

Characteristics, changes and influence of body composition during a 4486km transcontinental ultramarathon. Results from the TransEurope FootRace mobile whole body MRI-Project.

Schütz UHW^{1,2}, Billich C¹, König K³, Würslin C³, Wiedelbach H¹, Brambs HJ¹, Machann J^{3,4}

¹ Department of Diagnostic and Interventional Radiology, University Hospital of Ulm, Germany

² Outpatient Rehabilitation Centre at University Hospital of Ulm, Germany

³ Section on Experimental Radiology, Department of Diagnostic and Interventional Radiology, University Hospital of Tübingen, Germany

⁴ Institute for Diabetes Research and Metabolic Diseases (IDM) – Metabolic Imaging – of the Helmholtz Center Munich at University of Tübingen (Paul Langerhaus Institute Tübingen), Germany

Address of correspondence:

Uwe H.W. Schütz, M.D.

Department of Diagnostic and Interventional Radiology, University Hospital Ulm
Albert-Einstein-Allee 23, 89081 Ulm, Germany

Outpatient Rehabilitation Centre at University Hospital of Ulm, Germany
Pfarrer-Weiβ-Weg 10, 89073 Ulm, Germany

Phone: ++49 731 50061192, ++49 731 964293 112, ++49 171 7628003
e-mail: uwe.schuetz@rocketmail.com

Abstract

Background. Nearly 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 Transeurope Footrace 2009 using a mobile whole body magnetic resonance (MR) imager are presented and 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 4486 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 10mm, slice distance 10mm (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 (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), somatic adipose soft tissue (SAST). Specific volume changing was tested on significance. Tests on difference and relationship regarding prarace and race performance and non-finishing were done using statistical software SPSS.

Results. Total, somatic and visceral volumes showed a significant decrease throughout the race. Adipose tissue showed a significant decrease compared to start at all measurement times for TAT, SAST and VAT. Lean adipose tissues decreased until the end of the race, but not significantly. The mean relative volume changes of the different tissue compartments at last measurement compared to start were: TV -9.5% (SE 1.5%), TSV -9.4% (SE 1.5%), TVV - 10.0% (SE 1.4%), TAT -41.3% (SE 2.3%), SAST -48.7% (SE 2.8%), VAT -64.5% (SE 4.6%), IAAT -67.3% (SE 4.3%), MAT -41.5% (SE 7.1%), TLT -1.2% (SE 1.0%), SLT -1.4% (SE 1.1%). Before start and at early phase of TEFR09 the non-finisher group had significantly more percentage volume of TVV, TAT, SAST and VAT compared to finisher. VAT correlates significantly with prarace training volume and intensity one year before the race and with 50km- and 24hrs-race records. Neither prarace body composition nor specific tissue compartment volume changes showed a significant relationship to performance in the last two thirds of TEFR09.

Conclusions. With this mobile MRI field study the complex changes of body composition during a multistage ultramarathon could be demonstrated in detail in a new and differentiated way. Participants lost more than the half of their adipose tissue. Even lean tissue volume (mainly skeletal muscle tissue) decreased due to the unpreventable chronic negative energy balance during the race. VAT shows the fastest and highest decrease compared to SAST and lean tissue compartments during the race. It seems to be the most sensitive morphometric parameter regarding the risk of non-finishing a transcontinental footrace and shows a direct relationship to pre-race-performance. However, body volume or body mass and therefore fat volume has no correlation with total race performances of ultra-athletes finishing a 4500km multistage race.

Key words: magnetic resonance imaging; MRI; body mass; body volume; body composition; running; marathon; ultramarathon; performance; adipose tissue; body fat; lean tissue; visceral; somatic; topography; segmentation; mapping

Introduction

With the worldwide growing number of people running, endurance sports have experienced differentiation into multiple (sub-) disciplines in the last decades. Concerning distance run, the ultramarathon (UM) seems to be the greatest challenge in endurance running. The German Ultramarathon Association (DUV) defines foottraces 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 meets 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, nearly 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 foottraces 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] was the first observational cohort field study of a transcontinental MSUM, the Transeurope Footrace 2009 (TEFR09) [3]. A unique collective of 67 endurance runners (mean age 50.7 yrs., SD 10.5 yrs., range 26-74 yrs., m 56 (83.6%)) met the challenge and tried to cross 6 countries when running 4486 km in 64 stages (mean 70.1 km, min 44 km, max 95.1 km) without any day rest [4]. The central aspect of TEFR-project was the usage 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. Secondary it's supposed, that although the running distance of a transcontinental UM cannot be trained 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 rest implicates, 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 TEFR09, 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 4500 km race progresses.

