Subcutaneous fat patterning in athletes: selection of appropriate sites and standardisation of a novel ultrasound measurement technique

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APPENDIX

Ad Part A of the (printed) main publication: Ultrasound technique and accuracy

Important points for avoiding errors and for standardising the US technique:

1. Besides setting the sound speed correctly, it is also important to set all ultrasound (US) imaging parameters that determine the image quality adequately for a given anatomical structure. For example, using a too high gain, inadequate time-gain-compensation, inadequately positioned foci, or too low frequency, would broaden the borders and reduce image contrast. This would also decrease substantially the accuracy of tissue border detection.

2. The marker on one side of the linear US probe (a linear probe is necessary for accuracy reasons) is always directed upwards (cranially) or upwards-and-to-the-left. This corresponds to the usual application of diagnostic US probes in medicine.

3. Measurements are usually made on the right side of the participant's body. The middle of the probe is placed exactly above the landmarked site and held perpendicular to the skin. The investigator's hand may contact the participant's body in order to stabilise and guide of the probe, but there must be a minimum distance of 5 cm between the support hand and the probe. Otherwise, the pressure might distort the fat profile.

4. Always use a thick layer of US gel (at least 3 to 5 mm, avoid any air bubbles) between the probe and the skin. In the image, a black band above the skin corresponds to this gel layer. This is vital for the assessment of the US image because it ensures that there is no pressure on the skin which would compress the subcutaneous adipose tissue (SAT) layer. If the image quality is not good enough, it can be useful to restart the imaging with a new layer of gel on the probe. During US imaging, check that a dark band of about 3 to 5 mm above the skin can be seen on the screen.

5. The light should be dimmed in the examination room and the general gain and the depth gain compensation should be set such that the US image of the SAT is very dark and only the structures of interest can be seen clearly. Too much gain would

result in displaying "noise" in addition to real objects and in a decrease of resolution. Using "contour enhancement" image processing may improve the clarity of tissue borders.

6. The borders of SAT should be clear to the investigator when capturing the US image. The SAT layer should appear dark in the US image (otherwise the gain is too high); eventually some light lines which correspond to fibrous structures can be seen embedded in the fat. The investigator has to make sure that the borders of the SAT (end of skin on the upper side and muscle fascia on the lower side of the SAT layer) are clearly visible as white bands without any interruption; otherwise the algorithm will not do the image segmentation correctly.

7. In case of doubt about the location of the muscle fascia (i.e. the lower border of the SAT), compress the SAT with the US probe in order to distinguish between SAT and the muscle; this is essential in such cases to avoid erroneous image evaluation (misinterpretation of intermediate fasciae, like Camper's fascia in the abdomen).[14 15] When borders are clear, start the imaging again with a new 5 mm layer of gel.

8. Image evaluation should be done soon after having captured the US image. In case this is not possible, all US systems permit text to be inserted onto the US image. This feature can be used to mark the fascia of the muscle underneath the SAT (to prevent misinterpretations later). Of course, such annotations must be outside the region of interest.

9. In case a vein appears in the US image (black band without any grey speckle), move the probe beside the vein. For example, at the brachioradialis (BR) site, the vena cephalica is sometimes in the field of view. This might erroneously lead to a measurement of the vein thickness instead of the SAT thickness.

10. Establishing a reference data base requires a complete set of "meta information", including: an anonymous code for each athlete, gender, ethnicity, age (A), body mass (m), body height (h), sitting height (s), leg length (l). Additional anthropometric measures can be added optionally. Athletes are characterised by their sport, sport discipline, performance level, training frequency and years of training.

Analysis of US thickness measurement accuracy:

A typical US image from the site front thigh (FT) is shown in figure 7 in order to elucidate the resolution and accuracy limits of US imaging (18 MHz linear probe: GE L8-18i, operated in harmonic mode). The thicknesses d_{INCL} = 4.6 mm, d_{EXCL}= 4.2 mm, and the thickness of the dermis (1.0 mm) can be measured accurately with the evaluation software (FAT Software; rotosport.com) in the US image, but the value obtained for the epidermis thickness of 0.23 mm is too high; the thickness of the epidermis (measured microscopically) ranges typically between 0.08 to 0.10 mm. Accuracy of US distance measurements cannot reach microscopic levels. The accuracy of tissue border detection is determined by: first, the axial US resolution limit (approximately equal to the wavelength used: about 0.1 mm, 0.2 mm, and 0.3 mm at 18, 9, and 5 MHz, respectively), and second, the sound speed (*c*) used for calculating distances in a given tissue (d_{US}): $d_{US}=cT/2$ (T is the echo time). A sound speed of 1450 m/s was set in the inter-active evaluation algorithm for distance determination in SAT.[17]

The actual value might deviate slightly from the values determined in excised animal fat tissues (sound speed has not yet been measured in human SAT). A difference of 1% (e.g. 1465 m/s instead of 1450 m/s) would result in a measurement error of 0.1 mm in a 10 mm thick layer. In this case, this error is comparable to the border detection error (at 18 MHz). Conventional US machines use c = 1540 m/s for all tissues: in a 10 mm SAT layer, this wrong speed of sound would result in an error of 0.6 mm, which cannot be ignored when accurate measurements are desired. The image analysis software used here allows setting of the correct speed of sound for all biological tissues.

