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Original article

Novel approaches for the assessment of relative body weight and body fat in diagnosis and treatment of anorexia nervosa: A cross-sectional study

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SUMMARY

Background & aims: Anorexia nervosa (AN) is a severe psychosomatic disease that seriously affects nutritional status. Therapeutic approaches primarily aim for rapid weight restoration by high caloric diets and activity restriction. This often promotes abdominal body fat gain, which potentially negatively influences the patient's compliance and increases the risk of relapse. This study focused on the evaluation of body weight and subcutaneous adipose tissue (SAT) in AN patients by novel approaches.

Methods: The SAT of AN patients ($n = 18$, body mass index (BMI) 15.3 ± 1.3 kg/m^2) was determined by a highly accurate and reliable ultrasound method. The sum of SAT thicknesses of eight sites (D_{INCL}) was calculated. Individual metabolic profiles were analyzed. The mass index (MI), which considers body proportions, was used in addition to BMI. Additional to the standard laboratory diagnostics, dermal carotenoids measured by resonance Raman spectroscopy, leptin, and oxidative stress indicators were determined.

Results: The mean MI was 15.7 ± 1.4 kg/m^2. The D_{INCL} considerably differed between individuals with the same BMI. Half of the patients (Group 1) had low D_{INCL}: 1.3–28.4 mm, and Group 2 showed values up to 58.2 mm (corresponding to approximately 6 kg SAT mass). The two group means differed by more than 300% ($P < 0.001$). Accordingly, leptin levels significantly differed ($P < 0.001$). Mean SAT thicknesses were significantly higher in Group 2 at all eight sites. The groups also significantly differed in two oxidative stress parameters: total antioxidative capacity, malondialdehyde-modified low density lipoprotein immunoglobulin M (MDA-LDL IgM), and in the carotenoid level.

Conclusion: Half of the patients had sufficiently high fat mass, despite very low BMI. Consequently, their muscle (and other organ) masses must have been extremely low. Diagnostic criteria and treatment protocols for AN should consider each patient's body composition. In addition to dietary treatments, muscle training at low energy turnover rates may be essential for avoiding unnecessary body fat gain, better treatment results, and long-term recovery.

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1. Introduction

Anorexia nervosa (AN) is a severe psychiatric disease with a high mortality rate caused by severe malnutrition [1], a partly irreversible loss of body structure, and various other health consequences [2,3]. The causes of AN are complex, which makes effective therapeutic approaches difficult [4–9]. Current treatment strategies focus primarily on rapid weight restoration [7–9], and are based on two key determinants that influence energy metabolism [3]: increased energy intake taking into consideration any possible refeeding complications [7,10–12], including the administration of artificial nutrient-enriched dietary supplements to achieve the energy requirements for weight gain [2,7,9]; restrict physical activity and exercise to reduce energy expenditure [2,7–9], and avoid over-exercise [13], hyperactivity [14], hypermetabolism [7], and compulsive and compensatory behavior that may lead to an increased risk of adverse physiological outcomes [13–17]. However, some therapeutic approaches support supervised physical activity in the recovery process [3,16,18], since beneficial effects on the individual's well-being and positive influences on body composition have been reported [3,14,16,18–20]. This may contribute to more sustainable therapy [3,13,16], and does not necessarily impair weight gain [3,21–23]; on the contrary, it may reinforce it [3].

Most commonly, body mass index (BMI) and the speed of weight gain are used for assessing nutritional status and therapy progress [20,24,25]. Thus, the patient's body composition remains unconsidered, which is a substantial health criterion. Despite expected decreased muscle and fat masses in AN patients [16,26], an excessive gain in abdominal body fat is a known side effect of the current therapy strategies [3,27–31], and a major risk factor for relapse [7,27,30] since it enhances body image disturbances and concerns about body shape [32,33], which are diagnostic criteria for AN [34,35]. There is still a lack of satisfactory assessment tools for the therapy progress and body composition determination in therapy guidelines and clinical practice [8,9,36,37]. Widely used methods have known inherent problems [38]. Dual-energy X-ray absorptiometry (DXA) is considered to be the reference method; however, its application is not very feasible in clinical routine. Bioelectrical impedance analysis (BIA) is often used in the field [39–41], but it has limited reproducibility [42] and accuracy [38], and low validity [43], especially in AN patients [41,44,45].

The aim of the present study was to assess subcutaneous adipose tissue (SAT) in AN patients by a novel ultrasound method which allows fat thickness layer measurements with high accuracy and precision to gain detailed information on fat patterning in these patients [46]. Ultrasound results were compared with bioimpedance analysis. Additionally, a new measure for relative body weight, the mass index (MI) [47,48], which considers individual body proportions, was applied in addition to BMI. It was hypothesized that AN patients have very low SAT values, and that both measures for relative body weight do not sufficiently predict body fat.

2. Materials and methods

2.1. Participants

2.1.1. Recruitment and selection criteria

Female patients with AN ($n = 18$) according to International Classification of Diseases (ICD-10) criteria [35], aged between 18 and 40 years, were recruited from three psychiatric clinics in Graz, Austria. Exclusion criteria were: acute or chronic diseases or infection, alcohol or drug abuse, major cognitive deficits, life-threatening conditions during AN, history of digestive diseases (e.g. inflammatory bowel diseases and irritable bowel syndrome), history of gastrointestinal surgery, treatment with antibiotics and intake of pre- or probiotics within the previous 2 months, pregnancy, or breastfeeding. The study population was a subgroup of a larger cross-sectional study (five groups of different energy status $n = 107$). The study was conducted according to the Helsinki Declaration and approved by the ethics committee of the Medical University of Graz (MUG-26-383ex13/14). All participants gave their written informed consent for anonymous use of their data.

