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Abstract

Background: Almost nothing is known about the medical aspects of runners doing a transcontinental ultramarathon over several weeks. The results of differentiated measurements of changes in body composition during the Transeurope Footrace 2009 using a mobile whole body magnetic resonance (MR) imager are presented and the proposed influence of visceral and somatic adipose and lean tissue distribution on performance tested.

Methods: 22 participants were randomly selected for the repeated MR measurements (intervals: 800 km) with a 1.5 Tesla MR scanner mounted on a mobile unit during the 64-stage 4,486 km ultramarathon. A standardized and validated MRI protocol was used: T1 weighted turbo spin echo sequence, echo time 12 ms, repetition time 490 ms, slice thickness 10 mm, slice distance 10 mm (breath holding examinations). For topographic tissue segmentation and mapping a modified fuzzy c-means algorithm was used. A semi-automatic post-processing of whole body MRI data sets allows reliable analysis of the following body tissue compartments: Total body volume (TV), total somatic volume (TSV) and total visceral volume (TVV), total adipose (TAT) and total lean tissue (TLT), somatic (SLT) and visceral lean tissue (VLT), somatic (SAT) and visceral adipose tissue (VAT) and somatic adipose soft tissue (SAST). Specific volume changes were tested on significance. Tests on difference and relationship regarding prerace and race performance and non-finishing were done using statistical software SPSS.

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Background

With the worldwide growing number of people running, endurance sports have experienced differentiation into multiple (sub-) disciplines in the last decades. Concerning distance running, the ultramarathon (UM) seems to be the greatest challenge in endurance running. The German Ultramarathon Association (DUV) defines footraces of 50 km or longer as UM. However, as in every field of human physical activities, some people try to push themselves to the limits and beyond. For these ultra-athletes a multistage ultramarathon (MSUM) is the ultimate test of endurance performance. Sometimes, the worldwide small group of ultra-endurance runners meet with each other trying to achieve the impossible: finishing a multistage transcontinental footrace over thousands of kilometers. These most extreme multistage endurance competitions in the world take the runner to a different level, where nutrition, sleep, energy and psychological states have to be carefully managed. Besides a few case reports, almost nothing has been reported about the medical aspects of runners doing a transcontinental extended MSUM over several weeks [1].

Until now, there have been no series published regarding UM running over more than 1,500 km. However, prolonged ultra-endurance footraces offer the best opportunity to study physical adaptations and the relationships of the physiological parameters in endurance athletes.

The Transeurope Footrace Project (TEFR-project) [2] is the first observational cohort field study of a transcontinental MSUM, the Transeurope Footrace 2009 (TEFR09) [3]. A unique group of 67 endurance runners (mean age 50.7 years, standard deviation (SD) 10.5 years, range 26 to 74 years, m 56 (83.6%)) met the challenge and tried to cross six countries while running 4,486 km in 64 stages (mean 70.1 km, min 44 km, max 95.1 km) without any day of rest [4]. The central aspect of the TEFR-project was the use of a mobile magnetic resonance imaging (MRI) scanner accompanying the TEFR09 participants on a truck trailer over 64 days under their ‘natural’ conditions [2].

One focus of this presentation is on the descriptive presentation of characteristics and changes in body composition during TEFR09 in a new way, differentiating between somatic and visceral and segmental volumes of defined fat and lean tissue compartments measured by continuous mobile whole body MRI. In addition, possible associations of body volume composition and prerace and race performance were analyzed to test the following hypotheses: it is hypothesized, that prerace endurance running performance is related to specific body fat and lean tissue composition in ultra-athletes. Secondly, it is supposed, that although the running distance of a transcontinental UM cannot be trained for concerning the running volume (km), participants need specific prerace performance skills and fat and lean tissue volume distribution, if they want to finish such a race. Due to the expected huge energy burden a transcontinental footrace without any day of rest...
implies, another assumption is that it is mandatory for every participant to lose body mass and total body volume (TV) due to massive adipose tissue decrease and more or less lean tissue catabolism. At least, with the continuous differentiated measurement of body tissue compartments throughout the entire TEF09, it should be shown indirectly, that although the participants are preselected in regard to their ultra endurance running expertise, they will develop further economical adaptations as the 4,500 km race progresses.

**Methods**

**Subjects**

Every TEF09 participant was asked to join the TEF09-project, which was approved by the local ethics committee of the University Hospital of Ulm (UHU, No.: 270/08-UBB/se) in accordance with the Declaration of Helsinki, regarding the study design, risk management plan and individual protocols [2]. Forty-four participants (67%) were recruited for the study and gave their informed written consent. Every second subject (n = 22, 20 men, mean age 49.1 years, SD 11.5 yrs., range 27 to 69 years) was randomly selected for whole body MRI measurements regarding body composition. According to project protocol these subjects underwent a whole body MRI before the start at Bari (South Italy) and during the race in measurement intervals of approximately 800 km. Due to various reasons, deviations from planned measurement intervals (MI: t0 to t6) occurred. The mean deviation of actual from planned measurement intervals was 187.8 km (SD = 141.3 km) [2].

**Prerace performance**

Before the start of TEF09 all subjects filled out specific questionnaires concerning their prerace experience in endurance running. This history includes the years of regular endurance running (PRY), the number of finished (nF) marathons (M), UM and MSUM, and the prerace records (PRR) for marathon and specific UM (50 km, 100 km, 6 hours, 12 hours, 24 hours) races within the last decade before TEF09. It also includes the extent of prerace training (PRT) 16 months before TEF09: training volume (Vol: km/week), training duration (Time: hours/week) and the training intensity (Int: km/hour). The self-disclosures about nF and PRR were cross checked with the archive of the DUV and discrepancies were clarified. However, for PRT and PRY we had to rely solely on the self-disclosures; these could not be compared with any official lists.

**Body composition analysis**

Different techniques for quantification of body fat are described and more or less commonly used in the literature: *In vivo*, two-compartment model methods are the hydrodensitometry [5] and the body fat percentage and muscle mass calculation from anthropometric data such as skinfold thickness (SF) calipimetry and/or segmental body circumferences (CF) [6-8]. Three-compartment methods are the bioelectrical impedance analysis (BIA) [9] and dual-energy X-ray absorptiometry (DEXA) [10]. Using these methods, indirect measurement, approximated calculation or simple estimation of total, regional or local adipose or lean tissue [11-14] is possible. In contrast, a whole body MRI assessment of adipose tissue as a multi-compartment method is the only method enabling exact topographic tissue mapping and tissue segmentation. Therefore, it is the gold standard imaging tool for differentiated assessment of adipose or lean tissue distribution in the body [15-18].

Subjects who finished TEF09 had whole body MRI measurements a 1.5 Tesla MRI scanner (Magnetom Avanto™, Siemens Ltd., Erlangen, Germany) mounted on a mobile unit (MRI-Trailer, SMIT Mobile Equipment B.V., Great Britain) was used. The total 45 tonnes of equipment (MRI-trailer, truck tractor, external 105KVA diesel-generator, and material van) was built up and taken down daily at each stopover of TEF09, requiring daily checks and support of all technical systems [2].

Several MRI techniques have been described for the measurement and quantification of body fat composition: T1-weighted imaging by spin-echo or gradient-echo techniques [14,16,19], chemical shift selective (CHESS) imaging [20-22], or DIXON techniques [23,24]. All of them have specific advantages and disadvantages, the details of which are beyond the scope of this article. For analysis of body composition a standardized assessment of whole body adipose tissue measurement based on a MRI protocol according to Machann et al. [25] was used. A two-dimensional T1-weighted turbo spin echo sequence with an echo train length of seven was applied (Siemens Ltd.). Measurement parameters were set to be: flip angle 180°, echo time 12 ms, repetition time 490 ms, slice thickness 10 mm, slice distance 10 mm, 5 slices per sequence, field of view 1,991 cm², matrix size 256 × 196 was recorded in a measuring time of 12 seconds (allowing breath holding examinations in the trunk area), bandwidth 120 Hz/pixel. A total of 90 to 120 images were generated, depending on the size of the
subject. Total examination time was between 20 and 25 minutes including one rearrangement of prone positioned subject (head forward and arms extended for upper body, feet forward for lower body), as total table feed of the MR-imager is limited to 110 cm. In order to guarantee identical slice positions after reposi-
tioning, the subjects were marked at the iliac crest. A body coil was used.

Image post-processing
For topographic tissue segmentation and mapping of the athletes body a fuzzy c-means algorithm according to Würslin et al. [26] was used. This approach provides a simple and time-saving strategy for assessment and standardization of individual adipose tissue distribution in the entire body. Due to standardization using defined internal markers, it enables a completely automatic, reliable analysis and creation of adipose tissue distribution profiles of the whole body from the multislice MR datasets and makes a reliable comparison of subjects with different body structure possible [25,26].

