in the leg measurement parameters with a millimeter measuring tape at thigh level compared to calf level (P<0.01). The mean thigh circumference was 54.3±6.3 cm (minimum 38.8-62.8 maximum) in lipedema, 48.7±2.9 cm (minimum 45.1- 55.0 maximum) in controls; P was 0.001. The mean calf circumference was 31.8±4.4 cm (minimum 22.4- 38.3 maximum) in lipedema, 29.5±1.3 cm (minimum 27.1- 32 maximum) in the control; P was 0.02. Right and left side mean calf/thigh ratios were 58.5±5.7 cm (minimum 47.7- 70.2 maximum) in lipedema, 60.7±2.5 cm (minimum 55.8- 65.3 maximum) in control; P value was 0.10 and there was no significant difference between the groups.

In patients with lipedema, no significant difference was found between the right and left side skin and subcutaneous total thickness and measure measurements. The involvement was similar on all sides (P>0.05). Right-left thigh total skin and subcutaneous thickness difference P:0.13; right-left calf total skin and subcutaneous thickness difference P:0.77; right-left measure measurement of thigh difference P: 0.29, right-left measure measurement of calf difference P: 0.44. The cases had similar bilateral thickening, consistent with the clinical involvement of lipedema.

When the mean skin and subcutaneous total thickness of the right and left sides, thigh and calf values

were compared with ultrasound in lipedema, no significant correlation was found between them (r: 0.43/P: 0.06). On the other hand, a significant correlation was found between thigh and calf circumferences in measuring with a measuring tape (r: 0.56/P: 0.01).

Table 1. Right and left side mean skin, subcutaneous and total thicknesses and calf/thigh ratios of the lipedema and control group

	Right + left mean lipedema	Right + left mean control	P value
Thigh skin (mm) Mean±SD (Min-Max.)	1.8±0.4 (1.1-3.2)	1.6±0.1 (1.4-1.9)	0.08
Thigh subcutaneous(mm) Mean±SD (Min-Max.)	26.3±9.3 (9.5-50.2)	15.6±1.7 (13.0-19.4)	0.001*
Thigh total (mm) Mean±SD (Min-Max.)	28.5±9.5 (11.2-52.6)	17.2±1.8 (14.5-21.2)	0.000**
Calf skin (mm) Mean±SD (Min-Max.)	1.6±0.3 (0.8-2.7)	1.4±0.1 (1.2-1.6)	0.006*
Calf subcutaneous (mm) Mean±SD (Min-Max.)	17.7±5.4 (5.3-29.4)	9.8±1.5 (7.3-12.3	0.000**
Calf total (mm) Mean±SD (Min-Max.)	19.1±5.3 (7.2-31.0)	11.2±1.6 (8.5-13.7)	0.000**
Total calf/thigh (%) Mean±SD (Min-Max.)	70.7±18.7 (32.8-110.2)	64.8±6.0 (54.8-76.9)	0.23

Table 2. Mean values of adiponectin, ghrelin, resistin and visfatin in serum of lipedema and control group

	Lipedema	Control	P value
Adiponectin (ng/mL) Mean±SD (Min-Max.)	10.9±0.49 (9.7-11.5)	10.9±0.51 (9.9-11.5)	0.61
Ghrelin (ng/mL) Mean±SD (Min-Max.)	6.07±1.4 (2.7-6.8)	6.6±0.1 (6.3-6.7)	0.40
Resistin (pg/mL) Mean±SD (Min-Max.)	304.1±186.8 (-24.1-575.3)	392±369.3 (37.5-1530.7)	0.84
Visfatin (ng/mL) Mean±SD (Min-Max.)	0.16±0.04 (0.1-0.2)	0.16±0.05 (0.1-0.3)	0.78

In the control group, the mean skin and subcutaneous total thickness measured by ultrasound in the thigh and calf showed a significant relationship with each other (r: 0.81/P: 0.000). Similarly, measuring tape measure and circumference were found to be significantly correlated with each other between thigh and calf (r: 0.67/P: 0.006). Mean skin and subcutaneous ultrasound measurements of the right and left sides of the thigh and calf in patients with lipedema were compared with each other. There was no significant relationship between skin and subcutaneous thickness (r/p >0.05), (thigh; r: 0.33/P: 0.16 and calf; r: 0.03/ P: 0.88). In controls, there was no difference between skin and subcutaneous ultrasound measurements in the calf (r/p>0.05), while skin involvement in the thigh (0.49/ 0.06) was related to subcutaneous involvement (r/p <0.01), (0.71/0.003).