2.1.2. Additional information on the study population

Dieticians provided dietary advices. The nutritional treatment was based on high-caloric diets and recommended reduction of physical activity. Additional information on physical activity (International Physical Activity Questionnaire IPAQ Score) [49], nutritional intake (repeated 24-h recalls analyzed by a national specific software) [50], history of weight cycling [51], family history, and depression status were evaluated [52,53]. Demographic and clinical data (education, marital status, medication, smoking status) were collected [54]. Information on the disease and treatment history of the patients are provided in the appendix. Patient numbers were ordered according to their D_{INCL} values (P1–P18). D_{INCL} represents the sum of SAT thicknesses of eight standardized body sites measured by ultrasound. The patients were assigned to two groups: Group 1 (lower SAT) with D_{INCL} values below and Group 2 (higher SAT) above the D_{INCL} median.

2.2. Laboratory assessments

Standard blood values were determined and dermal carotenoids were assessed at the palm (resonance Raman spectroscopy) [55,56]. Oxidative stress parameters were determined in serum: total peroxides (TOC®) [57], endogenous peroxidase-activity (EPA®), total antioxidative capacity (TAC®) (Labor Diagnostic Nord, Nordhorn, Germany). Titers of autoantibodies against oxidized LDL (oLAb®) (Biomedica, Vienna, Austria) and malondialdehyde-modified LDL (MDA-LDL IgM®) (Omnignostica GmbH, Höflein/Klosterneuburg, Austria) were measured by ELISA [58,59]. Plasma leptin levels were measured by ELISA (BioVendor, Brno, Czech Republic).

2.3. Anthropometry

Measurements of body mass (m), body height (h), and sitting height (s) were performed in accordance with the International Society for the Advancement of Kinanthropometry (ISAK) [60]. The BMI (m/h^2) and MI = 0.53 $m/(h\,s)$ were calculated [47,48,61]. The MI considered individual sitting height for assessing relative body weight: in individuals with long legs, the MI was higher than the BMI and vice versa. The SAT mass (m_{SAT}) was calculated according to: m_{SAT} (kg) = 0.65 d_8 S ρ, with d_8 being the mean of D_{INCL}, S the body surface area (according to Du Bois: $S = 0.20247\ h^{0.725}\ m^{0.425}$) [62], and ρ the density of fat (0.92 kg/m^3) [63]. The calibration factor of 0.65 resulted from comparative measurements at 216 randomly distributed sites in a set of test persons [64]. The SAT thicknesses were accurately measured values that should primarily be used, whereas the calculated SAT mass included model assumptions.

2.4. Ultrasound imaging technique

Brightness mode ultrasound (US) accurately measures SAT thicknesses and is reproducible in all groups from extremely lean to obese people [38,46,65,66]. The standardized eight sites (upper abdomen (UA), lower abdomen (LA), erector spinae (ES), distal triceps (DT), brachioradialis (BR), lateral thigh (LT), front thigh (FT),

and medial calf (MC)) were measured [66]. Lateral thigh was replaced by external oblique (EO) because LT was defined after the study data were collected [67,68]. These body sites represent the body parts, trunk, arms, and legs. Sites are defined with respect to the person's body height, which ensures interpersonal comparability. A thick gel layer between the probe and the skin was used to prevent SAT compression. Figure. 1 shows a typical US image with clearly visible SAT layer borders. For US imaging, a conventional US system (GE Logiq-e, General Electric) with a linear probe (L8-18i RS) operated at 8–16 MHz was used. The US images were evaluated with semiautomatic evaluation software (Rotosport, Stattegg, Austria). The software provides information on D_{INCL}, D_{EXCL} (SAT thicknesses without embedded tissues, e.g. fibrous structures) and calculates embedded structures ($D_{ES} = D_{INCL} - D_{EXCL}$). The US measurement was performed by two certified investigators (iasms.org, 2-day course and supervised post-course training).

2.5. Bioelectrical impedance analysis

Single-frequency bioelectrical impedance analysis (BIA) (BIA 101, Akern) was conducted according to recommended procedures [44,45], and analyzed with the BodyComposition–Professional software v9.0.14325, which uses equations from Sun et al. for calculating fat free mass (FFM) and total body water [69], and Sergie et al. for extracellular water calculation [70]. Total body fat (TBF) was calculated by subtracting FFM from body mass (m): TBF = m − FFM. Resistance (R), reactance (Xc), and phase angle (PA) were measured at 50 kHz. This software was used for the comparison with the US measurement because it is commercially available and therefore widely used in clinical practice. Additionally, other BIA equations that are supposed to be more appropriate in AN patients were applied. For example, the equations from Deurenberg and Kushner were used [71,72], which Matter et al. suggested for AN patients [73].