Material and Methods

Subjects. Every TEFR09 participant was asked to join the TEFR-project, which was approved by the local ethics committee of the University Hospital of Ulm (UHU, No.: 270/08-UBB/se) in accordance to the Declaration of Helsinki, regarding the study design, risk management plan and individual protocols [2]. Forty-four participants (67%) could be recruited for the study and gave their informed written consent. Every second subject (n=22,

male 20, mean age 49.1 yrs., SD 11.5 yrs., range 27-69 yrs.) was randomly selected for whole body MRI measurements regarding body composition. According to project protocol these subjects underwent a whole body MRI before 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-t6) occurred. Mean deviation of actual from planned measurement intervals was 187.8 km (SD=141.3 km) [2].

Prerace performance. Before the start of TEFR09 all subjects filled out specific questionnaires concerning their prerace experience in endurance running. This request of history includes the years of regular endurance running (PRY), the number of finished (n_F) marathons (M), UM and MSUM, and the prerace records (PRR) for marathon and specific UM (50 km, 100 km, 6 hrs, 12 hrs, 24 hrs) races within the last decade before TEFR. It also includes the extent of prerace training (PRT) 16 months before TEFR09: training volume (Vol: km/week), training duration (Time: hrs/week) and the training intensity (Int: km/h). The self-disclosures about n_F 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 literature: In vivo, two-compartment model methods are the hydrodensitometry [5] and the body fat percentage and muscle mass calculation from anthropometric data like skinfold thickness (SF) calipometry or/and segmental body circumferences (CF) [6,7,8]. Three-compartment methods are the bioelectrical impedance analysis (BIA) [9] and the dual-energy X-ray absorptiometry (DEXA) [10]. With these methods indirect measurement, approximated calculation or simple estimation of total, regional or local adipose or lean tissue [11,12,13,14] 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 [15,16,17,18].

Subjects who finished TEFR09 got whole body MRI six times during TEFR09 (7 measurements in total). Body mass (BM) weightening was done at the same time as MRI and every 4th day: BIA balance Tanita BC-545 to the nearest 0.1 kg (Tanita, Arlington Heights, IL, U.S.A.). Body height was measured with a wall-mounted stadiometer (to the nearest 5 mm, standing barefoot) and body mass index (BMI) could be calculated.

Mobile whole body MRI. For whole body magnetic resonance (MR) measurements a 1.5 Tesla MRI scanner (Magnetom AvantoTM, 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 down daily at each stopover of TEFR09, requiring daily checks and support of all technical systems [2].

Several MRI techniques have been described for 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 [20,21,22], 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 2D T1-weighted turbo spin echo sequence with an echo train length of 7 was applied (Siemens Ltd.). Measurement parameters were set to be: flip angle 180°, echo time 12 ms, repetition time 490 ms, slice thickness 10mm, slice distance 10mm, 5 slices per sequence,

field of view 1991 cm², matrix size 256 x 196 was recorded in a measuring time of 12 seconds (allowing breath holding examinations in the trunk area), bandwidth 120 Hz/pixel. 90-120 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 repositioning, 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].

Signal of intestinal content with short T1 can be interpreted as visceral adipose tissue (VAT) in absence of intraluminal gastroenteric nutrition fat (INF). If 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 to lose too much energy in progress 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 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 MR table and follow the breath commands exactly. These specific circumstances resulted in an image post-processing analysis being less automated than 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 start of TEFR09, ABM and INF together account for 13.2% of total adipose tissue. Due to continuous loss of adipose body tissue, this ratio rises up to 28.2% till the end of race. For visceral adipose tissue, the account for INF rises from 3% at 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 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): TV can be subdivided in total somatic volume (TSV) and total visceral volume (TVV, figure 2) or can be subdivided in total adipose tissue (TAT; without INF) and total lean tissue (TLT). TLT can be separated in somatic (SLT) and visceral lean tissue (VLT). Subtraction of ABM from TAT leads to total adipose soft tissue (TAST). TAST can be subdivided in 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 in 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). Total volume (TV), lean tissue (LT) and adipose soft tissue (AST) volume was calculated for

the upper and lower extremities (UE and LE) and for the trunk (TR), additionally: for nomenclature of specific segmented tissues see table 1.