The signal of intestinal content with a short T1 can be interpreted as visceral adipose tissue (VAT) in the absence of intraluminal gastroenteric nutrition fat (INF). If the scanned subject is in a non-fasting condition, the visceral T1 signal occurs from both, VAT and INF. Reliability (mean absolute deviation of three repeated measurements) is noted with 3.08% for total volume (TV), 1.48% for total adipose tissue (TAT) and 1.13% for visceral adipose tissue (VAT) [26].

Due to their immense mental and physical stress caused by the daily ultra-endurance burden, the runners’ biggest fear was losing too much energy over the course of TEFR09. Their primary effort after stage finishing was to get as much nutrition and calories as possible before falling asleep. Therefore, it was not always possible to ensure fasting conditions of the subjects for mobile MRI measurements. Some subjects were motivated enough to do the MR examination directly after the daily stage before eating in a fasting but exhausted condition, so they sometimes were not able to lie absolutely still on the MR table and follow the breath commands exactly. These specific circumstances resulted in the image post-processing analysis being less automated than that mentioned by Machann and Würslin [25,26]; Movement artifacts had to be cleared manually more often before automatic post-processing. Compared to normal or overweight patients, in thin and lean bodies the amount of adipose bone marrow (ABM) and INF is more relevant in relation to whole body adipose and lean tissue. At the start of TEFR09, ABM and INF together accounted for 13.2% of total adipose tissue. Due to the continuous loss of adipose body tissue, this ratio rises up to 28.2% until the end of the race. For visceral adipose tissue, INF rose from 3% at the start up to 65.4% at the end of TEFR09. Therefore, a manual separation of ABM (Figure 1) and INF (Figure 2) was done on all MR slices of the subjects. Looking at the mean differences, Würslin et al. [26] calculated between manual tissue segmentation and their automatic procedure (2.07% for TV, 8.13% for TAT, 3.21% for VAT), the described additional manual corrections regarding the small volumes of ABM and INF are appropriate.

After these procedures a specific and extensive topographic mapping and segmentation of body tissue was possible (Table 1). Total volume (TV) can be subdivided into total somatic volume (TSV) and total visceral volume (TVV, Figure 2) or can be subdivided into total adipose tissue (TAT; without INF) and total lean tissue (TLT). TLT can be separated into somatic (SLT) and visceral lean tissue (VLT). Subtraction of ABM from TAT leads to total adipose soft tissue (TAST). TAST can be subdivided into VAT and somatic adipose soft tissue (SAST). Therefore, somatic adipose tissue (SAT, Figure 1) is the same as SAST plus ABM or TAT minus VAT, respectively. VAT can be subdivided into intraabdominal (retro- and intraperitoneal) adipose tissue (IAAT) and intrathoracic, mainly mediastinal adipose tissue (MAT).

Body segmentation was done into upper extremities (UE), trunk (TR) and lower extremities (LE). TV, lean tissue (LT) and SAST volume was calculated for the upper and lower extremities (UE and LE) and for the trunk (TR). For nomenclature of specific segmented tissues see Table 1.

Statistical analysis
For data elaboration specific software was used: Microsoft™ Office Excel™ (Release 12.0.6665.5003, Microsoft Home and Student Suite, 2007, Microsoft Inc.) for data documentation, SPSS (IBM™ SPSS™ Statistics, Release 19.0.0, 2010, SPSS Inc.) for statistical analysis and SigmaPlot for Windows Version 11.0 (Release 11.2.0.5, 2008, Systat Software Inc.) for graphical data presentation.

The measured volumes of tissue compartments are presented as percentage volumes (vol%) and as absolute [1] and relative differences (%) to start. For every measurement interval (t0 to t5) the dispersion measures are presented graphically in box plot figures (median, 25th/75th percentile, 10th/90th percentile and all outliers) for all subjects (finishers and non-finishers) and location measures (mean and standard error, SE) are presented graphically in line figures for finishers only. Calculated total changes (t5 versus t0) of volumes and volume percentages are presented in text as means and standard deviation (SD) with minimum (min) and maximum (max) as appropriate.

Analyses on volume changes during TEFR09
For analysis of the significance regarding volume changes of the specific tissue compartments during TEFR09, a
univariate variance analysis (ANOVA) for repeated measurements was preferred (only subjects who had the whole body MRI at every measurement interval (t₀ to t₅): n = 12). Therefore, a common linear model for repeated measurements (with post hoc analysis on the significance between the different times of measurement) was chosen. For correction of accumulation of the alpha level due to multiple testing (of hypothesis: “The means at stage intervals are significantly different to means at start”), the Bonferroni-
procedure for confidence-interval (CI) adaption was applied. For the univariate ANOVA model, one precondition, the sphericity of data (homogenity between the variance of differences of two measurements) is necessary and was proven by the Mauchly-Test. Because of the small number of subjects, the power of the Mauchly-Test regarding sphericity is low. Therefore, the ‘Greenhouse-Geisser’ (SPSS) correction procedure was used. Looking at result reliability and test power, in cases of severe injury of the sphericity assumption, a multivariate ANOVA test was used. In cases of missing values, the specific dependent variable (specific tissue compartment) was excluded from ANOVA analysis.

Analyses of difference
For dependence analysis including all stages of and total TEFR09, analyses of difference between the dichotomous nominally scaled dependent variables of the sample finishing status (finisher/non-finisher: F/NF) regarding prerace running performance history and regarding total, lean and adipose tissue volumes were carried out. Depending on normal or free distribution of the independent interval scaled variables, the parametric independent t-test (variance homogeneity was calculated with Levene’s test) or nonparametric Mann–Whitney-U-test was used. Due to the higher power in small cohorts, the Shapiro-Wilk [27,28] test (and not the Kolmogorov-Smirnov statistic [29]) was used to check for normal distribution of the independent prerace variables of performance (PRY, PRT, PRR).

Analysis of relationships
For analyses of relationships, the Pearson correlation coefficient (CCp) and Spearman-rho correlation coefficient (CCs) were calculated for parametric and non-parametric parameters, respectively, using bivariate (two-sided) or univariate (one-sided) testing as appropriate:

- BM versus TV and its distribution throughout the race: bivariate CCs
- Prerace performance versus percentage total, lean and adipose volumes: univariate CCs / CCp
- Race performance versus percentage total, lean and adipose volumes: univariate CCp

For interpretation of CC-values the effect size according to Cohen (r = 1: low, r = 3: medium, r = 5: high) was used [30]. For all tests, an alpha level (P-value) of 0.05 was used to indicate significance.

Results
Case presentation
Figure 3 shows the topographical mapping of changes of lean and adipose tissue of a subject (male, 32 years, finisher) with one of the largest decreases of SAST and VAT during TEFR09. Runners often had discomfort or pain after stage finishing, so the investigators tried to adapt body positioning in the MR scanner to the athletes’ current problems to make it as comfortable as possible for them. Therefore, a reliable and strictly standardized lying
position on the MRI table was not possible at each time of measurement. Sometimes, knees or elbows were positioned more or less straightened. This explains the sometimes visible but small topographical phase shifting between different times of measurement in Figure 3.

**Total body volume versus body mass/body mass index**

The absolute volumes of all investigated body tissue compartments and segments are shown in Table 2. Over all subjects, mean loss of BM and BMI at the end of the race was 5.23 kg (SD 3.72 kg) and 1.49 kg/m$^2$ (SD 1.18 kg/m$^2$), respectively (Tables 3 and 4). There was a high correlation (CC$_S$: 0.978, P <0.001) between BM (kg) and TV [1] regarding mean absolute value changes throughout the race (Figure 4).

**Percentage body composition**

At the start of the TEFRO9, the mean percentage volume of TSV was 84.8 (SD 1.36 vol.%). TSV could be differentiated into mean SLT 65.0 vol% (SD 5.33 vol%), mean ABM 3.2 vol% (SD 0.89 vol%) and mean SAST 16.6 vol% (SD 5.58 vol%). The mean TVV of 15.2 vol% (SD 1.36 vol%) is consistent and divides into mean VLT 12.3 vol% (SD 1.23 vol%) and mean VAT 2.9 vol% (SD 1.37 vol%). From these data the changes in mean vol% of tissue compartments regarding the overall population of ultra-runners could be calculated for transcontinental MSUM races (Figure 5).

**Total volumes**

Percentage volume changes of TSV and TVV were not significant (Table 3, Figure 6). For absolute volumes (TV,
TSV, TVV), however, a significant change could be evaluated with a very high test power (Table 5). Except for TSV at the first measurement interval, significant decreases for TV, TSV and TVV could be shown at all MIs throughout TEFR09 (Figure 7). Paired comparison of MI after the start showed no significant difference for TVV but partial differences for TV and TSV (Figure 7).