2.6. Statistics

SPSS Statistics v23 software (IBM, Armonk, NY, USA) was used for statistical analysis. Shapiro-Wilk tests revealed that not all distributions were normal. Descriptive parameters were presented as mean ± standard deviation (SD) when data were normally distributed, otherwise the median and interquartile ranges (IQR) were used. For group comparisons Student's *t*-test and Mann-Whitney U test were used, respectively. Chi-squared test was used for qualitative variables. The Pearson correlation coefficient (r) was used for data in Fig. 5 and Supplemental Fig. 1 (appendix). The significance level was set at $P < 0.05$. All analyses were explanatorily interpreted.

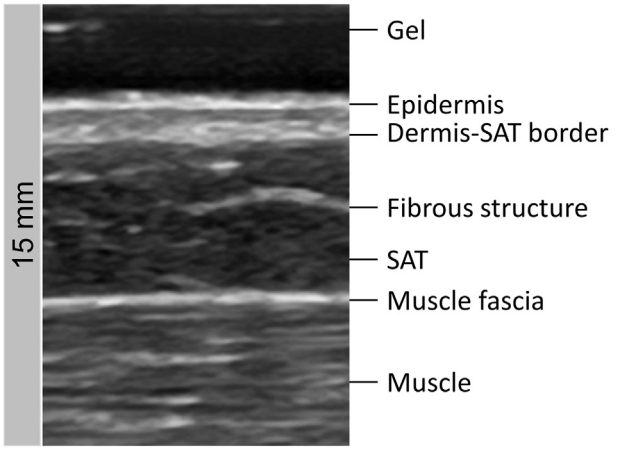

Fig. 1. Example of a typical ultrasound image. Ultrasound site: brachioradialis; subcutaneous adipose tissue (SAT) layers can be detected uncompressed due to the application of a thick gel layer between the probe and the skin. The upper and lower borders of SAT (dermis and muscle fascia, respectively) are clearly visible. The area in-between represents the SAT layer. Embedded fibrous structures are also visible. SAT thicknesses were calculated via the semiautomatic image segmentation software. Speed of sound in fat: c = 1450 m/s. SAT, subcutaneous adipose tissue.

3. Results

3.1. Body mass index and body fat

The study population had a mean BMI of 15.3 ± 1.3 kg/m^2 and a mean D_{INCL} of 29.13 ± 18.69 mm. Some patients had almost the same BMI, but D_{INCL} (and D_{EXCL}) differed substantially (Fig. 2, Table 1).

In some individuals, SAT thickness was quite high, although the BMI was extremely low (Fig. 3A, appendix). The thickness of the fat layers varied by several hundred percent at a given BMI. For instance, two patients with BMIs of 13.2 kg/m^2 and 13.3 kg/m^2 had D_{INCL} values of 1.3 mm and 24.4 mm, respectively. Another example: BMIs of 15.2 kg/m^2 and 15.1 kg/m^2 and according D_{INCL} values of 10.2 mm and 43.9 mm, respectively. The correlation of D_{INCL} and BMI was $r\,(18) = 0.741$, $P < 0.001$.

3.2. Measures for relative body weight: body mass index vs mass index

When using mass index (MI) instead of BMI, patient P17 would not be classified as an AN patient (BMI ≤ 17.5 kg/m^2, ICD-10). In 10 patients the MI differed by ≥0.5 kg/m^2 to the BMI. Figure. 3B shows the participants' relative body weights in terms of BMI and MI [47,48]. In this group of AN patients, the mean MI (15.7 kg/m^2) was larger than the mean BMI (15.3 kg/m^2) [38,61].

3.3. Body mass index and subcutaneous fat thickness

The group of investigated AN patients showed a large range of D_{INCL} from 1.3 mm to 58.2 mm. This indicates enormously divergent body compositions between patients. A median D_{INCL} = 30.17 mm was used to divide the group of AN patients into two subgroups. Detailed descriptive data of the groups are shown in Table 1. One subgroup of patients had extremely low SAT values (M = 13.7 mm, SD = 9.6), whereas the other subgroup had surprisingly high amounts of SAT (M = 44.6 mm, SD = 10.6), despite extremely low BMIs. Comparisons of D_{INCL} and D_{EXCL} showed significant differences ($t(16) = -6.498$, $P < 0.001$) and $t(16) = -6.384$, $P < 0.001$), respectively). The relative amount of embedded structures ($D_{ES} = D_{INCL} - D_{EXCL}$) decreased with increasing SAT thickness. The mean percentage of D_{ES} of Group 2 (higher SAT) was 9%, whereas the mean percentage in Group 1 (lower SAT) was 14%.

3.4. Fat patterning in anorexia nervosa patients

Fat patterning of the subgroups was compared. SAT layers significantly differed between the two groups at every single site ($P < 0.01$) (Fig. 4, Table 1). The SAT layers were extremely low at all sites in Group 1 (lower SAT): medians ranged from 0.4 to 2.1 mm. In Group 2 (higher SAT), the median SAT thickness was >5 mm at four sites: UA, LA, FT, MC (appendix).