The severity of lipedema determined by ultrasonography; analyzed by dividing them into two groups as mild-moderate and widespread-severe. The mean skin thickness of the thighs (P<0.05), (P: 0.04) and the subcutaneous mean of the calves (P<0.05), (P: 0.01) were significantly different between the two groups. The increase in thickness was more pronounced in the extensive and severe group. Calf subcutaneous thickness measurements showed a more significant increase with the severity of lipedema.

There was no significant difference between lipedema cases and controls in terms of adiponectin, ghrelin, resistin and visfatin values. Although there was no significant difference between them, the levels of resistin among the investigated adipokines were found to be lower in lipedema (Table 2).

No significant correlation was found between adiponectin, ghrelin, resistin and visfatin and ultrasound skin, subcutaneous and total skin and subcutaneous thickness measurements in patients with lipedema. Although not statistically significant in patients with lipedema, negative correlations were found with adipokine, ghrelin and visfatin in all measurements and all regions (Table 3). On the other hand, positive correlations were found with resistin in all measurements and all regions, although it was not significant in patients with lipedema (Table 3). Although it is not significant in cases with lipedema, it can be said that adiponectin, ghrelin and visfatin decrease as the skin, subcutaneous and total thickness increases, while resistin increases.

No correlation was found between adiponectin, ghrelin, resistin ultrasound skin, subcutaneous and total skin and subcutaneous thickness measurements in controls. In the control group, adiponectin was negatively correlated with the mean calf skin thickness on both sides (Table 3). Right and left side mean values of calf skin thickness

were negatively correlated with adiponectin. Apart from this, although all measurements and regions were not significant, they showed a positive correlation in contrast to lipedema. The mean skin thickness on both sides of the thigh and calf was negatively correlated with ghrelin (Table 3). Apart from this, all regions and measurements were positively correlated. Resistin showed a negative correlation with the mean skin thickness of both calves. Apart from this, all regions and measurements were positively correlated. As a result, adiponectin, ghrelin and resistin showed a negative correlation with the mean skin thickness of the calves, similar to each other. Unlike lipedema, adiponectin and ghrelin were mostly positively correlated. As adiponectin and ghrelin increased, subcutaneous thickness and total thickness were increasing positively rather than skin thickness. On the contrary, in cases with lipedema, adipokine and ghrelin tended to decrease as skin, subcutaneous and total thickness increased. Resistin showed mostly positive associations in lipedema and controls. A negative correlation was found between visfatin and mean subcutaneous and cutaneous-subcutaneous total thicknesses of both calves (Table 3). All measurements, especially in the thigh, showed a positive correlation with visfatin. Visfatin, like adiponectin, ghrelin and resistin, also showed negative associations in the calf, but this association was with subcutaneous thickness as opposed to skin thickness. While visfatin showed a negative relationship in lipedema, it showed a negative relationship with the calf and subcutaneous and total thickness in the controls. Visfatin showed significant dissociation between lipedema and controls, although not statistically significant. In contrast to lipedema in controls, the relationship between mean skin, subcutaneous and total thickness of the thighs and visfatin was positive (Table 3). Similar dissociation was observed in adipokine, adiponectin and visfatin correlated inversely (lipedema-negative vs. control-positive) with mean thickness measurements on both sides of the thigh in lipedema and controls. Although there was no statistically significant difference, there were different negative/positive relationships between skin-subcutaneous and total measurements in lipedema and controls, especially in adipokine and visfatin, and in ghrelin (Table 3).

In lipedema, no correlation was found between the measurement of thigh and calf circumference with a measuring tape and adiponectin, ghrelin, resistin and visfatin. Although there was no significant difference between adiponectin, ghrelin and visfatin, mean circumference measurements were negative; positive relations were found with resistin (Table 3).

Table 3. The relationships between the mean values of adiponectin, ghrelin resistin and visfatin in serum of the lipedema and control groups and the mean values of ultrasound and circumference measurements (r/p)