Total volume
After more than 4,000 km of running the mean TV showed a mean decrease of 9.5% (SD 5.1%, min −2.7%, max −17.9%) compared to the start. Depending on the total sample the mean TV decrease for the overall population of ultra-runners ranges between 8% to 11% (SE 1.5%), (Figure 8). Looking only at the group of finishers, the absolute amount of mean TV loss at the last MI was 6.1 L (SD 3.4 L, min−2.5 L, max −12.6 L (Figure 9). Mean loss of TV per km was 3.5 ml (SD 2.9 ml/km) in the beginning and became smaller, like a reversed parabolic function, during TEFR09 down to 1.5 ml/km (SD 0.8 ml/km) at the end of the race (Figure 10).

Total somatic volume
The relative decrease of TSV during TEFR09 showed a nearly similar curve as TV (Figure 8), but was less pronounced (mean −9.4% after more than 4,000 km, SD 5.3%, min −2.1%, max −18.1%) with the same SE of 1.5%. For the finisher group the absolute loss of TSV increased to 5.2 L in the mean (SD 3.0 L, min −1.7 L, max −11.1 L) at the end of the race (Figure 9). This is consistent with a mean TSV loss of 1.3 ml/km (SD −0.7 ml/km) at the end of TEFR09, starting with 2.7 ml/km (SD 2.7 ml/km) in the first eight stages of TEFR09 (Figure 10).

Total visceral volume
Compared to TV and TSV, the relative decrease of TVV occurred much faster but ended in a nearly similar

### Table 2 Mean volumes (l) of body compartments and segments (all subjects)

| Abbreviation | t0: start | t1: 317 to 789 km | t2: 1,003 to 1,635 km | t3: 2,516 to 2,738 km | t4: 3,234 to 3,669 km | t5: 4,037 to 4,440 km |
|--------------|-----------|-------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Abbreviation | TV        | TVV               | TSV                   | TLT                   | VLT                   | SLT                   |
| Abbreviation | 57.70     | 56.63             | 54.75                 | 53.39                 | 54.16                 | 52.65                 |
| Abbreviation | 8.74      | 8.34              | 7.93                  | 7.64                  | 7.65                  | 7.58                  |
| Abbreviation | 48.97     | 48.29             | 46.82                 | 45.74                 | 46.51                 | 45.08                 |
| Abbreviation | 44.34     | 44.61             | 45.07                 | 45.38                 | 46.20                 | 45.52                 |
| Abbreviation | 7.02      | 7.04              | 7.01                  | 7.10                  | 7.14                  | 7.12                  |
| Abbreviation | 37.32     | 37.56             | 38.46                 | 38.28                 | 39.06                 | 38.40                 |
| Abbreviation | 13.36     | 12.02             | 9.68                  | 8.00                  | 7.96                  | 7.14                  |
| Abbreviation | 11.65     | 10.73             | 8.76                  | 7.46                  | 7.45                  | 6.68                  |
| Abbreviation | 11.52     | 10.19             | 7.66                  | 6.05                  | 6.01                  | 5.19                  |
| Abbreviation | 9.81      | 8.89              | 6.84                  | 5.51                  | 5.50                  | 4.73                  |
| Abbreviation | 1.71      | 1.30              | 0.92                  | 0.54                  | 0.51                  | 0.45                  |
| Abbreviation | 1.55      | 1.15              | 0.77                  | 0.39                  | 0.36                  | 0.33                  |
| Abbreviation | 0.17      | 0.15              | 0.15                  | 0.15                  | 0.14                  | 0.13                  |
| Abbreviation | 0.05      | 0.21              | 0.36                  | 0.57                  | 0.70                  | 0.86                  |
| Abbreviation | 1.98      | 1.93              | 1.92                  | 1.95                  | 1.95                  | 1.95                  |

| Abbreviation | t0: start | t1: 317 to 789 km | t2: 1,003 to 1,635 km | t3: 2,516 to 2,738 km | t4: 3,234 to 3,669 km | t5: 4,037 to 4,440 km |
|--------------|-----------|-------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Abbreviation | ABM       | IAAT              | MAT                   | INF                   | SAST                  | SAST                  |
| Abbreviation | 23.51     | 22.49             | 21.46                 | 21.65                 | 22.02                 | 20.60                 |
| Abbreviation | 28.83     | 28.63             | 27.86                 | 26.57                 | 27.03                 | 27.00                 |
| Abbreviation | 5.79      | 5.89              | 5.75                  | 5.73                  | 5.64                  | 5.60                  |
| Abbreviation | 17.15     | 16.97             | 16.82                 | 17.45                 | 17.83                 | 16.97                 |
| Abbreviation | 23.22     | 23.12             | 23.46                 | 23.02                 | 23.39                 | 23.39                 |
| Abbreviation | 4.79      | 4.62              | 4.70                  | 4.81                  | 4.74                  | 4.77                  |
| Abbreviation | 4.84      | 4.36              | 3.43                  | 2.96                  | 2.96                  | 2.40                  |
| Abbreviation | 4.31      | 3.47              | 2.12                  | 1.02                  | 0.99                  | 0.81                  |
| Abbreviation | 0.98      | 0.95              | 0.72                  | 0.57                  | 0.54                  | 0.48                  |

ABM, adipose bone marrow; IAAT, intraabdominal adipose tissue; INF, intraluminal nutrition fat; LE, lower extremities; MAT, mediastinal adipose tissue; SAST, somatic adipose soft tissue; SAT, somatic adipose tissue; SLT, somatic lean tissue; TAT, total adipose tissue; TLT, total lean tissue; TR, trunk; TSV, total somatic volume; TV, total volume; TVV, total visceral volume; UE, upper extremities; VAT, visceral adipose tissue; VLT, visceral lean tissue.
amount with a mean of 10.0% (SD 4.9%, min −3.8%, max −19.3%) in a negative parabolic graph shape (see Figure 8). The mean loss of absolute TVV was 0.9 L (SD 0.5 L, min −0.3 L, max −1.7 L) for finishers (Figure 9).

Mean absolute TVV loss per km during TEFR09 had a maximum of 0.75 ml/km (SD 0.5 ml/km) at the beginning and 0.2 ml/km (SD 0.1 ml/km) at the end (Figure 10).

Adipose tissue
In total (TAT), somatic (SAST) and visceral (VAT) adipose tissue, a significant change of absolute volumes (Table 5) and percentage volumes (Table 3) could be evaluated with a very high test power at the different MIs during TEFR09. A significant decrease for TAT, SAST and VAT could be shown at all MIs throughout TEFR09 compared to the start and for TAT and SAST compared to the first MI after the start (stage 5 to 12) (Figure 11). For other MIs the paired comparison showed no significant change.

Total adipose tissue
There was a continuous decrease of TAT that ended in a relative mean loss of 41.3% (SD 8.0%, min −25.4%, max −38.2%) in a negative parabolic graph shape (see Figure 8). The mean loss of absolute TVV was 0.9 L (SD 0.5 L, min −0.3 L, max −1.7 L) for finishers (Figure 9). Mean absolute TVV loss per km during TEFR09 had a maximum of 0.75 ml/km (SD 0.5 ml/km) at the beginning and 0.2 ml/km (SD 0.1 ml/km) at the end (Figure 10).

Table 4 Significance of topographic tissue volume changes regarding percentage volume (vol. %)

| (vol.%) | Mauchly-Test | Univariate ANOVA | Multivariate ANOVA |
|---------|--------------|------------------|--------------------|
|         | P-value      | F value          | P value            | test power | F value | P value | test power |
| TSV     | 0.322        | 2.565            | 0.058              | 0.640      | -       | -       | -          |
| TVV     | 0.322        | 2.565            | 0.058              | 0.640      | -       | -       | -          |
| TLTa    | 0.000        | 44.605           | 0.000              | 1.000      | 51.592  | 0.000   | 1.000      |
| SLTa    | 0.000        | 43.573           | 0.000              | 1.000      | 19.556  | 0.001   | 1.000      |
| VLTa    | 0.005        | 22.980           | 0.000              | 1.000      | 6.699   | 0.013   | 0.884      |
| TATa    | 0.000        | 44.655           | 0.000              | 1.000      | 52.762  | 0.000   | 1.000      |
| SASTa   | 0.000        | 44.721           | 0.000              | 1.000      | 57.109  | 0.000   | 1.000      |
| VATa    | 0.000        | 23.718           | 0.000              | 1.000      | 8.598   | 0.007   | 0.950      |

*Mean differences are significant; **Greenhouse-Geisser** correction procedure was used; †Pillai-Spur, Wilks-Lambda, Hotelling-Spur and 'biggest characteristical root of Roy'. ANOVA, analysis of variance; SAST, somatic adipose soft tissue; SLT, somatic lean tissue; TAT, total adipose tissue; TLT, total lean tissue; TSV, total somatic volume; TVV, total visceral volume; VAT, visceral adipose tissue; VLT, visceral lean tissue.

Bold numbers mark the preferred P-value of the prevered ANOVA regarding sphericity assumption proven by Mauchly-Test.
max −53.2%) with a small SE of 2.3% (Figure 8) at the last MI. Looking at the absolute loss of TAT, a finisher lost 5.3 L in the mean (SD 2.6 L min −2.7 L, max −9.8 L) until the end of the race (Figure 9). The mean TAT loss per km in finishers was 3.2 ml (SD 1.7 ml/km) at the beginning and 1.2 ml (SD 0.6 ml/km) at the end of TEFRO9 (Figure 10).