3.5. Bioelectrical impedance analysis measurement of body fat

Figure. 5 depicts contradictory results obtained with the US method compared to BIA (TBF). For example, BIA assessed 1 kg of

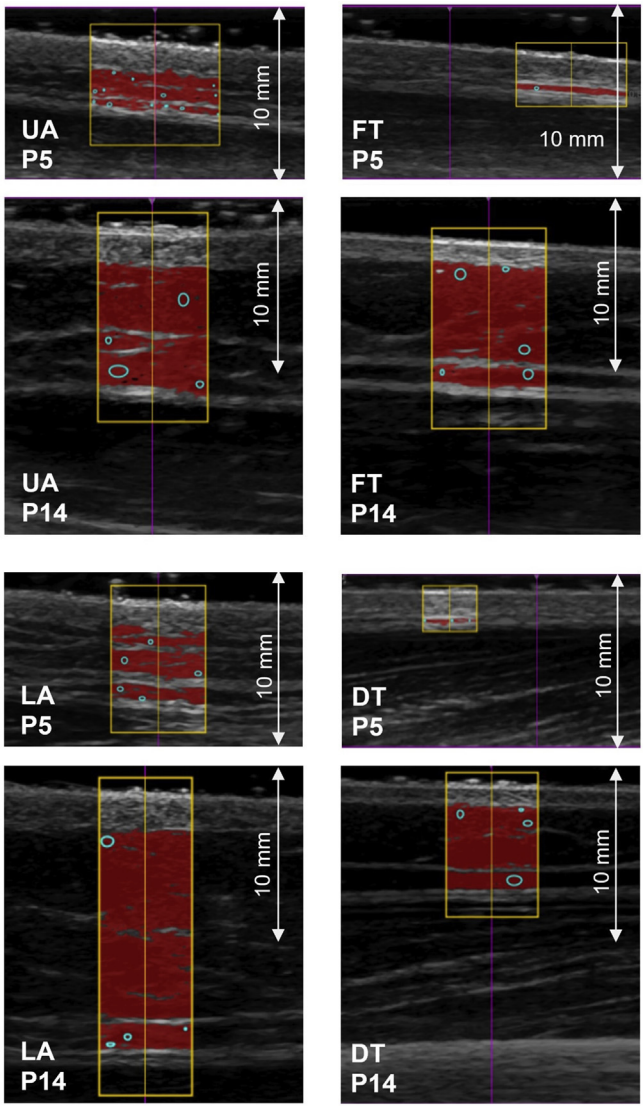

Fig. 2. Comparison of fat patterning in two anorexia nervosa patients with the same BMI (four selected sites). A series of subcutaneous adipose tissue (SAT) ultrasound measurements at the four selected sites upper abdomen (UA), lower abdomen (LA), front thigh (FT) and distal triceps (DT) of patients P5 and P14 who had the same BMI is shown here. Additional anthropometric data and information on the measurement and evaluation procedure are provided in the appendix. Mean SAT thicknesses (in mm) of P5 are: UA: 1.94, LA: 3.65, FT: 0.28, DT: 0.17. The sum of the eight standardized site collection (not all are shown here) D_{INCL} = 10.15 mm. Mean SAT thicknesses (in mm) of P14 are: UA: 6.54, LA: 11.89, FT: 6.51, DT: 4.50, D_{INCL} = 43.88 mm. SAT mass resulted in 1.2 kg for P5 and 4.5 kg for P14. Similar cases of great variations of SAT layer thickness at every single site and D_{INCL} were also found in several other patients with almost the same BMI. MI, body mass index; D_{INCL}, the sum of SAT layers at the eight standardized ultrasound sites; DT, distal triceps; FT, front thigh; LA, lower abdomen; P, patient; SAT, subcutaneous adipose tissue; UA, upper abdomen.

TBF for four patients, but D_{INCL} varied from 1.3 to 24.4 mm. Six patients with a mean TBF of 3.9 ± 0.3 kg according to BIA had D_{INCL} values ranging from 10.2 to 43.9 mm. The correlation of D_{INCL} and BIA TBF (kg) was $r(18) = 0.759$, $P < 0.001$.

3.6. Additional information

3.6.1. Energy and nutrient intake

Although the patients received similar dietary treatment, the two groups reported significant differences in energy intake: Group 1 (lower SAT): M = 10,100 kJ, SD = 3100/day (2400 ± 700 kcal; 59 ± 14 kcal/kg body mass); Group 2 (higher SAT): M = 6200 kJ, SD = 3800/day (1500 ± 900 kcal; 35 ± 22 kcal/kg body mass). Besides the higher energy intake, Group 1 (lower SAT) also reported a higher protein and fat intake than Group 2 (higher SAT). The intake of other macronutrients such as carbohydrates, monosaccharides, fiber, saturated fatty acids and water showed no significant differences. Group 1 (lower SAT) reported having more frequently consumed high energy supplements than Group 2 (higher SAT) (not significant).

3.6.2. Lifestyle information

Physical activity level, smoking habits and other assessed patients' data (appendix) did not differ significantly between the two groups. SAT thicknesses were not correlated with physical activity as determined by International Physical Activity Questionnaire.

3.6.3. Laboratory assessments

Laboratory chemistry revealed no deviation from the reference blood values, including parameters of lipid and carbohydrate metabolism, kidney, liver and thyroid function. However, Alanin-Aminotransferase (ALT) ($t(16) = 2.130$, $P = 0.049$) and lactate-dehydrogenase (LDH) ($t(16) = 2.411$, $P = 0.028$) were significantly higher in the lower SAT group (Group 1) (Table 1).