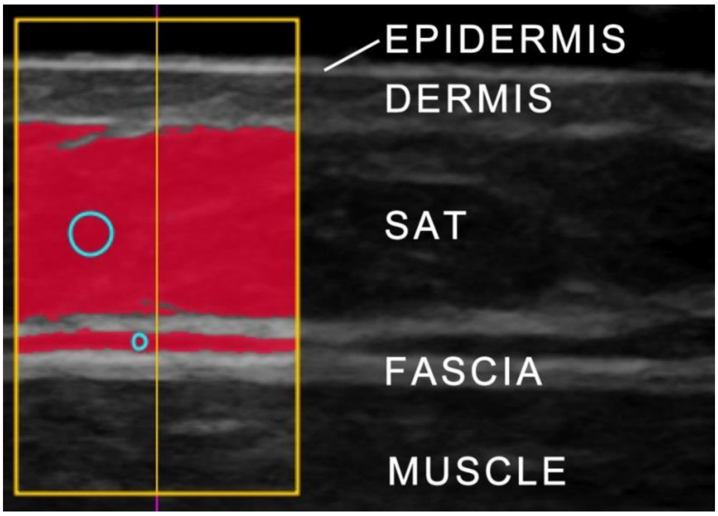

Figure 7: The semi-automatic image segmentation and thickness measurement algorithm was applied to determine the SAT thickness at the site FT (red area). Within the region of interest (yellow frame), 156 thickness measurements resulted in an average value of d_{INCL} = 4.64 mm and d_{EXCL} = 4.20 mm (c_{FAT} was set to: 1450 m/s).[17] The mean thickness of the embedded fibrous structure is 0.44 mm. The US thickness measurements of the dermis and epidermis resulted in 0.96 mm and 0.23 mm, respectively (c_{SKIN} was set to 1540 m/s).[17] The two borders of the epidermis cannot be resolved separately (just one white band of 0.23 mm mean thickness appears in the image). All US system parameters were adequately set to obtain optimal image resolution and contrast (gain, time gain compensation, foci, frequency, 18 MHz probe was used in harmonic mode).

In obese persons, SAT layers are several centimetres thick. There, accuracy is limited by incorrect sound speed rather than by US resolution. The *relative* distance measurement error would not increase with thickness; when sound speed is set correctly, it can even be smaller than in thin layers. For instance, when measuring a 100 mm thick SAT layer, it does not matter whether the borders are detected with a resolution of 0.1 or 0.3 mm (corresponding to 0.1% or 0.3% error, respectively). Therefore, it is also possible to obtain accurate thickness measurements in obese persons where lower US frequencies have to be used. The percentage deviation from the actual sound speed equals the relative distance measurement error because the technical border resolution error can be neglected in such thick layers.

Ad Part B of the (printed) main publication:
Selection and standardisation of ultrasound sites

Some of the new US sites are still in the vicinity of ISAK sites (DT, UA, LA, EO, FT, and MC) but with a much easier marking protocol, whereas some sites were added in order to receive SAT thickness information from body segments that were not included in the ISAK selection: ES for the trunk, LT for the fat depot at the lateral side of the thigh, and BR to represent the lower arm. The ISAK triceps site has been replaced by the distal triceps (DT) at which SAT thickness is more consistent than at the ISAK triceps site (where the curvature of the triceps muscle influences the SAT thickness). In order to sample SAT of the lower arm, the new site brachioradialis (BR) was introduced (instead of the ISAK biceps site, where SAT thickness is very thin in most athletes). Front thigh (FT) and medial calf (MC) remained close to the ISAK sites, but marking precision is much better with the new protocol.