Lipedema	Adiponectin (ng/mL)	Ghrelin (ng/mL)	Resistin (pg/mL)	Visfatin (ng/mL)
Thigh (mm)				
Skin	-0.21/0.37	-0.30/0.19	0.18/0.43	-0.02/0.91
Subcutaneous	-0.24/0.30	-0.29/0.21	0.05/0.83	-0.05/0.82
Total thickness	-0.24/0.30	-0.40/0.08	0.12/0.60	-0.009/0.97
Calf (mm)				
Skin	-0.05/0.82	-0.28/0.24	0.26/0.27	-0.14/0.54
Subcutaneous	-0.42/0.07	-0.35/0.13	0.43/0.06	-0.03/0.90
Total thickness	-0.29/0.21	-0.20/0.40	0.38/0.10	-0.13/0.58
Thigh/calf(%)	-0.06/0.78	0.30/0.21	0.09/0.70	-0.19/0.42
Thigh (cm) circumference	-0.39/0.09	-0.35/0.13	0.16/0.50	-0.09/0.69
Calf (cm) circumference	-0.36/0.12	-0.27/0.25	0.13/0.57	-0.06/0.80
Control				
Thigh (mm)				
Skin	0.01/0.95	-0.03/0.89	0.06/0.81	0.16/0.55
Subcutanenous	0.28/0.29	0.24/0.37	0.27/0.31	0.11/0.69
Total thickness	0.31/0.26	0.28/0.30	0.28/0.30	0.06/0.81
Calf (mm)				
Skin	-0.08/0.76	-0.07/0.80	-0.009/0.97	0.31/0.24
Subcutaneous	0.31/0.24	0.14/0.61	0.07/0.78	-0.10/0.71
Total thickness	0.30/0.26	0.13/0.63	0.09/0.74	-0.09/0.74
Calf/Thigh(%)	0.25/0.36	-0.12/0.65	-0.06/0.82	-0.03/0.90
Thigh (cm) circumference	-0.22/0.41	-0.04/0.86	0.02/0.91	-0.03/0.88
Calf (cm) circumference	-0.26/0.34	0.13/0.62	-0.37/0.16	-0.42/0.11

In the controls, no correlation was found between the measurement of thigh and calf circumference with a

measuring tape and adiponectin, ghrelin, resistin and visfatin. Although not statistically significant, the associations with circumference measurements in adipokine and visfatin in controls were negative as in lipedema. Although the relationships between the mean circumference of both thighs and between ghrelin and resistin were determined as in lipedema, it was found in the opposite direction of lipedema in the calf (Table 3).

There was no significant difference between the groups in terms of severity of lipedema (mild-moderate and widespread-severe) and adipokines (P: adiponectin 0.46; ghrelin 0.87; resistin 0.14 ve visfatin 0.68; >0.05). Although there was no significant difference between patients with lipedema, pain was defined more frequently in patients with widespread-severe (7.6±1.5) compared to mild-moderate (6.4±1.5).

3.2.Discussion

In our cases, subcutaneous and total skin-subcutaneous thickness values were found to be significantly increased in the thigh and calf, excluding skin thickness in the thigh, compared to the controls. In other words, skin thickness in the thigh (mean of both sides) in lipedema cases did not differ significantly compared to the controls, unlike the calf (P>0.05). There were significant differences in the mean values of both sides between the groups in terms of skin thickness on the calf (P: 0.002 and P: 0.006). The significant difference detected in the skin and subcutaneous total value in the thigh was due to the significant thickening under the skin rather than the skin thickness. The fact that the thickening of the skin and subcutaneous total value in the thigh is more pronounced in lipedema compared to the controls can be explained by the fact that the subcutaneous rather than the skin involvement is more in the thigh. In lipedema, thickening is more prominent in the subcutaneous subcutaneous tissue rather than the skin, and this difference in involvement occurs in the thigh, not the calf. The difference in tape measurements between the groups was found in the thigh, not the calf. Ultrasonography reveals the thickening that is more prominent in the thigh in lipedema, beyond the tape measurement; showed the difference in skin and subcutaneous tissue. Tape measurements do not show tissue properties compared to ultrasonography, they evaluate all tissue components of skin, subcutaneous, muscle and bone in full thickness.

No significant correlation was found between thigh and calf values in skin and subcutaneous total thicknesses with ultrasound in lipedema. On the other hand, a significant relationship was found between thigh and calf circumferences in measuring with a measuring tape. In lipedema, the total skin and subcutaneous thickness measured by ultrasound in the thigh is unrelated to the thickness in the calf, and the difference in lipedema-specific involvement was reflected in the ultrasound measurements. However, tape measurements could not show the difference in thigh and calf involvement in lipedema. The fact that ultrasound provides a more precise and detailed evaluation and its superiority over relatively coarse tape measurement can be effective in the inconsistency between them. All tissue components (skin, subcutaneous, muscle and bone) in the extremity are included in tape measurement. In contrast, ultrasound provides the opportunity to evaluate only the subcutaneous tissue that is expected to be affected in lipedema. In our findings, there was no significant difference in thigh skin thickness compared to the control group. The difference in skin and subcutaneous involvement was observed in the thigh, as opposed to the calf, when compared to the controls. In the control group, the skin and subcutaneous total thickness measured by ultrasound in the thigh and calf showed a significant relationship with each other. Similarly, a significant correlation was found between circumference of the thigh and calf measured with measuring tape. Contrary to lipedema, in controls if there is an increase in the total thickness of the skin and subcutaneous tissue in the thigh or an enlargement in the circumference, it also occurs in the calf.