Statistical Analysis. For data elaboration specific software were used: MicrosoftTM Office ExcelTM (Release 12.0.6665.5003, Microsoft Home and Student Suite, 2007, Microsoft Inc.) for data documentation, SPSS (IBMTM SPSSTM 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 [l] and relative differences [%] to start. For every measurement interval (t0 – 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 present graphically in line figures for finishers only. Calculated total changes (t5 vs. 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 on significance regarding volume changes of the specific tissue compartments during TEFR09, a univariate variance analysis (ANOVA) for repeated measurements was preferred (only subjects who got the whole body MRI at every measurement interval (t0-t5): n=12). Therefore, a common linear model for repeated measurements (with post hoc analysis on significance between the different times of measurement) was chosen. For correction of accumulation of the alpha niveau due to multiple testing (of hypothesis: “The means at stage intervals are significant 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 (homogeneity 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 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 on difference. For dependence analysis including all stages of and total TEFR09, analyses on difference between the dichotomous nominally scaled dependent variables of the sample finishing status (finisher/non-finisher: F/NF) regarding pre-race running performance history and regarding total, lean and adipose tissue volumes were done. 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 of distribution of the independent pre-race variables of performance (PRY, PRT, PRR),

Analysis on relationship. For analyses on relationships the Pearson correlation coefficient (CC_P) and Spearman-rho correlation coefficient (CC_S) were calculated for parametric and non-parametric parameters, respectively, with using bivariate (two-sided) or univariate (one-sided) testing as appropriate:

- BM vs. TV and its distribution throughout the race: bivariate CC_S
- Prerace performance vs. percentage total, lean and adipose volumes: univariate CC_S / CC_P
- Race performance vs. percentage total, lean and adipose volume_s: univariate CC_P

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 alpha level (p-value) of 0.05 was used to indicate

significance.

Results

Casuistry. Figure 3 shows the topographical mapping of changes of lean and adipose tissue of a subject (male, 32 yrs, 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 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.

TV vs. BM/BMI. 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/m² (SD 1.18 kg/m²), respectively (table 3). There was a high correlation (CC_S: 0.978, p<0.001) between BM [kg] and TV [l] regarding mean absolute value changes throughout the race (figure 4).

Percentage body composition. At start of TEFR09, 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 splits in mean VLT 12.3 vol% (SD 1.23 vol%) and mean VAT 2.9 vol% (SD 1.37 vol%). From these data the changes of mean volumes percentages of tissue compartments regarding the overall population of ultra-runners could be calculated for transcontinental MSUM races (figure 5).

Total volumes. Percentage volumes changes of TSV and TVV were not significant (table 3, figure 6). But for absolute volumes (TV, TSV, TVV), a significant change could be evaluated with a very high test power (table 4). Except TSV at first MI, significant decreases for TV, TSV and TVV could be shown at all MI throughout TEFR09 (figure 7). Paired comparison of MI after start showed no significant difference for TVV but partial differences for TV and TSV (figure 7).

TV. After more than 4000 km of running the mean TV showed a mean decrease of 9.5% (SD 5.1%, min -2.7%, max -17.9%) compared to start. Depending on the total sample the mean TV decrease for overall population of ultra-runners ranges between 8 to 11% (SE 1.5%); see figure 9. Looking only at the group of finishers, the absolute amount of TV loss in mean at the last MI was 6.1 l (SD 3.4 l, min -2.5 l, max -12.6 l) (figure 10). Mean loss of TV per km was 3.5 ml (SD 2.9 ml/km) in the beginning and got 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 11).

TSV. Relative decrease of TSV during TEFR09 showed a nearly similar curve as TV (figure 9), but was less pronounced (mean -9.4% after more than 4000 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 mean (SD 3.0 l, min -1.7 l, max -11.1 l) at the end of race (figure 10). This is consistent with a mean TSV loss of 1.3 ml/km (SD -0.7ml/km) at the end of TEFR09, starting with 2.7 ml/km (SD 2.7 ml/km) in first 8 stages of TEFR09 (figure 11).

TVV. Compared to TV and TSV, the relative decrease of TVV occurred much faster but ended in nearly similar amount with a mean of 10.0% (SD 4.9%, min -3.8%, max -19.3%) in a negative parabolic graph shape (see figure 9). 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 10). 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 11).

Adipose tissue. For adipose tissue, in total (TAT), somatic (SAST) and visceral (VAT), a significant change of absolute volumes (table 4) and percentage volumes (table 3) could be evaluated with a very high test power at the different MI during TEFR09. Significant decrease for TAT, SAST and VAT could be shown at all MI throughout TEFR09 compared to start and for TAT and SAST compared to first MI after start (stage 5 to 12) (figure 8). For other MI the paired comparison showed no significant change.

TAT. A continuous decrease of TAT occurred and ended in a relative mean loss of 41.3% (SD 8.0%, min -25.4%, max -53.2%) with a small SE of 2.3% (figure 9) at last MI. Looking at absolute loss of TAT, a finisher lost 5.3 l in mean (SD 2.6 l, min -2.7 l, max -9.8 l) until the end of race (figure 10). 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 TEFR09 (figure 11).