The ISAK trunk sites caused major US imaging problems.[14 15] Subscapular was eliminated from the set of trunk sites because of the complex underlying anatomical structures and because of large SAT thickness gradients within the image. This site is replaced by the erector spinae (ES), where identification of SAT was always clear and thickness gradients were very small. The ISAK sites of abdominal, iliac crest, and supraspinale were also replaced for the same reasons. Three sites on the anterior side of the trunk were used in the inter-observer study described in part C: upper abdomen (UA), lower abdomen (LA), and external oblique (EO). For marking EO, the *anterior superior iliac spine (ASIS)* needs to be marked beforehand: this landmark is at the lower edge where the bone can just be felt. Lifting the right heel assists palpation of the most anterior point of the bone where the sartorius muscle originates (this can be traced when rotating the femur outward). For marking the EO site, it is important to find the ASIS point accurately. The ASIS point can also be used to measure the leg length (l). Most recently performed measurements in heavy weight athletes and in obese persons (manuscript in preparation) have shown that lateral thigh (LT) should be preferred to the external oblique (EO) site. In this group, marking EO was sometimes associated with difficulties because the bony landmarks were hard to identify and in obese persons folds of fat and skin prevented precise marking. Therefore, LT is suggested as one of the eight core sites, in place of EO. From this site, interesting information concerning fat patterning in different somatotypes and between men and women can be expected.[26 27 29]

When using sites in the abdomen region, the measurer has to be aware that an intermediate fascia (Camper's fascia) may be embedded in the SAT layer. In case of doubt about the lower border of the subcutaneous tissue, the US probe can first be put with some pressure on the skin. This enables the operator to distinguish visually between fat and muscle, since the fat is highly compressible and the border to the fascia of the clearly identifiable muscle becomes evident. After this pre-test, the US measurement should be resumed at this site with a new 5 mm layer of gel between the probe and the skin. It is of paramount importance to identify the muscle and its fascia without doubt, otherwise severe measurement mistakes will result.[14 15]

Ad Part C of the (printed) main publication: Inter-observer reliability

Statistics

The Kolmogorov-Smirnov test was used to test for normal distribution. Box-plots are used to visualise the distribution of each observer's measurement differences from their mean when absolute values of deviations are of interest (figures 6A and 6B), and when data are not normally distributed (figures 10A-D). Statistical analyses included the determination of standard errors of estimate (SEE), and linear regressions including coefficients of determination (R^2) and significances. Inter-observer correlations were determined by calculating Spearman’s rank correlation coefficients (ρ), and intraclass correlation coefficients (ICC).

Participants and observers

All participants (or their parents) completed a written consent form and had the opportunity to discuss methods and aims of the study with the investigators. Ethical approval was given by the Ethics Commission of the Medical University of Graz (20-295ex08/09). Data of participants are shown in table 3. The three observers performed the measurements in accordance with the site marking description and the US measuring and image evaluation technique as described in parts A and B of this publication. Two observers had performed US SAT measurements on more than 50 participants previously, while the third observer had undertaken a 2-day training course and had participated in a comparative test with five test persons.

Table 3: Participants

SAT patterning was measured by three observers in 12 national level athletes (females: 5 gymnasts, 1 swimmer; males: 4 gymnasts, 2 swimmers); age: 19.5 (±4.3) years, h = 1.70 (± 0.12) m, s = 0.90(± 0.06) m, m = 64.1 (±15.0) kg, BMI = 21.8 (± 2.4) kgm^{-2}, MI_1 = 21.8 (±2.3) kgm^{-2}.[3,4,12,31]

PARTICIPANT	age [y]	*sex*	***h*** **[m]**	***s*** **[m]**	***m*** **[kg]**	BMI	MI_1
1	18	m	1.698	0.862	60.0	20.8	21.7
2	19	m	1.664	0.909	61.7	22.3	21.6
3	15	f	1.540	0.812	43.1	18.2	18.3
4	19	m	1.895	0.999	84.8	23.6	23.7
5	22	m	1.773	0.918	66.3	21.1	21.6
6	26	m	1.733	0.958	73.2	24.4	23.4
7	16	f	1.562	0.836	46.7	19.1	19.0
8	20	f	1.734	0.934	61.3	20.4	20.1
9	17	f	1.635	0.869	56.0	21.0	20.9
10	18	f	1.704	0.894	59.6	20.5	20.7
11	29	m	1.908	1.009	97.0	26.6	26.7
12	15	f	1.580	0.847	59.4	23.8	23.5

Anthropometric data include: body mass *(m),* body height *(h),* sitting height *(s)* - measured in fully stretched position (compare to figure 1C), body mass index (BMI=m/h^2), and the mass index (MI_1). The MI_1 considers individual sitting height: $MI_1 = 0.53\ m/(hs)$; for persons with an average relative sitting height (i.e. a Cormic index of C=*s/h=0.53*), the BMI equals the MI, for persons with long legs, the MI is higher than the BMI.[3 4 12 31] MI_1 values differed less than 1 kgm^{-2} in this group of athletes. Leg length is measured in standing position from the floor to the anterior superior spine (ASIS; see, appendix, part B). In the standardised SAT measurement protocol, athletes are additionally characterised by their sex, ethnicity, sport, discipline, performance level, training volume per week, and years of training.