**Somatic adipose soft tissue**

The relative SAST decrease compared to the start showed a steeper graph than TAT and ended in a mean loss of 48.7% (SD 9.9%, min −25.9%, max −65.5%) after more than 4,000 km (Figure 8). The absolute SAST decrease in finishers showed a mean of 4.4 L (SD 2.2 L, min −2.2 L, max −8.4 L) at the end of TEFRO9 (Figure 9). This corresponds to a mean loss of SAST of 1.1 ml/km (SD 0.5 ml/km) at the end of TEFRO9 compared to 2.4 ml/km (SD 1.4 ml/km) at the start (Figure 10).

**Visceral adipose tissue**

The relative decrease of VAT occurred much more rapidly in the mean and ended in a relative VAT volume loss of 64.5% (SD 15.9%, min −27.7%, max −88.8%) at the end of the race (Figure 8) compared to the start and a SE up to 4.6%. The percentage volume of VAT decreased more quickly and severely compared to absolute VAT volume (Figure 12). In absolute values, this rapid and continuous loss of VAT ended in a mean of −0.9 L (SD 0.5 L, min −0.3 L, max −1.7 L) in the finisher group (Figure 9), which was nearly the same as the absolute TVV loss. Therefore, the mean VAT volume loss per km was the same as for TVV in the finishers (Figure 10).

The subdivision of VAT into IAAT and MAT shows that IAAT decreased a bit faster than VAT in total and ended in a relative volume loss of 67.3% (SD 14.8%, min −31.7%, max −88.8%) at the end (Figure 13). MAT initially decreased as rapidly as IAAT with respect to VAT but reached a plateau of 30% volume loss after nearly 1,000 km of running before it decreased again in the last third of the race up to 41.5% with a larger variance (SD 24.7%, min −0.1%, max −89.0%).

**Lean tissue**

Due to significant and continuous loss of different adipose tissue volumes the percentage volume of TLT, SLT and VLT increased during TEFRO9 significantly without relevant changes in absolute volumes, respectively (Table 3, Figure 12); analysis of the means of absolute volume showed no significant changes for total, somatic and visceral lean tissue at the different MIs during TEFRO9 (Table 5).

**Total lean tissue, somatic lean tissue, visceral lean tissue**

TLT volume showed undulating relative changes during TEFRO9 in the mean compared to the start. Just at the end, after more than 4,000 km running, the mean relative changes were −1.2% TLT (SD 3.3%, min 6.3%, max −5.5%) with an SE of 1.0% (Figure 8). Due to nearly stable volume regarding VLT during TEFRO9, the SLT data during TEFRO9 was similar to that of TLT relative to the start: mean −1.4% (SD 3.9%, min 7.5%, max −6.6%). Not every finisher showed a decrease of absolute TLT and SLT; some of them showed increases, some decreases: mean −0.91 (SD 1.2 L, min 1.1 L, max −2.8 L); see Figure 9. Mean loss of TLT and SLT per km changed between 0.3 and 0.2 ml with a wide range (SD at beginning 1.9 ml/km, at the end 0.3 ml/km), see Figure 10.

**Segmental volume analysis**

The significance of the volume changes in the different body segments is shown in Table 6. For the lower extremities the change of volumes was only significant for SAST_LE but not for TV_LE or LT_LE; for the trunk and upper extremities decreases were significant for adipose soft tissue volume (SAST_TR, SAST_UE) and total volume (TV_TR, TV_UE) but not for lean tissue volume.
Most decrease of somatic adipose tissue occurred in the trunk (t5: mean −50.3%, SD 12.0%), followed by the arms (t5: mean −39.1%, SD 8.3%); in the legs the adipose tissue loss was the smallest, but significant (t5: mean −29.2%, SD 13.4%), Figure 14. Although changes of lean tissue were not significant in any segment, mean values demonstrate a mean increase in the legs in the first half of TEFRO9, and in the trunk in the first third of the race, while in the arms lean tissue loss was already detectable at the first MI t1 (Figure 14).

Finisher/non-finisher
A total of 45.5% of the subjects did not finish the race. The dropout rate of subjects compared to all race participants is shown in Figure 15. The main reason (70%, n = 7) for premature dropping out of the race was intolerable pain in the legs due to an overload of muscle and tendons (soft tissues) leading to intermuscular and peritendinous inflammation (fasciitis): lower legs (40%), upper legs (30%). Other reasons were a high tibial stress fracture, a painful bunion and one rapidly progressing phlegmonia from the thumb up to the forearm needing immediate surgical intervention.

Figure 16 shows the distribution of percentage volumes for all tissue segments at the start time (t0) and MI t1 (317 to 789 km) for finishers (nF = 12) and non-finishers (nNF = 10) of TEFRO9. At both times the finisher group had significantly more percentage volume regarding total somatic tissue (mean TSV) than non-finishers of TEFRO9 (at t0 +1.8%: 85.5 vol% versus 84.0 vol%, at t1 +1.6%: 85.8% versus 84.4%) and, therefore, significantly less percentage volume of mean TVV (at t0 -10.5%: 14.5 vol% versus 16.0 vol%, at t1 -9.5%: 14.2 vol% versus 15.6 vol%), Table 7. The finisher group showed significantly less adipose tissue volume percentage

![Figure 6 Changes of somatic and visceral percentage volumes during TEFRO9 (finisher, nF = 12).](image)

**Table 5 Significance of topographic tissue volume changes regarding absolute volume measurements (l) (nF = 12)**

| (l)  | P value | F value | test power | F value | P value | test power |
|------|---------|---------|------------|---------|---------|------------|
| TV   | 0.000   | 20.162  | 0.999      | 5.758   | 0.020   | 0.828      |
| TSV  | 0.001   | 18.607  | 0.999      | 4.937   | 0.030   | 0.762      |
| TVV  | 0.000   | 21.516  | 0.999      | 8.678   | 0.007   | 0.952      |
| TLT  | 0.516   | 1.209   | 0.322      | 0.307   | -       | -          |
| SLT  | 0.516   | 1.209   | 0.322      | 0.307   | -       | -          |
| TAT  | 0.000   | 32.274  | 1.000      | 18.577  | 0.001   | 1.000      |
| SAST | 0.000   | 32.692  | 1.000      | 15.624  | 0.001   | 0.998      |
| VAT  | 0.000   | 21.607  | 0.999      | 8.594   | 0.007   | 0.950      |

a mean differences are significant; b Greenhouse-Geisser correction procedure was used; c Pillai-Spur, Wilks-Lambda, Hotelling-Spur and ‘biggest characteristical root of Roy’. ANOVA, analysis of variance; SAST, somatic adipose soft tissue; SLT, somatic lean tissue; TAT, total adipose tissue; TLT, total lean tissue; TSV, total somatic volume; TVV, total visceral volume; VAT, visceral adipose tissue.

Bold numbers mark the preferred P-value of the prevered ANOVA regarding sphericity assumption proven by Mauchly-Test.
than the non-finishers for TAT and VAT at t0 and t1, and also for SAST at t1 (Table 7). At the start, non-finishers had 71.5% more VAT volume percent (mean VAT at t0: 2.2 vol% versus 3.8 vol%), 28.0% more SAST volume percent (mean SAST at t0: 15.0 vol% versus 19.2 vol%) and in total 26.6% more TAT volume percent (mean TAT at t0: 20.6 vol% versus 26.1 vol%) than the finishers (Table 7). At the first MI, t1, the difference between finisher and non-finisher was significantly further on; non-finishers had 96.8% more VAT volume percent (mean VAT at t0: 1.6 vol% versus 3.2 vol%), 39.7% more SAST volume percent (mean SAST at t0: 13.3 vol% versus 18.5 vol%) and in total, 34.9% more TAT volume percent (mean TAT at t0: 18.3 vol% versus 24.7 vol%) than finishers (Table 7). These differences for adipose tissue compartments were no longer detectable as the race proceeded (t2 to t5); either there are not enough numbers to treat in the group of non-finishers for further analysis on the difference with finishers or no difference could be shown. Conversely, the lean tissue difference of percentage volume was significantly smaller in non-finishers compared to finishers for TLT (at t0: -6.9%, at t1: -7.8%) and SLT (at t0: -8.1%, at t1: -8.9%) (Table 7). VLT showed no significant difference between finishers and non-finishers at any MI (t0 to t5). Table 8 and Figure 17 demonstrate a significant relative volume loss at MI t1 and t2 compared to the start only for SAST and no other tissue compartment.