SAST. Relative SAST decrease compared to 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 4000 km (figure 9). 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 TEFR09 (figure 10). This corresponds to a mean loss of SAST of 1.1 ml/km (SD 0.5 ml/km) at the end of TEFR09 compared to 2.4 ml/km (SD 1.4 ml/km) at the start (figure 11).

VAT. The relative decrease of VAT occurred much more rapidly in mean and ended in a relative VAT volume loss of 64.5% (SD 15.9%, min -27.7%, max -88.8 %) at the end (figure 9) compared to start and a SE up to 4.6%. 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 10), which was nearly the same as absolute TVV loss. Therefore, the mean VAT volume loss per km was the same as for TVV in finishers (figure 11).

The subdivision of VAT in IAAT and MAT shows, that IAAT decreased a bit faster than VAT in total and ended in a relative volume loss 67.3% (SD 14.8%, min -31.7%, max -88.8%) at the end (figure 13). MAT initially decreased as rapid as IAAT resp. VAT but reached a plateau of 30% volume loss after nearly 1000 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 TEFR09 significantly without relevant changes in absolute volumes, respectively (table 3, figure 12); analysis of means of absolute volume showed no significant changes for total, somatic and visceral lean tissue at the different MI during TEFR09 (table 4).

TLT, SLT, VLT. TLT volume showed undulating relative changes during TEFR09 in mean compared to start. Just at the end after more than 4000 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 9). Due to nearly stable volume regarding VLT during TEFR09, SLT showed similar data during TEFR09 like TLT relative to 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 -0.9 l (SD 1.2 l, min 1.1 l, max -2.8 l); see figure 10. 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 11.

Segmental volume analysis. Significance of volume changes in the different body segments is shown in table 5: For the lower extremities the change of volumes was only significant for

SAST_{LE} but not for TV_{LE} and 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 (LT_{TR}, LT_{UE}). 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 lost 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 TEFR09, and in the trunk in the first third of the race, while in the arms lean tissue loss was detectable already at the first MI t1 (figure 14).

Finisher/Non-finisher. 45.5% of the subjects did not finish the race. Dropout rate of subjects compared to all race participants is shown in figure 15. The main reason (70%, n_i=7) for premature dropping out of the race was intolerable pain progress in the legs due to 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 thumb up to the forearm needing immediate surgical intervention.

Figure 16 shows distribution of percentage volumes for all tissue compartments at time of start (t0) and MI t1 (317-789 km) for finishers (n_F=12) and non-finishers (n_{NF}=10) of TEFR09. At both times the finisher group had significantly more percentage volume regarding total somatic tissue (mean TSV) than non-finishers of TEFR09 (at t0 +1.8%: 85.5 vol% vs. 84.0 vol%, at t1 +1.6%: 85.8% vs. 84.4%) and therefore significantly less percentage volume of mean TVV (at t0 -10.5%: 14.5 vol% vs. 16.0 vol%, at t1 -9.5%: 14.2 vol% vs. 15.6 vol%), table 6. The finisher group showed significant less adipose tissue volume percentage than non-finisher for TAT and VAT at t0 and t1, and also for SAST at t1 (table 6): At start, non-finishers had 71.5% more VAT volume percent (mean VAT at t0: 2.2 vol% vs. 3.8 vol%), 28.0% more SAST volume percent (mean SAST at t0: 15.0 vol% vs. 19.2 vol%) and in total 26.6% more TAT volume percent (mean TAT at t0: 20.6 vol% vs. 26.1 vol%) than finishers (table 6). At first MI t1 the difference between finisher and non-finisher was significant further on: non-finishers had 96.8% more VAT volume percent (mean VAT at t0: 1.6 vol% vs. 3.2 vol%), 39.7% more SAST volume percent (mean SAST at t0: 13.3 vol% vs. 18.5 vol%) and in total 34.9% more TAT volume percent (mean TAT at t0: 18.3 vol% vs. 24.7 vol%) than finishers (table 6). These differences for adipose tissue compartments were not detectable any more in the further racing run (t2-t5): either there are not enough numbers to treat in the group of non-finishers for further analysis on difference to finishers or no difference could be shown. Conversely, lean tissue difference of percentage volume was significant smaller in non-finisher compared to finisher for TLT (at t0: -6.9%, at t1: -7.8%) and SLT (at t0: -8.1%, at t1: -8.9%) (table 6). VLT showed no significant difference between finisher and non-finisher at any MI (t0-t5). Table 7 and figure 17 demonstrate a significant relative volume loss at MI t1 and t2 compared to 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 subjects group (table 8), every participant of TEFR09 had already finished nearly one UM and MSUM, but not every subjects had finished a single marathon. The endurance training extent one year and 3 months before TEFR09 varied also for training volume [km], time [h] and intensity [km/h] in the subject group (table 8). For number of finished marathons, UM and MSUM no difference between finisher and non-finisher could be evaluated (table 9). But regarding prerace training volume and intensity one year before TEFR09 and their 50km- and 24hr-race record, finishers had a significant higher prerace-performance compared to non-finishers (table 9). Only these five prerace-performance parameters (PRT_{Vol08/09}, PRT_{Int08},