Plasma leptin levels ranged from 1.0–7.7 ng/ml. Group 1 (lower SAT) had mean leptin values of 1.1 ± 0.3 ng/ml and Group 2 (higher SAT) a mean of 4.2 ± 2.3 ng/ml. Leptin differed significantly between the two groups (U = 1, $P < 0.001$).

Regarding oxidative stress, the TAC of AN patients (M = 0.95 mmol/l, SD = 0.37) was low (reference: > 1.3 mmol/l). In addition, TAC ($t(12.9) = -4.341$, $P = 0.01$) and MDA-LDL IgM (U = 15, $P = 0.024$) were significantly lower in the lower SAT Group 1 (Table 1). Although in Group 2 (higher SAT) the antioxidative levels with respect to TAC, TOC, EPA, oLAB and MDA-LDL IgM were lower compared to reference values, their concentrations were still higher compared to the levels of Group 1 (lower SAT). According to these indicators, Group 1 (lower SAT) had higher oxidative stress than Group 2 (higher SAT).

3.6.4. Dermal carotenoid level

The level of accumulated carotenoids in the skin at the palm measured by resonance Raman spectroscopy differed significantly between the groups ($t(16) = 2.978$, $P = 0.009$, Table 1).

4. Discussion

This study applied, for the first time, novel approaches for determining both relative body weight and body fat in AN patients.

4.1. Measures for relative body weight

According to ICD-10 (F.50.0) and DSM-V, a BMI cut-off is among the three main criteria for diagnosis of AN. Regarding BMI, the World Health Organization (WHO) points out that 'problems arise, however, in adults whose shape differs from the norm, particularly those whose legs are shorter or longer than might be expected for their height.' [74] Based on this remark, the MI, which considers individual leg lengths, was introduced [47,48,61]. The MI was larger than the BMI in 13 cases and lower in two cases (Fig. 3B) in the current study. Using the MI would shift some of the individuals' relative body weight beyond the AN criterion of <17.5 kg/m^2. In individuals with relatively long or short legs, the difference between BMI and MI can be a full unit or more (e.g. P8: BMI = 13.3 kg/m^2, MI = 14.4 kg/m^2). However, relative body weight without accurate and reliable assessment of body fat is a weak criterion for healthy weight [16,74,75].

Table 1
Anthropometric and body composition data, and striking blood values of the study population (Group 1 and Group 2) that was divided into the subgroups Group 1 below and Group 2 above the median of D_{INCL} (the sum of subcutaneous adipose tissue (SAT) of the eight body sites measured by ultrasound). When data were normally distributed, they were presented as means and standard deviation (SD), otherwise they were reported as median and interquartile range (IQR) in italic font.

N	Group 1 lower SAT		Group 2 higher SAT		Group 1 and Group 2		*P*
	9		9		18		
	Mean *Median*	SD *IQR*	Mean *Median*	SD *IQR*	Mean *Median*	SD *IQR*	
Age, years	22.7	3.6	22.2	3.0	22.4	3.2	0.778
Anthropometry							
Height, m	1.68	0.08	1.65	0.06	1.66	0.07	0.340
Sitting height, m	0.87	0.04	0.85	0.03	0.86	0.04	0.216
Body mass, kg	40.91	5.41	43.70	4.00	42.31	4.84	0.232
BMI, kg/m^2	14.5	1.1	16.1	0.9	15.3	1.3	0.003
MI, kg/m^2	14.8	1.2	16.6	0.8	15.7	1.4	0.002
Ultrasound measurement of subcutaneous adipose tissue							
D_{INCL}, mm	13.66	9.55	44.59	10.62	29.13	18.69	<0.001
D_{EXCL}, mm	11.76	8.62	40.57	10.44	26.17	17.49	<0.001
D_{ES}, mm	*2.11*	*1.75*	*3.75*	*0.96*	*3.08*	*1.75*	<0.001
D_{ES}, %	14%		9%		10%		
Fat patterning measured by ultrasound							
UA, mm	2.32	2.07	5.47	1.92	3.90	2.45	0.004
LA, mm	2.63	2.17	9.91	2.98	6.27	4.38	<0.001
ES, mm	2.01	1.69	4.44	1.72	3.23	2.02	0.008
DT, mm	1.69	1.80	6.14	1.65	3.92	2.76	<0.001
BR, mm	*0.38*	*0.63*	*2.58*	*1.78*	*1.10*	*2.28*	<0.001
EO, mm	*0.45*	*0.67*	*3.12*	*2.47*	*1.68*	*2.73*	<0.001
FT, mm	2.35	1.89	7.63	2.15	4.99	3.26	<0.001
MC, mm	*1.00*	*1.93*	*5.24*	*4.33*	*2.91*	*4.47*	0.002
Single frequency bioimpedance analysis							
R, ohm	690.0	60.3	767.2	55.2	728.6	68.7	0.012
Xc, ohm	56.1	9.7	72.8	10.4	64.4	13.0	0.003
PA, °	4.65	0.68	5.41	0.60	5.03	0.73	0.022
RI, cm^2/ohm	41.1	4.4	35.5	3.9	38.3	4.9	0.012
Plasma values – selection of striking parameters							
Leptin, ng/ml	*1.00*	*0.15*	*3.70*	*4.39*	*1.55*	*2.78*	<0.001
Oxidative stress							
TAC, mmol/l	0.69	0.31	1.21	0.18	0.95	0.37	0.001
MDA-LDL IgM, mU/ml	*87.87*	116.60	*302.85*	548.23	*137.79*	*407.08*	0.024
Liver							
ALT, U/l	26.11	6.58	19.11	7.34	22.61	7.66	0.049
Heart							
LDH, U/l	180.56	20.09	157.67	20.18	169.11	22.81	0.028
Nutritive aspects							
Carotenoid, Counts	43,889	12,211	26,333	12,797	35,111	15,127	0.009