Prerace performance
Although there is a wide range of finished long distance foot races in the subject group (Table 9), every participant of TEF09 had already finished nearly one UM and MSUM, but not every subject had finished a single marathon. The endurance training extent of one year and three months before TEF09 also varied for training volume (km), time (hours) and intensity (km/hour) in the subject group (Table 9). For the number of finished marathons, UM and MSUM no difference between finisher and non-finisher could be evaluated (Table 10). However, regarding prerace training volume and intensity one year before TEF09 and their 50 km- and 24 hour-race record, finishers had a significantly higher prerace-performance compared to non-finishers (Table 10). Only these five prerace-performance parameters (PRT\textsubscript{Vol08}, PRT\textsubscript{Vol09}, PRT\textsubscript{Int08}, PRR\textsubscript{50km}, PRR\textsubscript{24hr}) also showed a mainly high and medium correlation with the volume percentage of adipose tissue compartments (VAT, SAST, TAT), TLT and SLT (Figure 18).

Race-performance
No relevant correlation between percentage fat and lean volumes of different compartments at the start and the race performances of the subjects at TEF09 could be detected (Figure 19). For SAST at the beginning of TEF09 (stage 1 to 8) a significant correlation between percentage volume at the start and cumulative performance is given, but only at a medium to low effect size. For TAT, TLT and SLT the significance for such a correlation is shown at the first 12 to 15 stages and during the last third of TEF09 at a medium effect size (Figure 19). A correlation of percentage fat and lean volumes to performance at the individual stages can only be shown for
a few stages at a middle to low effect size. None of the relative changes in the investigated volumes during TEFR09 were significantly correlated with performance.

**Discussion**

Almost nothing is known about the influence of the endurance burden on the specific changes in body composition regarding distribution of adipose and lean tissues in somatic and visceral compartments and in the body segments. Field studies on this topic mostly use methods which only allow indirect measurements and approximated calculations or simple estimations of total or local adipose or lean tissue proportions [11-14]. For TAT and subcutaneous adipose tissue (SCAT = SAST without intermuscular adipose tissue (IMAT) [31]), some of these indirect methods show more or less correlation to MRI findings [17]. These methods are not able to predict the amount of visceral (VAT) or somatic adipose tissue (SAT) in the body [16,32]. Being the first investigation in endurance field studies using the gold standard method [18] whole body MRI for such analyses, our results provide new data on the volume changes of fat and lean tissue in these different parts of the athlete’s body.
Age and gender related differences
Bale et al. [33] found a lower percentage of body fat in female elite marathon runners. In obese patients (BMI >27 kg/m²) Machann et al. [25] found that the amount and distribution of adipose tissue correlated with age (VAT increasing with age) and with gender (%SAT female > male, % VAT male > female). They found no consistent differences in TAT profiles between the selected age groups for both females (n = 40, mean age 45 years, SD 12 yrs., range 23 to 64 yrs.) and males (n = 40, mean age 45 years SD 12 yrs., range 24 to 65 yrs.) in their group. Naturally, our group of ultra-runners with a comparable age distribution (n = 22, mean age 49 years, SD 12 years, range 27 to 69 years) showed a very low absolute mean volume of VAT at the start of TEFRO9 (females: 0.5 L, males 1.8 L) compared to obese patients (females 1.5 to 4 L, males 4 to 6.8 L) [25].
Statistical analysis of gender-related differences was not possible (only two females) in our group, but even these data indicate that a difference in VAT between men and women is not only visible in obese people, but is also visible

Figure 9 Absolute changes of specific tissue volume during TEFRO9 compared to the start (finisher, nF = 12). TEFRO9, Transeurope Footrace 2009.
in thin ultra-endurance athletes. Analysis based on age showed no correlation to fat distribution at the start (TAT, SAST, VAT) or to volume changes of lean and adipose tissue during TEFR09.

Changes in body composition
Different effects of endurance performance on body composition are described in the literature. Beyond dispute is the fact that endurance performance leads to a decrease in body mass, mainly body fat. Body fat is the main energy-rich substrate for endurance performance [34-37]. Therefore, endurance exercise leads to a reduction of subcutaneous tissue as demonstrated in several field studies [34,36,38].

The specific influence on energy turnover seems to depend on the type of endurance burden [1,39]. In general, non-stop ultra-endurance races over hours, days or weeks without a break result in a decrease in body mass [1,36,40,41] in which body fat as well as skeletal muscle seems to decrease [1,36,40-42]. In ultra-endurance performances with defined breaks, body mass may remain stable [43-45] or even increase [34] and body fat is reduced [34,46,47], whereas skeletal muscle mass seems to be spared [35,43,47] or may even increase [46]. Our
Figure 11 Post hoc analysis of the significance of the paired comparison of total fat tissue measurements at different time intervals (finisher, \( n_f = 12 \)).

Figure 12 Changes of adipose and lean tissue percentage volumes during TEFRO9 (finisher, \( n_f = 12 \)). TEFRO9, Transeurope Footrace 2009.
whole body MRI results show comparable results for an ultra-long MSUM over 64 days without any day rest; every subject decreased in BM(I), TV, TSV and TVV due to massive loss of TAT, SAT and VAT, respectively. Not every runner lost TLT and SLT during the TEFR09. Some of them showed increases, some decreases. Knechtle et al. found the same individual differences for lean tissue in ultra runners during a 1,200 km MSUM across Germany [48]. If there are not sufficiently long breaks in ultra-endurance races, some participants might not find enough time for regeneration and restoration of their energy depots before the next stage. As the race progresses this leads to the utilization of muscle tissue for energy provision.

Mass loss
Raschka and Plat observed a mean loss of 1.75 kg body mass in an ultra-endurance run over 1,000 km within 20 days [34]. In their investigation, there was a statistically significant decrease in body mass after day 8 until day 11, which then remained stable until the finish. In another investigation of 10 ultra runners (BIA), the mean loss of BM after a 1,200 km footrace was also not significant, but the loss of 3.9 kg fat mass was [48]. Unfortunately, the authors gave no information about the relative changes of fat and lean body mass. Our results determined that a transcontinental ultra-long MSUM of 64 stages leads to a significant three times higher loss of body volume (9.5%) than published for body mass loss in deca-triathlons or 20 stage MSUMs [34,47].

Visceral adipose tissue
Mediastino-abdominal lipomatosis is described as being associated with exertional dyspnea [53], non–insulin-dependent diabetes, type IV hyperlipidemia, and type IV hyperlipidemia, and

**Table 6 Significance of segmental volume changes regarding repeated absolute volume (l) measurements (n<sub>F</sub> = 12)**

|                  | Mauchly-Test | Univariate ANOVA<sup>a</sup> | Multivariate ANOVA<sup>b</sup> |
|------------------|--------------|----------------------------|-------------------------------|
| (I)              | P value      | F-value                    | P value                        | F-value | P value | test power |
| TV_LE            | 0.003        | 7.763                      | 0.002                          | 0.946   | 2.341   | 0.149      | 0.423 |
| TV_TR<sup>c</sup> | 0.133        | 6.549                      | 0.003                          | 0.918   | -       | -          | -    |
| TV_U<sup>c</sup> | 0.001        | 27.504                     | 0.000                          | 1.000   | 13.942  | 0.002      | 0.996 |
| SLT_LE           | 0.003        | 6.411                      | 0.20                           | 0.733   | 14.587  | 0.095      | 0.597 |
| SLT_TR           | 0.252        | 5.534                      | 0.21                           | 0.769   | -       | -          | -    |
| SLT_U<sup>c</sup> | 0.700        | 3.128                      | 0.29                           | 0.734   | -       | -          | -    |
| SAST_LE<sup>c</sup> | 0.000        | 63.294                     | 0.000                          | 1.000   | 20.644  | 0.000      | 1.000 |
| SAST_TR<sup>c</sup> | 0.000        | 17.388                     | 0.000                          | 0.996   | 13.087  | 0.002      | 0.995 |
| SAST_U<sup>c</sup> | 0.000        | 16.151                     | 0.000                          | 0.987   | 3.389   | 0.041      | 0.584 |
| TV_LE<sup>c</sup> | 0.001        | 27.504                     | 0.000                          | 1.000   | 13.942  | 0.002      | 0.996 |
| TV_TR<sup>c</sup> | 0.133        | 6.549                      | 0.003                          | 0.918   | -       | -          | -    |
| TV_U<sup>c</sup> | 0.003        | 7.763                      | 0.002                          | 0.946   | 2.341   | 0.149      | 0.423 |

<sup>a</sup> Mean differences are significant; <sup>b</sup>Greehouse-Geisser correction procedure was used; <sup>c</sup>Pillai-Spur, Wilks-Lambda, Hotelling-Spur and ‘biggest characteristical root of Roy’. ANOVA, analysis of variance; LE, lower extremities; SAST, somatic adipose soft tissue; SLT, somatic lean tissue; TR, trunk; TV, total volume; UE, upper extremities. Bold numbers mark the preferred P-value of the preferred ANOVA regarding sphericity assumption proven by Mauchly-Test.
hyperuricemia. The abdominal VAT is an important independent risk factor for metabolic diseases in the older patient [54] and there is evidence that mainly abdominal VAT, which is morphologically and functionally different from abdominal SAST, is associated with the metabolic syndrome (insulin resistance, dyslipidemia, hypertension, obesity) and hyperinsulinemia [55-60], as well as linked inflammatory diseases [61]. The real mean loss of relative IAAT while running a MSUM of nearly 4,500 km, was more than two thirds compared to the start in our group (Figure 13). We showed that endurance running also has a direct influence on intrathoracic fat, especially MAT, which decreased up to more than 40% in the mean (Figure 13). MAT is associated with hypertension, obesity, and iatrogenic Cushing syndrome [57,62-65].