$\text{PRR}_{50\text{km}}$, $\text{PRR}_{24\text{hr}}$) showed also a mainly high and medium correlation with 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 start and race performances of subjects at TEFR09 could be detected (figure 19). For SAST at the beginning of TEFR09 (stage 1 to 8) a significant correlation between percentage volume at start and cumulative performance is given, but only on a medium to low effect size. For TAT, TLT and SLT significance for such a correlation is shown at the first 12 to 15 stages and at the last third of TEFR09 on a medium effect size (figure 19). Correlation of percentage fat and lean volumes to performances at individual stages can only be shown for very few stages on middle to low effect size. For none of the investigated volumes the relative changes during TEFR09 show a significant correlation to performance.

Discussion

Nearly nothing is known about the influence of endurance burden on the specific changes of 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 [11,12,13,14]. 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 showed new data on 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 lower percentage body fat in female elite marathon runners. In obese patients ($\text{BMI} > 27 \text{ kg/m}^2$) Machann et al [25] found correlation of amount and distribution of adipose tissue to age (VAT increasing with age) and to gender (%SAT female > male, %VAT male > female). But they found no consistent differences in TAT profiles between the selected age groups for both females ($n=40$, mean age 45 yrs., SD 12 yrs., range 23-64 yrs.) and males ($n=40$, mean age 45 yrs., SD 12 yrs., range 24-65 yrs.) in their collective. Naturally, our collective of ultra-runners with comparable age distribution ($n=22$, mean age 49 yrs., SD 12 yrs., range 27-69 yrs.) showed a very low absolute mean volume of VAT at start of TEFR09 (female: 0.5 l, males 1.8 l) compared to obese patients (females 1.5 – 4 l, males 4-6.8 l) [25]. Statistical analysis on gender related differences was not possible (only 2 females) in our collective, but even these data indicate that a difference in VAT between male and female is not only visible in obese people, but is also visible in thin ultra-endurance athletes. Analysis on age showed no correlation to fat distribution at start (TAT, SAST, VAT) or to volume changes of lean and adipose tissue during TEFR09.

Changes in body composition. Different effects of endurance performances on body composition are described in literature. Beyond dispute is the fact that endurance performance leads to a decrease of body mass, mainly body fat. Body fat is the main energy-rich substrate for endurance performance [34,35,36,37]. Therefore, endurance exercise leads to a reduction of subcutaneous tissue as demonstrated in several field studies [34,36,38].

But specific influence on the 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] where body fat as well as skeletal muscle

seems to decrease [1,36,40,41,42]. In ultra-endurance performances with defined breaks body mass may remains stable [43,44,45] 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 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 TEFR09Some of them showed increases, some decreases. Knechtle et al. found the same individual differences for lean tissue in ultra runners during a 1200 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. With progression of the race this is leading to implementation of muscle tissue for energy provision.

Mass loss. Raschka and Plat a mean loss of 1.75 kg body mass in an ultra-endurance run over 1000 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 on 10 ultra runners (BIA) the mean loss of BM after a 1200 km footrace was also not significant, but the loss of 3.9 kg fat mass was [48]. Unfortunately authors gave no information about relative changes of fat and lean body mass. Our results determine, 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 stages MSUM [34,47].

The relation of water and lipid to the density of human adipose tissue ranges from 0.925 to 0.97 kg/l [49]. Assuming the middle (0.948 g/l), in our investigation the ultra-athletes lost a total fat mass (TAT) of 4.8 kg in mean (SAST 4.0 kg, VAT 0.8 kg), resembling the main part (91.8%) of body mass loss of 5.2kg. The lean tissue of the human body has a higher density than adipose tissue and muscle tissue (range 1.05 to 1.06 g/l) and varies with age [49,50], ranging between 1.10 and 1.11 g/l [51,52]. With these data and knowing the mean relative reduction of TLT (1.2%), the mean loss of lean body mass can be calculated about -0.67 kg at the end of TEFR09 in our subject group.