ALT, alanine aminotransferase; BMI, body mass index; BR, brachioradialis; DES, embedded fibrous structures; DEXCL, the sum of subcutaneous adipose tissue (SAT) layer thicknesses without embedded fibrous structures (DES = DINCL – DEXCL); DINCL, the sum of SAT layers at the eight standardized ultrasound sites; DT, distal triceps; EO, external oblique; ES, erector spinae; FT, front thigh; LA, lower abdomen; LDH, lactate dehydrogenase; MC, medial calf; MDA-LDL IgM, malondialdehyde-modified low density lipoprotein immunoglobulin M; MI, mass index (0.53 × body mass/(body height × sitting height)); PA, phase angle; R, resistance; RI, resistance index (height^2/resistance); SAT, subcutaneous adipose tissue; TAC, total antioxidative capacity; UA, upper abdomen; Xc, reactance.

4.2. Body composition assessment

The US method revealed great variability of SAT in AN patients [46,66]. Half of the patients (Group 2, higher SAT) had SAT amounts comparable with healthy normal weight women (Fig. 2, Fig. 3A) [76]. According to preliminary reference values for D_{INCL} [76], values from 35 to 50 mm are considered as 'desirable range' for athletes, and 35–80 mm for all other women. The median of the current group (30.2 mm) was close to the lower border of the 'desirable range'. This was found, although EO was used instead of LT. In women SAT thickness is substantially higher in LT compared to EO; using LT would shift the median towards even higher values. The two subgroups' BMI medians differed by 12%, whereas D_{INCL} values differed by 330%, indicating that the large difference in SAT (and thus also of TBF [77]) cannot be captured by the BMI or the MI. Plasma leptin was expectantly low in all AN patients; however, it also significantly differed between the groups, underpinning the observed differences in body composition. Additionally, the two groups showed great differences in the fat patterning (Fig. 4). Several individuals had almost the same BMI although their SAT amount differed enormously (Fig. 2, Fig. 3A, B).

Yager and Anderson mentioned a loss of SAT as a common sign and symptom of AN [2]. However, this study found that the reduced weight did not indicate low SAT in all patients. In Group 2 (higher SAT, $D_{INCL} > 30$ mm) the patients' extremely low weight must have resulted from losses of other body structures, particularly muscle, organ and bone mass. Four patients had D_{INCL} values > 50 mm, which is considered as 'ballast fat' in competitive female sports [76].

Group 1 (lower SAT) reported significantly higher energy intake than Group 2 (higher SAT). However, AN patients may overestimate portion sizes according to their altered perception. Furthermore, the lower SAT Group 1 ($n = 7$) received energy dense sip food more often than the higher SAT Group 2 ($n = 3$). The generally increased oxidative stress status indicates the body's challenge in severe catabolic metabolism [78]. Hypercarotenemia and altered lipid metabolism occur in some AN patients [78,79]. The observed differences in oxidative stress and carotenoid concentration (Table 1) may indicate various metabolic disturbances due to body fat content.

However, there are currently no established threshold values available for minimum fat [80]. This is partly the case because