Until now, a specific treatment for the selective reduction of VAT is not known [66] and as our MR analyses showed that VAT decreased much more rapidly and
vigorously than SAST (Figure 8), a very good and effective way to reduce the risk of metabolic disease is endurance running. As VAT decreases much faster and more than SAST, our investigation indicates that three-compartment measurement methods, such as SF-analyses and BIA, cannot give accurate assumptions or calculations for IAAT and MAT. Even the results of the four-compartment method cadaver study are false, when post mortem findings are transferred to physiological effects which occur from the impact of long lasting running on fat and lean tissue in vivo [67].

Finishers versus non-finishers
55% (n = 12) of the 22 ultra-runners treated with mobile whole body MRI for this study reached the last measurement interval; 10 dropped out earlier. In contrast, the dropout rate for all starters at TEFR09 and all subjects taking part in the TEFR project was 31% [2]. Reasons for dropping out of this transcontinental MSUM race were overuse reactions of the musculoskeletal system of the lower extremities (80%, Figure 15), mainly concerning the myotendinous fascial system.

In a 17-day MSUM (1,200 km) Knechtle et al. found no differences between finishers and non-finishers regarding the anthropometric parameters, BMI, SF, CF, estimated skeletal muscle mass (estimated from SF and CF) and percent body fat (BIA) [68]. With whole body MRI for differentiated body composition analysis, however, we found significant differences between finishers and non-finishers between both somatic and visceral volumes and between adipose and lean tissue volumes at the start and early beginning of the 4,500 km MSUM TEFR09 (Figure 16). Out results indicate that the risk of dropping out of such an ultra-long transcontinental footrace is significantly higher when the total body fat percentage is more than 21% to 25% at the start, in which the visceral fat percentage (VAT) shows a higher difference between finishers and non-finishers (71.5% in the mean) than the somatic fat compartment (SAST, 28.0%). Because VAT is affected by the endurance running burden most quickly and most deeply compared to somatic fat and other lean tissue (Figure 9) and is highly correlated with prerace performance regarding training volume and intensity and specific ultramarathon race-performance (50 km-race), our results indicate, that VAT is the most sensible predictor for the risk of non-finishing a transcontinental MSUM, such as the TEFR09. In ultra-runners there is not a high SAST or TAT, if the VAT is low.

Although training a distance of 4,500 to 5,000 km is not possible, participants of such MSUMs should
acquire specific characteristics and levels regarding body composition and performance skills even before the race if they want to have a good chance to finish: VAT near 20% to 21%, training volumes of more than 100 km/week one year before the race and the performance intensity of 7.5 km/hour at a minimum allowing specific ultra-race records of less than 5 hours in 50 km-races or more than 178 km in 24-hour races. In other words, if these levels of prerace performance are reached for at least 15 months before the transcontinental race, the VAT (and SAST, TAT) as the sensible marker for specific body composition adaptation is also in an optimal range for low risk of non-finishing, because these parameters correlate in a mostly high level.

Because the subjects mainly fall out of the race due to overuse injuries in the myotendinous fascial system of the lower extremities, we tend to assume that the mentioned interdependent parameters of body composition and prerace ultra-running performance, lead to overuse injuries in the main stressed musculoskeletal organs, if they are not highly adapted as mentioned above; too little specific ultra endurance adaption and too much of VAT (and SAST) results in a high risk of severe soft tissue overuse in the legs and mostly happens in the early phase (Figure 15) of a transcontinental foot race.

Table 7 Analysis of the difference of percentage volume (vol%) between F/NF at the start (t0) and MI (t1, t2) for total, lean and adipose tissue compartments

|       | t0: F = 12, NF = 10 |       | t1: F = 12, NF = 8 |       | t2: F = 12, NF = 6 |
|-------|---------------------|-------|---------------------|-------|---------------------|
|       | Mean difference of percentage volume (vol.%) | P value (ITT) | Mean difference of percentage volume (vol.%) | P value (ITT) | Mean difference of percentage volume (vol.%) | P value (ITT) |
| TSV   | 1.52                | 0.005<sup>a</sup> | 1.35                | 0.036<sup>a</sup> | 1.07                | 0.182 |
| TVV   | -1.52               | 0.005<sup>a</sup> | 1.35                | 0.036<sup>a</sup> | 1.07                | 0.182 |
| TLT   | 4.04                | 0.031<sup>a</sup> | 6.39                | 0.015<sup>a</sup> | 5.05                | 0.088 |
| SLT   | 4.16                | 0.014<sup>a</sup> | 6.18                | 0.010<sup>a</sup> | 4.65                | 0.058 |
| VLT   | 0.032               | 0.014<sup>a</sup> | 0.21                | 0.707 | 0.39                | 0.604 |
| VAT   | -5.47               | 0.031<sup>a</sup> | -6.39               | 0.015<sup>a</sup> | -5.04               | 0.088 |
| SAST  | -4.21               | 0.080 | -5.27               | 0.032<sup>a</sup> | -3.64               | 0.184 |
| TAT   | -1.60               | 0.006<sup>a</sup> | -1.56               | 0.214<sup>a</sup> | -1.46               | 0.060 |

<sup>a</sup>Mean differences between finishers and non-finishers are significant. t0: at start of TEF09, t1: stage 5 to 12 (317–789 km), t2: stage 15 to 24 (1,003 to 1,635 km). F, finishers; ITT, t-test for independent samples; MI, magnetic imaging; NF, non-finishers; SAST, somatic adipose soft tissue; SLT, somatic lean tissue; TAT, total adipose tissue; TEF09, Transeurope Footrace 2009; TLT, total lean tissue; TSV, total somatic volume; TV, total visceral volume; VAT, visceral adipose tissue; VLT, visceral lean tissue.

Table 8 Analysis of the difference in relative volume changes (%) at MI t1 and t2 compared to start between F/NF for total, lean and adipose tissue compartments

|       | t1 versus t0: F = 11, NF = 9 | t2 versus t0: F = 11, NF = 7 |
|-------|-------------------------------|-------------------------------|
|       | Mean diff. of relative changes (%) | P value (ITT) | Mean diff. of relative changes (%) | P value (ITT) |
| TV    | 0.47                          | 0.608 | -1.49                        | 0.306 |
| TSV   | 0.41                          | 0.690 | -2.06                        | 0.190 |
| TVV   | 0.69                          | 0.640 | 1.35                         | 0.410 |
| TLT   | 1.09                          | 0.332 | -0.18                        | 0.913 |
| SLT   | 1.34                          | 0.319 | -0.21                        | 0.917 |
| VLT   | 0.07                          | 0.923 | -0.24                        | 0.545 |
| VAT   | -4.86                         | 0.086 | -0.952                       | 0.078 |
| SAST  | -6.93                         | 0.031<sup>a</sup> | -14.27                     | 0.046<sup>a</sup> |
| VAT   | -8.92                         | 0.191 | -8.88                       | 0.276 |

<sup>a</sup>Mean differences between finishers and non-finishers are significant. F, finishers; ITT, t-test for independent samples; measurement interval; NF, non-finishers; SAST, somatic adipose soft tissue; SLT, somatic lean tissue; t0, at start of TEF09; t1, stage 5 to 12 (317–789 km); t2, stage 15 to 24 (1,003–1,635 km); TAT, total adipose tissue; TEF09, Transeurope Footrace 2009; TLT, total lean tissue; TSV, total somatic volume; TV, total volume; TVV, total visceral volume; VAT, visceral adipose tissue; VLT, visceral lean tissue.

Bold numbers mark significant P-values.