No significant correlation was found between the mean skin and subcutaneous ultrasound measurements of the right and left sides of the thigh and calf in patients with lipedema. In controls, there was no difference between skin and subcutaneous ultrasound measurements on the calf, while skin involvement in the thigh was found to be related to subcutaneous involvement (r/p <0.01), (0.71/0.003). The findings showed that the skin and subcutaneous involvement were independent of each other in lipedema in contrast to the controls. In controls without lipedema, the thickening of the skin is compatible with the subcutaneous tissue.

There is insufficient data in the literature comparing the cutaneous and subcutaneous ultrasonographic features with those of healthy controls in patients with lipedema. Studies have mostly been done in cases with lymphedema including lipedema.

Skin ultrasonography is a useful imaging technique in distinguishing between lipedema and lymphedema and showing tissue features of involvement [19]. The first study in which computer-assisted ultrasonographic measurement was performed for the differential diagnosis of lymphedema and lipedema was performed in a limited number of around 10 cases in 2019 [19]. Dermal and subcutaneous tissue thickness measurement and echogenicity in lymphedema, lipedema and controls were analyzed by software ImageJ. As a result, lymphedema causes an increase in total skin thickness and dermal hypoechogenicity, especially in the distal (ankle and calf). It affects the dermis more. Lipedema, on the other hand, causes a significant increase in the subcutaneous tissue in the thigh, and hypoechoicity in the distal (ankle and calf). Lipedema mostly involves the subcutaneous tissue. Hypoechogenicity occurs especially in the distal both of them while dermal tissue in lymphedema, subcutaneous tissue in lipedema [19]. Our findings also support the results of this study.

Naouri et al. [12] examined dermal edema and the difference between lymphedema and lipedema in cases with high frequency ultrasound imaging (20 MHz). As a result, while dermal thickness and echogenicity are normal in lipedema, it increases and echogenicity

decreases in lymphedema. They concluded that the hypodermal tissue is increasing in lipedema without true dermal edema. As a matter of fact, Iker et al. [19] showed that lipedema mostly affects the subcutaneous tissue. Our findings showed that lipedema causes thickening of the subcutaneous tissue, especially in the thigh and in contrast to the skin. Skin-subcutaneous echogenicity was not examined in our study.

In our cases, there was no significant difference between right and left side ultrasound and tape measurement, supporting the symmetrical effect of lipedema on the lower extremities. In addition to clinical diagnostic parameters, especially ultrasonographic cutaneous and subcutaneous involvements strengthened the diagnosis of lipedema in our cases and eliminated the diagnostic complexity of accompanying obesity.

There was no significant difference between lipedema cases and controls in terms of adiponectin, ghrelin, resistin and visfatin values. Adipokines, except resistin, were found at similar levels with healthy controls. Our findings can be interpreted in two ways. First, there may not be a significant change in serum levels of metabolites secreted from adipose tissue or affecting adipose tissue such as ghrelin in lipedema. In this respect, by looking at serum levels, metabolic properties of lipedema and subcutaneous adipose tissue may be different and specific from other adipose tissue disorders.

The second interpretation of our findings can be made that although lipedema is a specific disorder in adipose tissue, dysfunctional adipose tissue enlargement is not reflected in serum levels of metabolites. In this context, the different perspective that ultrasonographic evaluation brings to the findings can be discussed. No significant correlation was found between adiponectin, ghrelin, resistin and visfatin and ultrasound skin, subcutaneous and total cutaneous-subcutaneous thickness measurement in patients with lipedema. Although not statistically significant in patients with lipedema, all measurements and relationships found in all regions were negative with adiponectin, ghrelin and visfatin, and positive with resistin. Although it is not significant in cases with lipedema, it can be said that adiponectin, ghrelin and visfatin decrease as the skin, subcutaneous and total thickness increases, while resistin increases. Studies investigating the relationship between ultrasonographically measured body fat and adipokines are insufficient in the literature. There is only one animal study on this subject in pony mares. In this study, subcutaneous subcutaneous fat thickness was measured monthly ultrasonographically from the shoulder, dorso-lumbar, and rump-gleteal regions in animals fed during april- october. Adipokines leptin, adiponectin, visfatin and resistin levels were also evaluated monthly. During the follow-up, the body did not increase significantly, but subcutaneous fat thickness increased significantly at all measured levels. While adipose tissue expression and plasma levels increased significantly in leptin and resistin, adiponectin decreased significantly and visfatin did not change. In the lumbar region and rump-gluteal ultrasound measurements, negative correlations with adiponectin and positive correlations with resistin and leptin were determined ($P<0.001$).