VAT. Mediastino-abdominal lipomatosis is described as being associated with exertional dyspnoe [65], non-insulin-dependent diabetes, type IV hyperlipidemia, and hyperuricemia. The abdominal VAT is an important independent risk factor for metabolic diseases in the older patient [53] and there is evidence that mainly abdominal VAT, which is morphologically and functionally different from abdominal SAST, is associated with metabolic syndrome (insulin resistance, dyslipidemia, hypertension, obesity) and hyperinsulinemia [54-59], as well as linked inflammatory diseases [60]. The real mean loss of relative IAAT while running a MSUM of nearly 4500 km, was more than two third compared to start in our collective (figure 13). We showed that endurance running also has an direct influence on intrathoracic fat, especially MAT, which decreased up to more than 40% in mean (figure 13). MAT is associated with hypertension, obesity, and iatrogenic Cushing syndrome [56,61-64].

Until now, a specific treatment for 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 9), a very good and effective way of metabolic disease risk reduction is endurance running. As VAT decreases much faster and more than SAST, our investigation indicates that three-compartment measurement methods like SF-analyses and BIA cannot give accurate assumption 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 long lasting running impact on fat and lean tissue *in vivo* [67].

F vs. NF. Fifty-five percent (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 at 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 myotendinofascial system.

In a 17day MSUM (1200 km) Knechtle et al found no differences between finishers and non-finishers regarding the anthropometric parameters body mass index, SF, CF, estimated skeletal muscle mass (estimated from SF and CF) and percent body fat (BIA) [68]. But with whole body MRI for differentiated body composition analysis, we found significant difference between finishers and non-finishers between both, somatic and visceral volumes and between adipose and lean tissue volumes at start and early beginning of the 4500 km MSUM TEFR09 (figure 16). Our results indicate, that the risk of dropping out of such an ultra-long transcontinental footrace is significantly higher, when total body fat percentage is more than 21-25% at start, in which the visceral fat percentage (VAT) shows more higher difference between finishers and non-finishers (71.5% in mean) than the somatic fat compartment (SAST: 28.0%). Because VAT is affected by endurance running burden most fast and most hardest 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 (50km-race), our results indicate, that VAT is the most sensible predictor for the risk of non-finishing a transcontinental MSUM like TEFR09. In ultra-runners there is not a high SAST or TAT, if VAT is low.

Although training a distance of 4500 to 5000 km is not possible, participants of such MSUM's 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 nearby 20-21%, training volumes of more than 100 km/week one year before the race and the performance intensity of 7.5 km/h in minimum allowing specific ultra-race records of less than 5 hrs in 50km-races or more than 178 km in 24hr-races. In other words, if these levels of prerace performances 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 myotendinofascial system of the lower extremities we tend to assume, that the mentioned interdependent parameters of body composition and prerace ultra-running performance, led to overuse injuries in the main stressed musculoskeletal organs, if they are not highly adapted as mentioned above: too less specific ultra endurance adaption and too much of VAT (and SAST) implements high risk of severe soft tissue overuse in the legs and mostly happens in the early phase (figure 15) of a transcontinental foot race.

But nearly every starter of TEFR09 showed more or less often overuse soft tissue problems of the myotendofascial 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 9 weeks can lead to these overuse syndromes without obligatory necessity of prevalent (intrinsic) factors like "overweight" (high VAT), suboptimal ultra-endurance pre-race 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 like pain resistance and personality traits are also relevant for finishing or non-finishing a transcontinental footrace [70]. One subject (male, 61 yrs.) has to stop the race after stage 38 (2.601 km run) due to a high tibial stress fracture which was detected in specific MRI at this

day (figure 15). The astonishing is not the stress fracture, because this can happen to every ultra runner when starting at a transcontinental race, but the fact that the major pain and massive performance (running velocity) loss started at stage 36 already. This subject ran 228 km (3 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 yrs.) 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 prarace 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 ultra-marathon performance needs specific running economy depending on ability of maximal oxygen uptake being highly correlated to citrate synthase activity and capillary network [71]. But these physiological factors are not investigated directly under race conditions in ultra-endurance events till now. Concerning this matter, only indirect parameters like 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 reasons 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 manifold 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, 10km) events [72]. Knechtle et al reported that anthropometry is not associated with performance in single, mono-stage UM races (24 hrs [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”: 1200 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 runner 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 3 races of Knechtle et al [76].