Reasons for suggesting the site lateral thigh instead of external oblique:

The lateral thigh (LT) site avoids marking problems that can occur at EO, particularly for heavy athletes and in participants with thick SAT layers. LT should, therefore, replace EO in future investigations. EO was used in this study because it is situated close to the ISAK site *supraspinale*. However, marking of EO necessitates the accurate identification of two anatomical bony landmarks, whereas all other sites do not. This may introduce a reduced precision, particularly for heavy athletes and obese persons. Preliminary results (this research is in progress) obtained with overweight persons indicate that thick layers of SAT do not cause noticeable measurement problems, except for the EO site. It is also useful to replace this site for athletes as the SAT is extremely thin in most cases (the mean contribution of EO to the average SAT thickness sum of the 12 athletes was only 6%). The low SAT thicknesses at EO (mean d_{INCL} in the 12 athletes was 1.8 mm, mean d_{EXCL} was 1.5 mm) can result in comparatively large relative errors δ/d. The athletes in tables 2 and 4 are ordered according to their sum of SAT thicknesses (D_{INCL}); this order does not change when the EO values are not considered. For these reasons, and because interesting information concerning fat patterning in different somatotypes and between men and women can be expected,[27 29] EO should be replaced by LT in the core set of sites.

Additional results

Sums of SAT thicknesses D_{EXCL} measured by three observers:

Figure 8 shows the results of thickness sums when fibrous structures were not included in the thickness measurements (according data can be found in table 4).

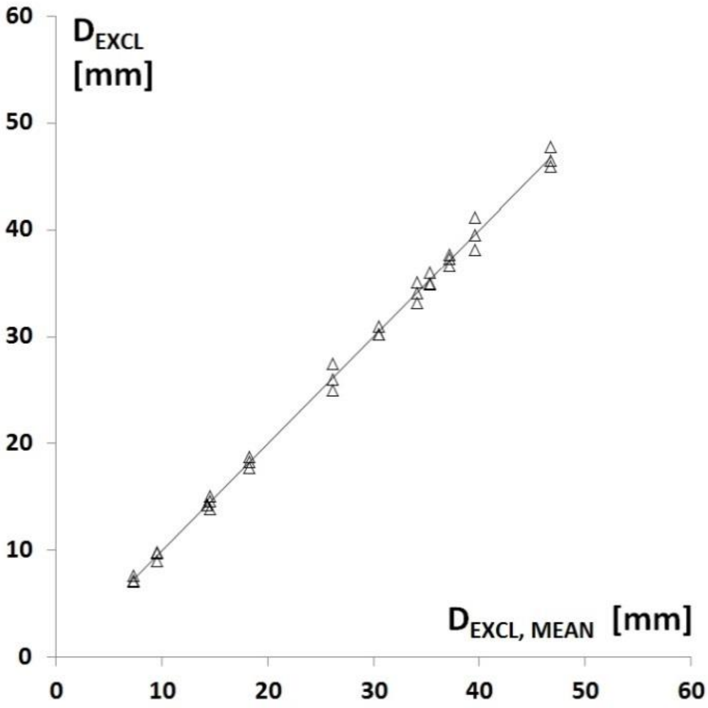

Figure 8: Sums of thicknesses from eight sites measured by the three observers in 12 athletes. The sums D_{EXCL} of the individual observers (in a given participant) are displayed over the mean value of the three observers. Statistics for sums of SAT thicknesses with the fibrous structures excluded (D_{EXCL}): $R^2 = 0.997$ ($p<0.01$), SEE = 0.66 mm, ICC = 0.996 (lower 95% confidence limit: 0.990; upper 95% confidence limit: 0.999).

Table 4: SAT thickness sums with fibrous structures excluded (D_{EXCL}), means of sums ($D_{EXCL,MEAN}$), , and differences (Δ_{EXCL}=D_{EXCL}- $D_{EXCL,MEAN}$) (see figures 8 and 9B).