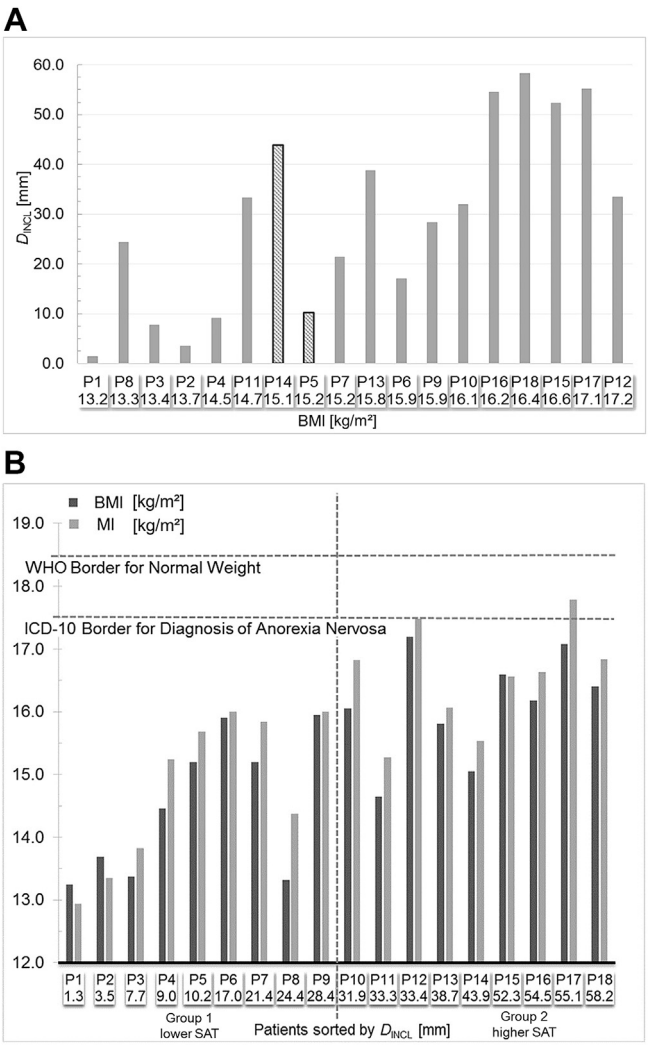

Fig. 3. A. Body mass index versus D_{INCL}. The patients (P1–P18) are ordered according to increasing BMI. Within this group of anorexia nervosa patients, the sums of eight subcutaneous adipose tissue (SAT) thicknesses (D_{INCL}) ranged from 1.3 to 58.2 mm. The patient numbers P1–P18 were assigned according to increasing D_{INCL} values. The two patients described in Fig. 2 are highlighted in this figure to point out the discrepancy for observed SAT and BMI classification. BMI, body mass index; D_{INCL}, the sum of SAT layers at the eight standardized ultrasound sites; P, patient; SAT, subcutaneous adipose tissue.
B. Body mass index and mass index. Patients P1–P18 are ordered according to increasing subcutaneous adipose tissue (SAT) thicknesses. MI = $0.53m/(hs)$; BMI = m/h^2. A BMI $\leq$17.5 kg/m^2 is one criterion of ICD-10 anorexia nervosa diagnosis, and the WHO underweight border $\leq$18.5 kg/m^2 are both highlighted with horizontal dashed lines. The vertical dashed line symbolizes the median of D_{INCL}. It divides the patients into subgroup Group 1 with low SAT layers and Group 2 with higher SAT amount. BMI, body mass index; D_{INCL}, the sum of SAT layers at the eight standardized ultrasound sites; ICD-10, International Classification of Diseases 10th revision; MI, mass index; P, patient; SAT, subcutaneous adipose tissue; WHO, World Health Organization.

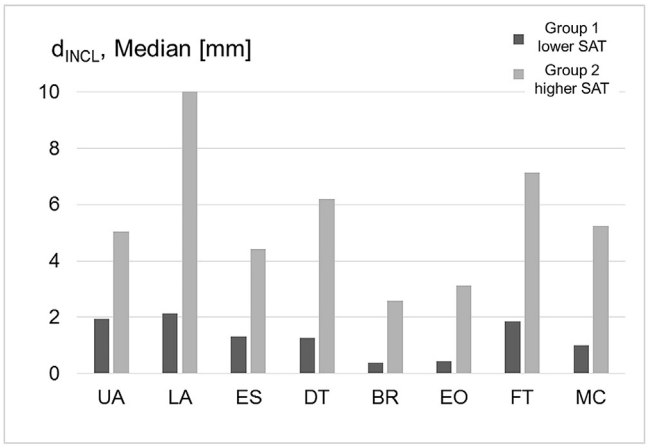

Fig. 4. Comparison of fat patterning between the two anorexia nervosa groups. Medians of d_{INCL} (SAT thickness at a single measurement site) of Group 1 with lower SAT (n = 9) and Group 2 with higher SAT (n = 9) are compared with each other. Group 2 has higher SAT thickness at every measured body site. BR, brachioradialis; d_{INCL}, SAT thickness at a single measurement site; DT, distal triceps; EO, external oblique; ES, erector spinae; FT, front thigh; LA, lower abdomen; MC, medial calf; SAT, subcutaneous adipose tissue; UA, upper abdomen.

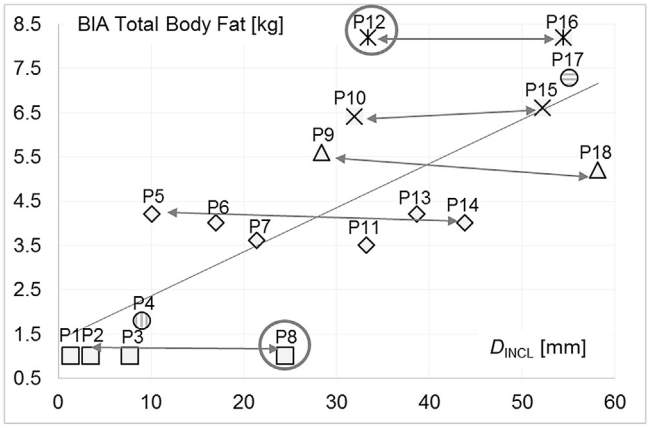

Fig. 5. Individual differences of D_{INCL} and total body fat with bioelectrical impedance analysis. Several patients with the same or similar total body fat (TBF) determined by bioelectrical impedance analysis (BIA) had totally different body fat measured by ultrasound. The arrows point out the great discrepancy between the D_{INCL} values and TBF determined by BIA. For example, TBF of all patients that are highlighted as squares resulted in 1 kg according to BIA, but ultrasound measurement revealed D_{INCL} values ranging from 1.3 to 24.4 mm (which amounts to about 0.1–2.6 kg SAT). Patients with similar TBF measured by BIA are labelled with the same symbols. Also, for patients with similar D_{INCL} values BIA results differed extremely. For example, the two encircled patients P8 and P12 had D_{INCL} values of 24.4 and 33.4 mm (2.6 kg and 3.7 kg SAT) and BIA measurement resulted in 1 kg and 8.2 kg TBF, respectively. BIA, bioelectrical impedance analysis; D_{INCL}, the sum of SAT layers at the eight standardized ultrasound sites; P, patient; SAT, subcutaneous adipose tissue; TBF, total body fat.