According to our results with a collective of 22 subjects and using gold standard whole body MRI, in athletes taking part at a 64-days MSUM there are no relevant correlations between total volume, percentage fat and lean volumes of different compartments at start and total race performances of subjects at TEFR09. For SAST a significant correlation between percentage volume at start and cumulative performance is given at the beginning of TEFR09 (stages 1 to 8), but only on a medium to low effect size. Correlation of percentage fat and lean volumes to performances at individual stages could only be shown in a few stages on middle 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 subcutaneous fat tissue amount (thickness or volume), being the main fat tissue compartment of the body, and performance in single or multiday ultramarathon races. But 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, like 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 had only a priority for a few of them. But 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 gets more significant influence, respectively.

Similar interpretation has to be done, when looking at segmental (somatic) tissue changes in the arm, legs and trunk during TEFR09. Like for adipose and lean total somatic and visceral volumes (figure 19) we also did an analysis on relationship between segmental tissue volume changes and race performance (results not demonstrated graphically) and detected only little to low medium effect size for correlations between SAST of all segments (UE, TR, LE) with cumulative race performance in first 8 stages of TEFR09. So in our investigation, all segments show similar significant relationship to race performance like SAST over all (figure 19) without any exceptional segment findings, which explains the inconstant finding in 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 at TEFR09 with muscle (SLT) increase in the legs, although they are already specialized on ultra running. These findings were not significant in mean. For the trunk mean increase of SLT could be detected also within 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 of 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 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 caloric 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 reasons: 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 are already shown by Millet et al [80,81,82]. 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 24hrs treadmill run [80]. Millet et al speculated that these changes in running mechanics are contributed to the overall limitation of the potentially harmful consequences of such a long-duration run on subjects' musculoskeletal system. But transferred to MSUM conditions, such changes of running mechanics may also be contributed to the necessity of the organism to optimize the running economy to a high-end level (as low energy consumption as possible) due to the massive negative energy burden a transcontinental race implicates. 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 the organism as well [85], even if the underlying mechanisms of relation between energy cost of running and step variability remains unclear till now. If running economy could not be sacrificed in ultramarathon [86,87] and the amount of running mechanic changes depends on duration of running and distance towards a fatigue state, respectively [81,85], it is even mandatory in transcontinental MSUM. Every subject of TEFR-project showed a significant loss of BM and TV throughout the race, independent from the prarace overall status of body composition and performance or nutrition behavior in the race. The massive negative energy burden of a 4500 km MSUM is indicated by the significant loss of the grey matter in the brain, also [88]. The analysis of specific lab markers of the required

blood and urine samples may give more data about the metabolic changes during TEFR09 in the nearby future.

Limitations. There was no general or individual nutrition plan offered or generated for the participants of TEFR09 or subjects of TEFR-project, respectively. The athletes got a breakfast and a dinner served in different locations at the stage destinations, but these meals were organized and oriented at the local conditions at the last minute. The food supply points during the stages also offered daily changing products and the athletes take additional individual food on their own throughout the way [2]. Therefore, documentation and measurement of nutrition and caloric intake was not really possible and a stringent documentation of nutrition by the subjects implicated the risk of compliance problems. Whole body mobile MRI protocols did not measure ectopic fat such as intracellular fat of organs (e.g. liver) and muscles (IMCL). For IMCL measurement specific mobile ¹H-MR-spectroscopy of muscles of the lower legs were implemented in TEFR-project [2], but 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 of 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 the half of their adipose soft tissue and even lean tissue volume decreased (mainly skeletal muscle tissue) and without any exception, every subject showed a significant loss of body volume. This indicates that running an MSUM of nearly 4500 km without any day 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 race during the first third in a MUSM due to overuse injuries of the myotendinofascial system of the legs. But 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 like bioelectrical impedance analysers and skinfold-equations cannot give estimations about relationship between visceral and somatic compartments and therefore cannot measure the most sensitive anthropometric predictor of non-finishing a MSUM: VAT. Running economy is mandatory for transcontinental MSUM races, and even in well trained ultra-athletes such events lead to further adaption of running mechanics and to metabolic changes as performance analysis compared to body composition changes throughout the race indicates.

Financial disclosure. This work is supported in part by the German Research Association (DFG: “Deutsche Forschungsgemeinschaft”), under Grants SCHU 2514/1-1 and SCHU 2514/1-2. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding was received for this study.

Competing interests. The authors declare that they have no financial or non-financial competing interests. There are no financial or non-financial competing interests of other people or organizations influencing our interpretation of data or presentation of information.

Author status. All authors of this manuscript had substantial contribution to conception and design or acquisition, analysis and interpretation of data; all revised it critically for important

intellectual content and did final approval of the version to be published: Schütz UHW conceived the study, implemented the project, participated in the data collection (MRI measurements), data evaluation with statistical analysis and drafted the manuscript. Billich C participated in the implementation of the project, in the data collection (MRI measurements) and in the implementation of the project. König K participated in the data evaluation. Würslin C participated in the data evaluation. Wiedelbach H participated mainly in the data collection (MRI measurements) and in the implementation of the project.. Brambs HJ participated in the implementation of the project. Machann J participated in the design of the study, its technical implementation and data evaluation. All authors read and approved the final manuscript.