		D_{EXCL}			Δ_{EXCL}		
PARTICIPANT	**$D_{EXCL, MEAN}$**	**OBS1**	**OBS2**	**OBS3**	**OBS1**	**OBS2**	**OBS3**
1	7.31	7.21	7.60	7.11	-0.10	0.29	-0.20
2	9.56	9.83	9.06	9.80	0.27	-0.50	0.24
3	14.27	14.28	14.30	14.24	0.01	0.03	-0.03
4	14.55	13.93	14.61	15.11	-0.62	0.06	0.56
5	18.22	17.70	18.72	18.25	-0.52	0.50	0.03
6	26.17	26.03	25.04	27.45	-0.14	-1.13	1.28
7	30.48	30.27	30.95	30.23	-0.21	0.47	-0.25
8	34.13	34.13	35.12	33.14	0.00	0.99	-0.99
9	35.33	34.90	36.03	35.05	-0.43	0.70	-0.28
10	37.19	37.29	36.63	37.66	0.10	-0.56	0.47
11	39.61	41.19	38.11	39.53	1.58	-1.50	-0.08
12	46.72	47.74	46.51	45.90	1.02	-0.21	-0.82

Observer differences from the mean:

Figures 9A and B show the differences of the individual observer's sums from their mean. Figure 9C and D show the relative observer differences in percent of $D_{INCL,MEAN}$ and $D_{EXCL,MEAN}$.