Visfatin was found to be the least relevant with subcutaneous adipose tissue. They found a negative, borderline significant relationship with visfatin only in the lumbar ($P<0.04$), and a negative insignificant relationship in other regions [20]. Although the results of our study were not statistically significant, they was in similar direction with the findings of the animal study by Staub et al. [20]. Adiponectin and visfatin were found negatively correlated as resistin was positively correlated. As subcutaneous adipose tissue thickness increases, adiponectin and visfatin tend to decrease and resistin to increase. As a result, although there was no statistically significant difference, there were divergent relationships in positive or negative directions between especially in adiponectin and visfatin levels and cutaneous-subcutaneous and total thickness measurements of lipedema and controls. It was suggested that the relationship between subcutaneous tissue thickness and adipokines was different from each other in lipedema and controls. The small number of our cases may be a factor in our inability to find statistically significant relationships and differences. Similar to our lipedema cases, findings of Staub ve ark. [20] in subcutaneous tissue thickness in the lumbar and rump-gluteal region and adiponectin, resistin and visfatin relationship did not overlaped with findings of their control group. Subcutaneous adipose tissue is mainly deposited in the femero-gluteal region, back, and abdominal wall [21]. Subcutaneous tissue and adipokines seem to differ from healthy controls in patients with lipedema. Although there was no significant difference between them and controls, resistin was found to be lower in patients with lipedema, while other adipokines were almost the same. Ultrasonographically measured skin-subcutaneous thickness might provide clues about adipokines, but studies with larger patient series are needed to clarify this relationship.

Thigh circumference measurements did not show divergent findings especially in adiponectin and visfatin as in ultrasonographic measurements. This may be related to the rougher assessment of tape measure measurements.

4. Conclusion

Compared to the controls, changes in the skin and subcutaneous tissue thickness specific to lipedema, especially in the subcutaneous tissue, were objectively demonstrated by ultrasonography. There was no statistically significant difference between lipedema cases and controls in terms of adipose tissue metabolites. Our finding can be interpreted that subcutaneous adipose tissue metabolites in lipedema differ from obesity in particular and show specificity in the light of general information. No statistically significant correlation was found between subcutaneous tissue thickness and adipose tissue metabolites. However, considering the findings of the lipedema and control groups, divergent relationships with negative/positive tendencies were

detected. Contrary to popular belief, not only visceral but also subcutaneous adipose tissue might affect metabolism. A mutual interaction between adipokines and subcutaneous adipose tissue may be involved in the etiopathogenesis of lipedema. However, in our study, the serum level of dysfunctional subcutaneous adipose tissue metabolites might not have been adequately reflected in ultrasonographic measurements of lipedema cases. The relatively low number of cases may also be a factor affecting our findings. Although white subcutaneous adipose tissue expresses molecules that trigger endocrine function, further studies are still needed to understand the interactions between hundreds of molecules produced in this tissue. Studies in larger case series will more clearly demonstrate the superiority of ultrasonographic examination in demonstrating fat tissue metabolism.