Figure 17 Difference between F and NF regarding relative volume changes of tissue compartments at first measurement interval (t1) of TEF09. F, finishers; NF, non-finishers; TEF09, Transeurope Footrace 2009.
fascial structures of the legs during the race, but the feet are not a region for problems for experienced endurance runners in a MSUM [69]. So the immense amount of mechanical stress on the musculoskeletal system when running nearly two marathons daily over a period of nine weeks can lead to these overuse syndromes without the obligatory necessity of prevalent (intrinsic) factors, such as ‘overweight’ (high VAT), suboptimal ultra-
endurance prerace performance or mal-alignment of the legs (which was only seen in one female subject suffering from a bunion). The majority of the participants was able to ‘overrun’ more or less severe overuse soft tissue syndromes in the legs and reached the finish line [2]. This indicates, that, despite the mentioned somatic parameters, other mentally based factors, such as pain resistance and personality traits, are also relevant for

| Years of regular endurance running | Mean | SD | 95%-percentile | Range |
|-----------------------------------|------|----|----------------|-------|
| M finished (number)              | 123.1| 218.2 | 2 to 297.8     | 2 to 988 |
| UM finished (number)             | 90.8 | 68  | 113.3 to 248.9 | 11 to 255 |
| MSUM finished (number)           | 6.3  | 2.9  | 1.3 to 13.6    | 1 to 14 |

### Endurance training extent 2008 (one year before TEFR)

| Annual running distance (km/year) | Mean | SD | 95%-percentile | Range |
|-----------------------------------|------|----|----------------|-------|
| M                                  | 5,468| 1,720 | 3,000 to 9,000| 2,580 to 9,152 |
| UM                                 | 105.1| 32.4 | 50.8 to 175.4 | 50 to 176 |
| MSUM                               | 12.5 | 3.1  | 7.1 to 19.6   | 7 to 20 |
| MSUM                               | 8.3  | 1.5  | 6.5 to 10.9   | 7 to 11 |

### Endurance training extent last two months before TEFR

| Total running distance (km)       | Mean | SD | 95%-percentile | Range |
|-----------------------------------|------|----|----------------|-------|
| M                                  | 898  | 267 | 500 to 1,260   | 500 to 1,500 |
| UM                                 | 110.5| 33.8 | 60.5 to 186   | 60 to 190 |
| MSUM                               | 13.2 | 3.5 | 8 to 21.6     | 8 to 22 |

### Table 9 Endurance running history of subjects (n = 22)

|                        | Mean | SD | 95%-percentile | Range |
|------------------------|------|----|----------------|-------|
| Years of regular endurance running | 17.4 | 7.6 | 6.3 to 31.8    | 6 to 32 |
| M finished (number)    | 123.1| 218.2 | 2 to 297.8     | 2 to 988 |
| UM finished (number)   | 90.8 | 68  | 113.3 to 248.9 | 11 to 255 |
| MSUM finished (number) | 6.3  | 2.9  | 1.3 to 13.6    | 1 to 14 |

### Table 10 Distribution type and analyses of the difference between F/NF regarding prerace performance indices

|                              | Valid | Missing | F         | NF         | statistic | P     | test type | P  |
|------------------------------|-------|---------|-----------|------------|-----------|-------|-----------|----|
| PRY (years)                  | 21    | 1       | 4.5       | 16.5       | 18.7      | 0.936 | 0.185     |    |
| nF M (n)                     | 19    | 3       | 13.6      | 81.5       | 194.3     | 0.481 | 0.000     | 0.211 |
| nF UM (n)                    | 22    | 0       | 0         | 94.8       | 86.0      | 0.858 | 0.005     | 0.895 |
| nF MSUM (n)                  | 22    | 0       | 0         | 7.0        | 5.4       | 0.936 | 0.165     | 0.146 |
| PRTVol08 (km/week)           | 21    | 1       | 4.5       | 117.9 (34.3)| 88.0 (20.8)| 0.959 | 0.505     | 0.032d |
| PRTTime08 (hour/week)        | 21    | 1       | 4.5       | 13.0       | 11.9      | 0.971 | 0.761     | 0.427 |
| PRTVol09 (km/week)           | 22    | 0       | 0         | 9.0 (1.47) | 7.5 (0.93)| 0.909 | 0.044     | 0.040d |
| PRTVol09 (km/week)           | 21    | 1       | 4.5       | 126.1 (35.2)| 89.7 (17.4)| 0.956 | 0.435     | 0.010d |
| PRTTime09 (hours/week)       | 21    | 1       | 4.5       | 14.2       | 12.0      | 0.947 | 0.303     | 0.159 |
| PRR (hours)                  | 15    | 7       | 31.8      | 3.1        | 3.1       | 0.851 | 0.018     | 0.676 |
| PRR50km (hours)              | 9     | 13      | 59.1      | 4.3 (0.57) | 5.1 (0.26)| 0.914 | 0.345     | 0.026d |
| PRR100km (hours)             | 17    | 5       | 22.7      | 9.5        | 10.4      | 0.933 | 0.248     | 0.241 |
| PRR (km)                     | 13    | 9       | 40.9      | 67.9       | 59.7      | 0.918 | 0.238     | 0.218 |
| PRR12hr (km)                 | 10    | 12      | 54.5      | 99.9       | 87.3      | 0.940 | 0.548     | 0.558 |
| PRR24hr (km)                 | 16    | 6       | 27.3      | 199.8 (22.5)| 168.5 (26.0)| 0.961 | 0.672     | 0.036d |

aITT: independent t-test; bbilateral (asymptotic) test; csignificance not shown (P >0.05): normal distribution accepted; dmean differences between finishers and non-finishers are significant. F, finishers; M, marathon; MSUM, multistage ultramarathon; MUW: Mann–Whitney-U test; n, number; NF, non-finishers; PRR, prerace records; PRT, prerace training; PRY, years of regular endurance running; IM, ultramarathon.

Bold numbers mark significant P-values of the test on difference between F and NF.
Figure 18 Correlation (one-tailed test) of percentage volumes and prerace performance.

Figure 19 Correlation of adipose and lean volumes at start with performance at TEF09. TEF09, Transeurope Footrace 2009.
finishing or non-finishing a transcontinental footrace [70]. One subject (male, 61 years old) had to stop the race after stage 38 (2,601 km run) due to a high tibial stress fracture which was detected in a specific MRI on this day (Figure 15). The astonishing thing is not the stress fracture, because this can happen to every ultra runner when starting a transcontinental race, but the fact that the major pain and massive performance (running velocity) loss had already started at stage 36. This subject ran 228 km (three stages) with a complete high tibial fracture before stopping the race, because he interpreted the pain as a soft tissue injury due to overuse and tried to ‘overrun’ it before he asked for MRI control. Another participant (female, 46 years old) showed the same behavior when running 208 km (stage 46 to 48) with a ventral pelvic ring stress fracture before diagnosis could be done with mobile MRI [2]. These examples and our prerace test on pain tolerance demonstrate that the resilience of the ultra athletes regarding pain is significantly higher than in a normal control group [70].

Body composition and performance

In specific treadmill investigations under laboratory settings, Millet et al. showed that a good single ultramarathon performance needs specific running economy depending on the ability of maximal oxygen uptake being highly correlated with citrate synthase activity and capillary network [71]. These physiological factors have not been investigated directly under race conditions in ultra-endurance events until now. Concerning this matter, only indirect parameters, such as anthropometric characteristics, are examined.

Several anthropometric factors are reported to affect performance in runners, but the presented data are inconsistent and often contradictory. Such differences are also present in the specific literature regarding anthropometrical predictors of performance outcome in ultra-marathons. Several factors are responsible for this. The numbers of volunteers are different, and in most reports they are limited and differ in gender and ethnic origin. Furthermore, the investigations are based on manifoldly different types of UM races. They can differ in the distance of running and number of stages, but also in altitude and/or external conditions.

Anthropometric parameters related to good performance are different in marathons and middle distance (half-marathon, 10 km) events [72]. Knechtle et al. reported that anthropometry is not associated with performance in single mono-stage UM races (24 hours [73]).

In MSUM Knechtle et al. found no correlation between BM or body fat (BIA) and race performance in a 17-stage MSUM (‘Deutschlandlauf 2007, 1,200 km) [68]. In a cohort of 392 athletes, Hoffman found a significant relationship of BMI to finishing times in mono-stage UM running (161 km UM) [74]. In single marathon runners abdominal and front thigh SF are correlated [75]. The sum of eight SF-locations correlated significantly to 100 km race-time in a survey of three races in Knechtle et al. [76].

According to our results with a group of 22 subjects and using gold standard whole body MRI, in athletes taking part in a 64-day MSUM there are no relevant correlations between total volume, percentage fat and lean volumes of different compartments at the start and total race performance of subjects participating in TEF09. For SAST, a significant correlation between percentage volume at the start and cumulative performance is seen at the beginning of TEF09 (stages 1 to 8), but only at a medium to low effect size. Correlation of percentage fat and lean volumes to performance at the individual stages could only be shown in a few stages at a medium to low effect size. Looking at percentage volume distribution, the participants already started with a low percentage of body fat. Therefore, our results might confirm earlier findings of a negative relationship between the amount of subcutaneous fat tissue (thickness or volume), being the main fat tissue compartment of the body, and performance in single or multiday ultramarathon races. However, in a multistage ultramarathon over thousands of kilometers we found no relationship between body fat percentage or BM or BV and race performance using specific whole body MRI, as Knechtle et al. did with BIA [68]. The majority of transcontinental MSUM participants ran not for winning but for finishing the race; therefore, running velocity was a priority only for a few of them. For single UM races, the race time and, therefore, the performance plays a more important role for the ultra-athletes, and body composition and fat distribution have a more significant influence, respectively.