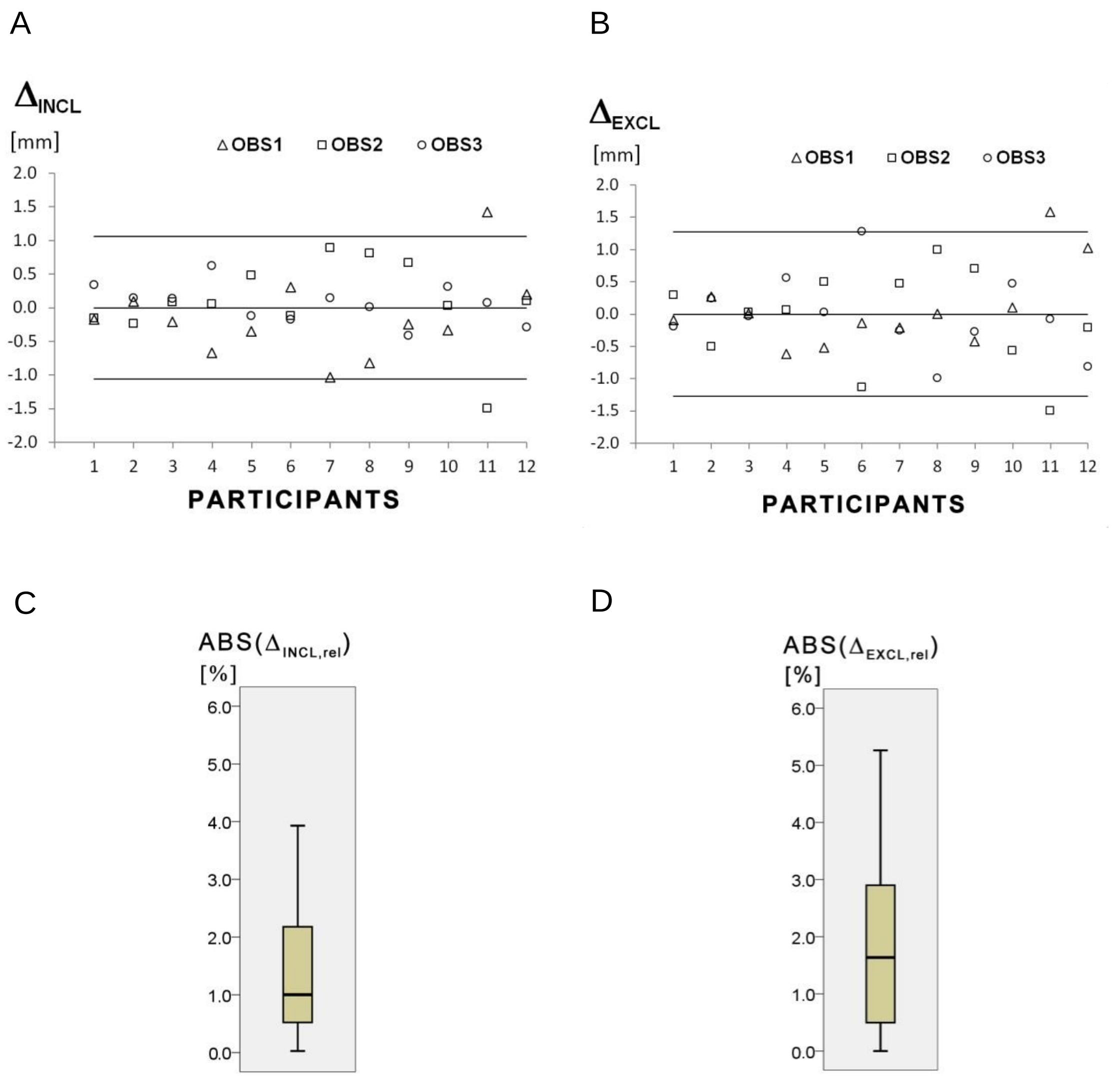

Figure 9: Observer differences from the mean.
Three observer measurements of the sums from the eight sites in each of the 12 athletes (N=36).
A.: The differences of the individual observer's sums from the means $\Delta_{INCL} = D_{INCL} - D_{INCL,MEAN}$ is shown for all 12 athletes (compare to table 2). The 12 athletes are ordered according to increasing values of D_{INCL}. SD is 0.54 mm. 95% of values are between upper and lower horizontal lines (±1.96·SD). Data is normally distributed.
B.: The differences of the individual observer's sums from the means of the three observers. $\Delta_{EXCL} = D_{EXCL} - D_{EXCL,MEAN}$ is shown for all 12 athletes (compare to table 4). SD is 0.65 mm. 95% of values are between upper and lower horizontal lines (±1.96·SD). Data is normally distributed.
C.: Relative observer deviations in percent of $D_{INCL,MEAN}$: $\Delta_{INCL,rel}=100 \cdot ABS(\Delta_{INCL})/D_{INCL,MEAN}$. Median: 1.0%; maximum: 3.9%; $D_{INCL,MEAN}$ ranged in the group of athletes from 10.2 mm to 51.2 mm.
D.: Relative observer deviation in percent of $D_{EXCL,MEAN}$: $\Delta_{EXCL,rel}=100 \cdot ABS(\Delta_{EXCL})/D_{EXCL,MEAN}$. Median was 1.6%; maximum: 5.3%; $D_{EXCL,MEAN}$ ranged from 7.3 mm to 46.7 mm.