sufficiently accurate measurement methods were missing, and the acceptable minimum of fat may be genetically predisposed [80]. Also in very lean people, US provides a reliable and accurate tool to measure SAT [66], whereas widely used methods like DXA [38], magnetic resonance imaging (MRI) scans, skin folds or BIA do not reach the necessary accuracy (see appendix) [38,68]. The current study compared fat mass assessed by BIA and US results (D_{INCL}). The TBF determined by BIA revealed inaccurate results in individuals (Fig. 5), although the correlation coefficient between D_{INCL} and BIA TBF was high. Also, the results obtained from formulas, suggested for AN patients [73], deviated substantially from D_{INCL}. The BIA equations often rely on comparisons with DXA with its known shortcomings (particularly in lean persons), instead of comparing it to multi-component methods [81]. However, no algorithm can compensate the basic shortcomings of the BIA method (appendix) [82].

Differences in body composition in AN patients at treatment baseline have previously been observed and a decrease in skeletal muscle and internal organ mass was associated with decreased fat mass [26,37,83]. Since the current study observed large differences in SAT at the same BMI, muscle and other organ masses must have independently decreased from fat mass. For example, participant P14 (BMI = 15.1 kg/m^2, m = 40.0 kg; Fig. 2, Fig. 3A) had a D_{INCL} of 43.9 mm. There was no need to increase the fat level of this patient

[76]. Her SAT mass amounted to 11.4% of her body mass; this percentage did not contain the visceral fat and fat embedded in other organs. In contrast to P14, patient P5 (BMI = 15.2 kg/m^2, m = 45.5 kg) had the extremely low SAT mass of 2.5% of her body weight; in this case, interventions focusing on increasing fat mass are obviously important. In Group 1 (lower SAT), percentage of fibrous structures was higher, which further reduced the pure amount of fat (Table 1, appendix).

4.3. Consequences for the therapeutic approach

In common treatment approaches, high caloric diet and limited physical activity is suggested to rapidly increase body weight [8,9,36]. Practice guidelines suggest that 'for severely underweight patient, exercise should be restricted and always carefully supervised and monitored' [9]; however, these authors state that further research is needed [36]. Rapid weight restoration is essential [4–9]; however, too quick weight gain at the beginning of treatment is considered to be unfavorable for later weight maintenance and long-term recovery [7,13,20]. Fast weight gain is often associated with abdominal fat accumulation [3,27,28,31], which can have negative metabolic effects [27]. AN is associated with body image disturbance [32], and body shape concerns [33], therefore, an inadequate gain of body fat can be expected to negatively influence compliance. However, El Gouch et al. did not find a connection between body fat gain and long-term outcome [84]. Mayer et al. observed that the accumulated body fat disappeared after long-term weight restoration [29,30]. Nevertheless, the relapse rate in AN patients is high [1], and thus long-term weight restored patients are rare. Strategies to increase muscle mass may enhance therapeutic success. It is indicated to hypothesize that strength training (few repetitions, thus low energy turnover) may be advantageous to increase muscle mass and avoid excessive gain of fat mass [85]. The restoration of lean body mass is a key determinant of outcome and quality of life [3]. Accurate body composition testing should be routinely implemented in the standard care of AN patients [37]. The potential therapeutic benefits have also been pointed out by Yamashita et al. [26].

4.4. Limitations of the study

In this study, 18 AN patients who had already received treatment were included. Further studies should include larger groups. Longitudinal studies will be necessary to test the suggested modifications of treatment practices. Information on nutrient intake and activity levels were based on self-assessment of the patients. The IPAQ could not map possible differences in physical activity habits because most participants were inpatients.

5. Conclusion

Ultrasound measurement enabled accurate monitoring of body fat. This method revealed enormous differences in SAT among AN patients with similar BMIs, and biochemical parameters (leptin, ALT, LDH, oxidative stress indicators, and carotenoid levels) corresponded with this finding. Although all patients were characterized by very low BMI, SAT thicknesses varied from extremely low to normal ranges. Half of the patients had sufficiently high fat mass. Body mass index is not an adequate criterion for classification of body fat mass in AN patients. The current data suggest a rethink of current treatment practice. For those with extremely low muscle mass, activity is recommended to increase muscle mass at a low energy turnover rate, which may also improve compliance and thus therapeutic outcome and long-term recovery.

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Statement of authorship

SL, SM, WM, and SH designed the project and the manuscript. WM introduced the mass index MI and together with AF supported the accurate application of the ultrasound fat measurement. Patients were recruited by SM, CB, OA and ML. The investigations were conducted by SL, SM, AO, CB and OA. Oxidative stress parameters were measured by WW, MM suggested to include dermal carotenoid status assessment, and chemical laboratory diagnostics were conducted by HM and SZ. All authors contributed to the final form of the manuscript.

Conflicts of interest

WM and AF contributed to developing the commercially available image evaluation software used here and participate in the returns. Except for this, all authors have declared that no competing interests exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2018.12.031.

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