Similar interpretation has to be done, when looking at segmental (somatic) tissue changes in the arms, legs and trunk during TEF09. As for adipose and lean total somatic and visceral volumes (Figure 19), we also did an analysis of the relationship between segmental tissue volume changes and race performance (results not demonstrated graphically) and detected only a small to low medium effect size for correlations between SAST of all segments (UE, TR, LE) with cumulative race performance in the first eight stages of TEF09. So, in our investigation, all segments show a significant relationship to race performance that is similar to that of SAST over all (Figure 19) without any exceptional segment findings, which explains the inconstant finding in the literature. Knechtle et al. [77] found an association between triceps SF thickness and performance in female 100 km ultra-runners. Tanaka and Matsuura mentioned this for CF of the thigh in the early eighties [78].

Some ultra athletes show adaption to the intense running burden of TEF09 with muscle (SLT) increase in
the legs, although they are already specialized in ultra running. These findings were not significant in the mean. For the trunk, a mean increase of SLT could also be detected in the first third of the race. This is explained by the gluteal and psoas muscles, which are part of the active motor system of the lower extremities but anatomically are placed in the trunk in our segmentation. All lean tissue segments showed a decrease in their volumes towards the end of TEFR09, indicating the high negative energy burden of transcontinental running.

**Metabolic changes**

After the first thousand kilometers the mean loss of TV per km, mainly caused by the SAST and VAT decrease, declined constantly up to more than half until the end of race (Figure 11). Despite lack of documentation of the nutrition and calorific intake but knowing that the subjects tried to ensure an optimum of energy intake, the decrease of fat volume loss can be explained by two factors: relevant metabolic changes regarding energy balancing [79] and improvement and optimization of running style during progression of the race. Not in multistage but in single stage ultra-running conditions such economical adaptations have already been shown by Millet et al. [80-82]. They could show significant changes of running mechanics and spring-mass behavior towards a higher mean step frequency (+4.9%) with shorter ground-feet contact time (−4.5%) and lower ground reaction force (−4.4%) due to functional leg length decrease (−13%) and increase of leg (+9.9%) and vertical stiffness (+8.6%) during the support phase of running between the early phase and the end of a 24-hour treadmill run [80]. Millet et al. speculated that these changes in running mechanics contributed to the overall limitation of the potentially harmful consequences of such a long-duration run on the subjects’ musculoskeletal system. Transferred to MSUM conditions, such changes in running mechanics may also contribute to the necessity of the organism to optimize the running economy to a high-end level (as low an energy consumption as possible) due to the massive negative energy burden a transcontinental race requires. The changes Millet et al. [80] and other researchers had measured [83,84] describe a running technique which requires only a low muscle power, because forceful eccentric load and step length are reduced. Besides the reduction of overuse risk for the musculoskeletal system this reduces the energy demand of the organism as well [85], even if the underlying mechanisms of the relation between energy cost of running and step variability remains unclear until now. If running economy could not be sacrificed in ultramarathons [86,87] and the amount of change in running mechanics depends on the duration of running and distance towards a fatigue state, respectively [81,85], it is even mandatory in transcontinental MSUM. Every subject in the TEFR-project showed a significant loss of BM and TV throughout the race, independent of the prerace overall status of body composition and performance or nutrition behavior during the race. The massive negative energy burden of a 4,500 km MSUM is also indicated by the significant loss of the grey matter in the brain [88]. The analysis of specific laboratory markers of the required blood and urine samples may give more data about the metabolic changes during TEFR09 in the near future.

**Limitations**

There was no general or individual nutrition plan offered or generated for the participants of TEFR09 or subjects of the TEFR-project, respectively. The athletes had a breakfast and a dinner served in different locations at the stage destinations, but these meals were organized and oriented at the local level at the last minute. The food supply points during the stages also offered products that changed every day and the athletes took additional individual food on their own throughout the race [2]. Therefore, documentation and measurement of nutrition and calorific intake was not possible and a stringent documentation of nutrition by the subjects implied the risk of compliance problems.

Whole body mobile MRI protocols did not measure ectopic fat such as intracellular fat of organs (for example liver) and muscles (intramyocellular lipids: IMCL). For IMCL measurement, specific protocols for mobile 1H-MR-spectroscopy of the muscles of the lower legs were implemented in the TEFR-project [2]. However, due to the dependence of this MR-method on a stable external magnetic field around the magnetom, the analysis of mobile 1H-MR-spectroscopy during TEFR09 did not lead to valid data and needed further development and implementation of post-imaging proof algorithms.

**Conclusions**

With this mobile MRI field study a complex change in body composition during an ultra-long MSUM could be demonstrated in detail. IAAT (VAT) shows the fastest and highest decrease compared to SAST and lean tissue compartments during TEFR09. Participants lost more than half of their adipose soft tissue and even lean tissue volume decreased (mainly skeletal muscle tissue). Without exception, every subject showed a significant loss of body volume. This indicates that running an MSUM of nearly 4,500 km without any day of rest is linked with an unpreventable chronic negative energy balance due to the massive running burden. The ratio of adipose tissue contribution between the visceral and somatic compartments has a significant influence on dropping out of the race during the first third in a MUSM due to overuse injuries of the myotendinous fascial system of the legs. Body volume or body mass and, therefore, fat
volume has no correlation with the performance of ultra-
athletes finishing a 64-stage UM. Two- and three-
compartment methods, such as bioelectrical impedance
analysers and skinfold-equations, cannot give estimations
about the relationship between the visceral and somatic
compartments and, therefore, cannot measure the most
sensitive anthropometric predictor of not finishing a
MSUM: VAT. Running economy is mandatory for trans-
continental MSUM races and, even in well trained ultra-
athletes, such events lead to further adaptation of running
mechanics and to metabolic changes as performance ana-
lysis compared to body composition changes throughout
the race indicates.

Abbreviations
ABM: Adipose bone marrow; ANOVA: Analysis of variance; BA: Bioelectrical
impedance analysis; BM: Body mass; BMI: Body mass index; CF: Body
circumference; CF: Body circumference; CHESS: Chemical shift selective
(imaging); CCP: Pearson correlation coefficient; CCS: Spearman-rho
correlation coefficient; DEXA: Dual-energy X-ray absorptiometry; F: Finisher;
IART: Intraabdominal adipose tissue: retroperitoneal and intraperitoneal fat
depots; IMAT: Intermuscular adipose tissue; IMCL: Intramyocellular lipids;
INF: Intraluminal nutrition fat in the gastrointestinal tract; LT-LE: Lean tissue
volume of lower extremities; LT-TR: Lean tissue volume of trunk; LT-UE:
Lean soft tissue volume of upper extremities; MAT: Intrathoracic,
volume of lower extremities; MAT: Intraabdominal, mainly mediastinal adipose tissue: max: Maximum; MI: Measurement interval;
min: Minimum; MR: Magnetic resonance; MRR: Magnetic resonance imaging;
MSUM: Multistage ultramarathon; NF: Non-finisher; PRR: Prerace years of
regular endurance running; PRR: Prerace records; PRRT: PRRue: Prerace record
in marathon; PRRT08: PRRue record in 50 km-races; PRRT09: PRRue record in
100 km-races; PRRT: PRRue record in 6 hours-races; PRRT: PRRue
record in 12 hours-races; PRRT: PRRue record in 24 hours-races;
PRRT: Prerace training; PRRinit: Prerace training intensity (km per week) in
2008; PRRT: PRRue: Prerace training volume (hours per week) in 2008;
PRRT: PRRue: Prerace training volume (hours per week) in 2009; PRRT: PRRue:
Prerace training hours per week in 2008; PRRT: PRRue: Prerace training hours per week in
2009; SAST: Somatic adipose soft tissue; SAT: Somatic adipose tissue;
SCAT: Subcutaneous adipose tissue; SD: Standard deviation; SF: Skinfold
thickness; SLT: Somatic lean tissue mostly muscles; TAST: Total adipose soft
tissue; TAT: Total adipose tissue; TEFR09: Transeroupe Footrace 2009;
TEFR-project: Transeroupe Footrace Project; TLT: Total lean tissue; TSV:
Total somatic volume; TV: Total volume of the body; TV-LE: Total volume of
lower extremities; TV-TR: Total volume of trunk; TV-UE: Total volume of upper
extremities; TVV: Total visceral volume; UM: Ultramarathon; VAT: Visceral
adipose tissue; VLT: Visceral lean tissue: includes lean tissue of intraabdominal
and intraabdominal organs.

Competing interests
The authors declare they have no competing interests.

Authors' contributions
All authors of this manuscript made substantial contributions to the
collection and design or acquisition, analysis and interpretation of data; all
revised it critically for important intellectual content and gave final approval
to the version to be published. SHW conceived the study, implemented
the project, participated in the data collection (MRI measurements), data
evaluation with statistical analysis and drafted the manuscript. BC
participated in the implementation of the project and in the data collection
(MRI measurements). KK participated in the data evaluation. WC
participated in the design of the study, its technical implementation and data
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