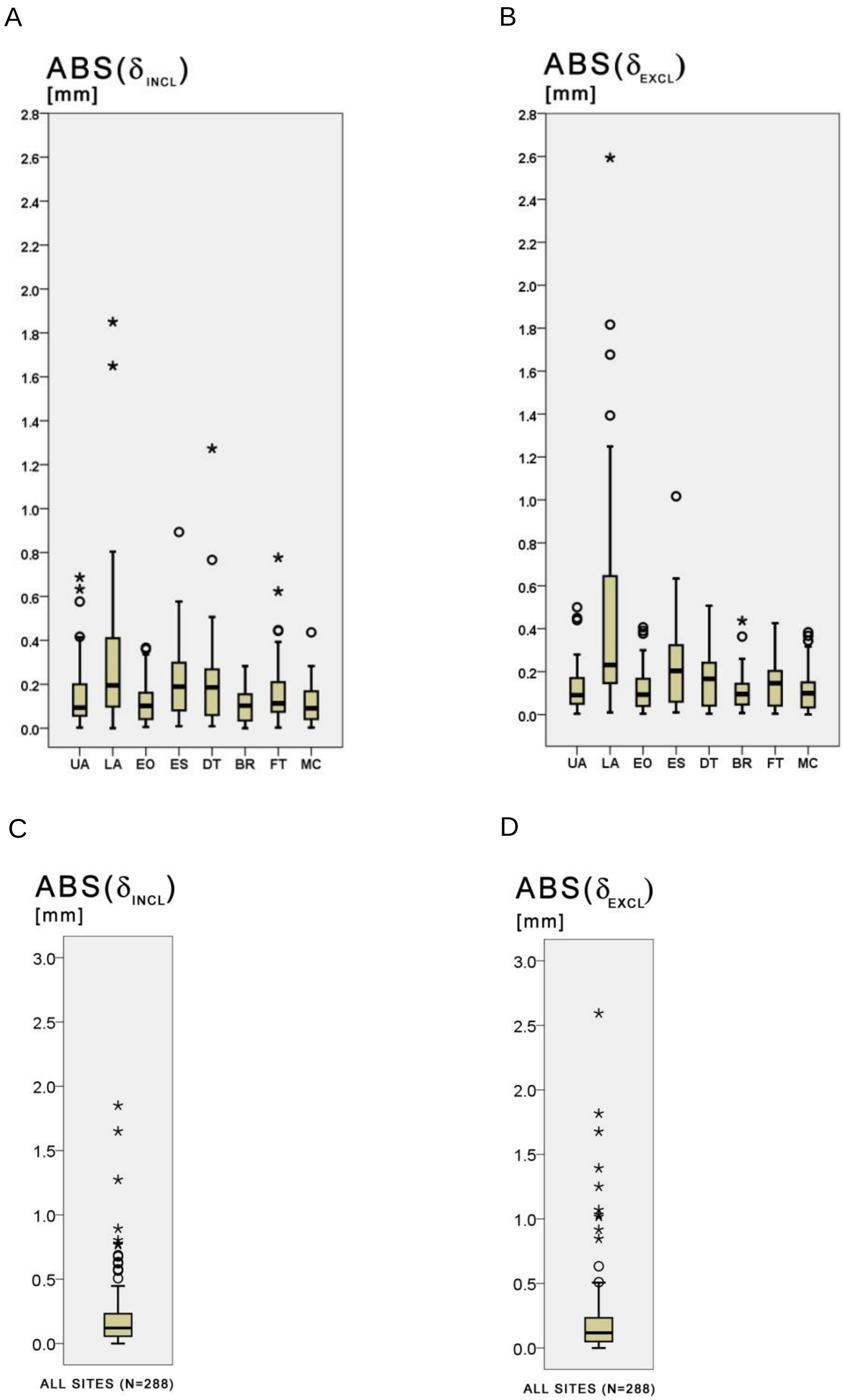

Figure 10: Observer differences from the mean at the individual eight sites.
Absolute values ABS(δ) are used. Number of comparisons at each of the eight sites: N=36 (twelve athletes were measured by three observers).
A: Absolute thickness value differences from the mean ABS(δ_{INCL}) for each of the eight sites. For characteristic box plot values see table 5A.
B: ABS(δ_{EXCL}), analogously to A (compare to table 5B).
C: Absolute thickness value differences from the mean ABS(δ_{INCL}) for all eight sites together. Median is 0.12 mm; 95% of values are below 0.58 mm.
D: Analogously to C, here for ABS(δ_{EXCL}). Median is 0.12 mm; 95% of values are below 0.50 mm.

Figures 10A and B show the absolute values (ABS) of observer deviations at each of the eight sites. The deviation δ is the individual observer value *d* minus the mean of the three observer values d_{mean} at the given site. The number of comparisons of ABS(δ) in each of the eight box plots was 36 (12 athletes were investigated by three observers). ABS(δINCL) refers to SAT thickness values with the fibrous structures included, and ABS(δEXCL) refers to SAT thickness values without fibrous structures. Tables 5A and B present the statistical characteristics related to the figures 10A and 10B. The deviations δ were small in most cases: Medians ranged between 0.09 mm and 0.23 mm, 95% percent of all ABS(δINCL) values (N=288) were below 0.58 mm, and 95% of ABS(δEXCL) values were below 0.50 mm (compare to figures 10C and 10D).

The deviations relative to the mean thicknesses $\delta_{INCL,rel}=100\cdot ABS(\delta_{INCL})/d_{INCL,MEAN}$, and $\delta_{EXCL,rel}=100\cdot ABS(\delta_{EXCL})/d_{EXCL,MEAN}$ can become large at sites where the biologically given thickness variation (in the close surrounding of the centre of the site) is comparable to the mean thickness in this area. This holds particularly true for very thin and inconsistent SAT layers. Large percentage deviations can also occur when, in very lean persons, the SAT thickness reaches the lower limit of the resolution of US (0.1 to 0.3 mm, depending on probe frequency). But such extremely thin fat layers at individual sites do not contribute substantially to the sum of SAT thicknesses.

Table 5: Characteristic values of box plots in figures 10A and 10B:
Absolute values of deviations from the mean at given sites are used.

A: Measurement deviations (fibrous structures included)	**ABS(δ_{INCL}) [mm]**							
	UA	**LA**	**EO**	**ES**	**DT**	**BR**	**FT**	**MC**
MEDIAN	0.09	0.20	0.10	0.19	0.19	0.10	0.11	0.09
INTERQUARTILE RANGE	0.15	0.32	0.12	0.22	0.21	0.13	0.15	0.13
MAXIMUM	0.69	1.85	0.37	0.89	1.27	0.28	0.78	0.44

B: Measurement deviations (fibrous structures excluded)	**ABS(δ_{EXCL}) [mm]**							
	UA	**LA**	**EO**	**ES**	**DT**	**BR**	**FT**	**MC**
MEDIAN	0.09	0.23	0.09	0.20	0.17	0.10	0.15	0.10
INTERQUARTILE RANGE	0.13	0.60	0.13	0.27	0.20	0.10	0.17	0.12
MAXIMUM	0.50	2.60	0.41	1.02	0.51	0.44	0.43	0.38

Inter-observer correlation matrix:

In table 6, inter-observer Spearman's rank correlation coefficients (ρ) are shown. Correlations at each individual site (three observers investigated 12 athletes, N=36), and for all eight sites summarised as well (N=288). At individual sites, ρ values (values above the diagonal in table 6 correspond to d_{INCL}) ranged from 0.88 to 0.99, and for all sites all three ρ values were 0.98. The ρ values below the diagonal refer to the measurements of d_{EXCL}: correlation coefficients ranged from 0.91 to 0.99 at individual sites, and from 0.98 to 0.99 for all sites together.