References

1. Lancha, A, Frühbeck, G, Gómez-Ambrosi J. Peripheral signalling involved in energy homeostasis control, *Nutrition Research Reviews*, 2012; 25(2): 223–48.
2. Unamuno, X, Gómez-Ambrosi, J, Rodríguez, A, Becerril, S, Frühbeck G, Catalán, V. Adipokine dysregulation and adipose tissue inflammation in human obesity, *European Journal of Clinical Investigation*, 2018, 48(9), e12997.
3. ´ Omez-Ambrosi, J.G, Cataí, V, Rodríguez, A, Andrada, P, Ramírez, B, Ibá~ P, et al., Increased Cardiometabolic Risk Factors and Inf lammation in Adipose Tissue in Obese Subjects Classified as *Metabolically Healthy*. 2014,, http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-0937/-/DC1.
4. Frühbeck G, Catalán, V, Rodríguez,, A, Ramírez, B, Becerril, S, Portincasa, P, et al., Normalization of adiponectin concentrations by leptin replacement in ob/ob mice is accompanied by reductions in systemic oxidative stress and inflammation, *Scientific Reports*, 2017; 7(1): 1–12.
5. Sethi, J.K, Vidal-Puig, A, Visfatin: the missing link between intra-abdominal obesity and diabetes? *Trends in Molecular Medicine*, 2005, 11(8), 344.
6. Giorgino, F, Laviola, L, Eriksson, J.W, Regional differences of insulin action in adipose tissue: insights from in vivo and in vitro studies, *Acta Physiologica Scandinavia*, 2005, 183(1), 13–30.
7. Castro, A, Kolka, C, Kim, S, Bergman, R, Obesity, insulin resistance and comorbidities? Mechanisms of association, *Arquivos Brasileiros de Endocrinologia e Metabologia*, 2014, 58(6), 600–9.
8. Crescenzi, R, Marton,, A, Donahue P, Mahany, H, Lants, S, Wang, P, et al., Tissue Sodium Content is Elevated in the Skin and Subcutaneous Adipose Tissue in Women with Lipedema, O*besity* 2018, 26(2), 310–7.
9. Wold, L, E.A, H, EV, A, Lipedema of the legs; a syndrome characterized by fat legs and edema, *Annals of Internal Medicine*, 1951, 34(5), 1243–50.
10. Halk, A, Damstra, R. First Dutch guidelines on lipedema using the international classification of functioning, disability and health, Phlebology, 2017, 32(3), 152–9.
11. Buso, G, Depairon, M, Tomson,, D, Raffoul W, Vettor, R, Mazzolai, L. Lipedema: A Call to Action! *Obesity*, 2019, 27(10), 1567–76.
12. Naouri, M, Samimi, M, Atlan M, Perrodeau, E, Vallin, C, Zakine G, et al. High-resolution cutaneous ultrasonography to differentiate lipoedema from lymphoedema. British Journal of Dermatology, 2010; 163(2): 296–301.
13. Marshall M, Schwahn-Schreiber ; C. Prävalenz des Lipödems bei berufs-tätigen Frauen in Deutschland (Lipödem-3-Studie). 2011; Available from: www.phlebologieonline.de
14. Reich-Schupke S, Altmeyer P, Stücker M. Thick legs - not always lipedema. *J Dtsch Dermatol Ges*, 2013, 11(3), 225–33.
15. Suehiro, K, Morikage, N, Murakami, M, Yamashita, O, Ueda, K, Samura, M, et al. Subcutaneous tissue ultrasonography in legs with dependent edema and secondary lymphedema, *Annals of Vascular Diseases*, 2014, 7(1), 21–7.
16. Suehiro, K, Morikage, N, Murakami, M, Yamashita, O, Samura, M, Hamano, K, Significance of ultrasound examination of skin and subcutaneous tissue in secondary lower extremity lymphedema, *Annals of Vascular Diseases*, 2013, 6(2), 180–8.
17. Suehiro, K, Morikage, N, Ueda, K, Samura, M, Takeuchi, Y, Nagase, T, et al., Aggressive Decongestion in Limbs with Lymphedema without Subcutaneous Echo-Free Space, *Annals of Vascular Surgery*, 2018, 53, 205–11.
18. Casley-Smith, J, Measuring and representing peripheral oedema and its alterations. Lymphology, 1994, 27(2), 56–70.
19. Iker, E, Mayfield, C.K, Gould, D.J, Patel, K.M, Characterizing lower extremity lymphedema and lipedema with cutaneous ultrasonography and an objective computer-assisted measurement of dermal echogenicity, *Lymphatic Research and Biology*, 2019, 17(5), 525–30.
20. Staub, C, Venturi, E, Cirot, M, Léonard, L, Barrière, P, Blard, P, et al. Ultrasonographic measures of body fatness and their relationship with plasma levels and adipose tissue expression of four adipokines in Welsh pony mares, *Domestic Animal Endocrinology*, 2019, 69: 75–83.
21. Störchle, P, Müller, W, Sengeis, M, Lackner, S, Holasek, S, Fürhapter-Rieger, A, Measurement of mean subcutaneous fat thickness: eight standardised ultrasound sites compared to 216 randomly selected sites, *Scientific Reports*, 2018, 8(1), 1–12.