Table 6: Inter-observer correlation matrices
Inter-observer Spearman's rank correlation coefficients (ρ) of observers OB1, OB2, and OB3 are presented for each individual site (N=36) and for all sites summarised as well (N=288). **Bold numbers** above the main diagonals in the matrices represent the ρ values corresponding to SAT thicknesses d_{INCL}, and numbers below the diagonal refer to d_{EXCL}.

UPPER ABDOMEN				**LOWER ABDOMEN**				**EXTERNAL OBLIQUE**			
	OB 1	OB 2	OB 3		OB 1	OB 2	OB 3		OB 1	OB 2	OB 3
OB 1	1.00	**0.97**	**0.98**	OB 1	1.00	**0.97**	**0.99**	OB 1	1.00	**0.97**	**0.96**
OB 2	0.97	1.00	**0.97**	OB 2	0.98	1.00	**0.96**	OB 2	0.96	1.00	**0.97**
OB 3	0.97	0.97	1.00	OB 3	0.99	0.96	1.00	OB 3	0.96	0.99	1.00
ERECTOR SPINAE				**DISTAL TRICEPS**				**BRACHIORADIALIS**			
	OB 1	OB 2	OB 3		OB 1	OB 2	OB 3		OB 1	OB 2	OB 3
OB 1	1.00	**0.94**	**0.88**	OBS 1	1.00	**0.99**	**0.98**	OB 1	1.00	**0.97**	**0.97**
OB 2	0.96	1.00	**0.91**	OBS 2	1.00	1.00	**0.97**	OB 2	0.92	1.00	**0.95**
OB 3	0.92	0.92	1.00	OBS 3	0.99	0.99	1.00	OB 3	0.99	0.91	1.00
FRONT THIGH				**MEDIAL CALF**				**ALL**			
	OB 1	OB 2	OB 3		OB 1	OB 2	OB 3		OB 1	OB 2	OB 3
OB 1	1.00	**0.97**	**0.97**	OB 1	1.00	**0.94**	**0.95**	OB 1	1.00	**0.98**	**0.98**
OB 2	0.98	1.00	**0.99**	OB 2	0.96	1.00	**0.94**	OB 2	0.98	1.00	**0.98**
OB 3	0.97	0.97	1.00	OB 3	0.96	1.00	1.00	OB 3	0.99	0.98	1.00

Comparison of this inter-observer study (using the new set of sites) to a previous study (where ISAK sites were used):

Figures 5 and 8 show the sums of thicknesses D measured by the three observers, and differences Δ of the sums (to the mean of the observers' D_{MEAN}) are given in tables 2 and 4 and in figures 6A-B and 9A-D. Most (95%) of the 36 sums D_{INCL} obtained by the three observers in the 12 athletes deviated less than 1.0 mm (figure 9A), and for D_{EXCL}, 95% of differences were less than 1.3 mm (figure 9B). The medians (of the absolute values of deviations) were: ABS(Δ): 0.24 mm (1.0% of $D_{INCL,MEAN}$) and 0.46 mm (1.6% of $D_{EXCL,MEAN}$), respectively (compare to figures 6A and 6B, and 9C and 9D). Only two outliers occurred (1.50 mm and 1.43 mm) which resulted from an erroneous US image of one of the observers at the abdomen site: the Camper's fascia had erroneously been identified as the lower border of SAT, instead of the muscle fascia.

The reduction of outliers is substantial when compared to the results described in a previous study.[15] In this previous study, which used the ISAK sites, only 89% of images could be evaluated (because 51 images out of a total of 456 were unclear): this was partly because the anatomical structures underneath the ISAK sites were complex and difficult to interpret, and partly because the US SAT measurement technique and training program have been further refined. The detailed methodical description and the definition of the new US sites are given in part B of the main text and in parts A and B of the appendix. In the previous inter-observer study,[15] 19 grossly incorrect measurements occurred; nine errors because images were not sufficiently clear (in addition to the 51 images that were excluded ex ante), and 10 due to misinterpretations of Camper's fascia. In the study presented here, all images (288) could be evaluated and there was only one erroneous measurement.

In both inter-observer comparison studies, precision tended to be better when fibrous structures were included in the distance measurements. This can be explained because for d_{EXCL} measurements several borders of SAT have to be determined by means of the interactive algorithm, whereas for determination of d_{INCL}, determination of just the upper and the lower border of SAT are sufficient. Validation studies will show whether d_{INCL} or d_{EXCL} has higher predictive value, and such studies will also clear whether or not a subset of the eight sites defined for patterning studies will be sufficient for total body fat assessment.