Acknowledgement. We really want to thank all the athletes of TEFR09 who took part at this project. Considering their immense physical and mental stress they showed an extraordinary compliance on every day of the race.

Literature / References

1. Knechtle B, Enggist A, Jehle T: **Energy turnover at the Race across America (RAAM): a case report.** *Int J Sports Med* 2005, **26**:499–503.
2. Schütz UH, Schmidt-Trucksäss A, Knechtle B, Machann J, Ehrhardt M, Wiedelbach H, Freund W, Gröninger S, Brunner H, Schulze I, Brambs HJ, Billich C: **The Transeurope Footrace Project: Longitudinal data acquisition in a cluster randomized mobile MRI observational cohort study on 44 endurance runners at a 64-stage 4,486km transcontinental ultramarathon.** *BMC Med* 2012, **19**:10:78.
3. **Transeurope Footrace** [<http://www.transeurope-footrace.org>]
4. Schulze I: *TransEurope-FootRace 2009: Bari - Nordkap - 4.487,7 km in 64 Tagesetappen.* Engelsdorfer Verlag; 1st edition; 2010
5. Brodie DA, Stewart AD: **Body composition measurement: a hierarchy of methods.** *J Pediatr Endocrinol Metab* 1999, **12(6)**:801-816.
6. Lee RC, Wang Z, Heo M, Ross R, Janssen I, Heymsfield SB: **Total-body skeletal muscle mass: development and cross-validation of anthropometric prediction models.** *Am J Clin Nutr* 2000, **72(3)**:796-803.
7. Janssen I, Heymsfield SB, Baumgartner RN, Ross R: **Estimation of skeletal muscle mass by bioelectrical impedance analysis.** *J Appl Physiol* 2000, **89(2)**:465-71.
8. Chan DC, Watts GF, Barrett PHR, Burke V: **Waist circumference, waist-to-hip ratio and body mass index as predictors of adipose tissue compartments in men.** *QJM* 2003, **96**:441–447.
9. Gualdi-Russo E, Toselli S: **Influence of various factors on the measurement of multifrequency bioimpedance.** *Homo* 2002, **53(1)**:1-16.
10. Lee SY, Gallagher D: **Assessment methods in human body composition.** *Curr Opin Clin Nutr Metab Care* 2008, **11(5)**:566-572.
11. Daniel JA, Sizer PS Jr, Latman NS: **Evaluation of body composition methods for accuracy.** *Biomed Instrum Technol* 2005, **39**:397–405.
12. Vasudev S, Mohan A, Mohan D, Farooq S, Raj D, Mohan V: **Validation of body fat measurement by skin folds and two bioelectric impedance methods with DEXA – the Chennai Urban Rural Epidemiology Study (CURES-3).** *J Assoc Physicians India* 2004, **52**:877–881.
13. Broeder CE, Burrhus KA, Svanevik LS, Volpe J, Wilmore JH: **Assessing body composition before and after resistance or endurance training.** *Med Sci Sports Exerc* 1997, **29**:705–712.

14. Vogt FM, Ruehm S, Hunold P, de Greiff A, Nuefer M, Barkhausen J, Ladd SC: **Rapid total body fat measurement by magnetic resonance imaging: quantification and topography.** *Rofo* 2007, **179**(5):480-486.
15. Ross R, Léger L, Morris D, de Guise J, Guardo R: **Quantification of adipose tissue by MRI: relationship with anthropometric variables.** *J Appl Physiol* 1992, **72**(2):787-795.
16. Thomas EL, Saeed N, Hajnal JV, Brynes A, Goldstone AP, Frost G, Bell JD: **Magnetic resonance imaging of total body fat.** *J Appl Physiol* 1998, **85**(5):1778-1785.
17. Ludescher B, Machann J, Eschweiler GW, Vanhöfen S, Maenz C, Thamer C, Claussen CD, Schick F: **Correlation of fat distribution in whole body MRI with generally used anthropometric data.** *Invest Radiol* 2009, **44**(11):712-719.
18. Abate N, Burns D, Peshock RM, Garg A, Grundy SM: **Estimation of adipose tissue mass by magnetic resonance imaging: validation against dissection in human cadavers.** *J Lipid Res* 1994, **35**(8):1490-1496.
19. Ross R, Shaw KD, Martel Y, de Guise J, Avruch L: **Adipose tissue distribution measured by magnetic resonance imaging in obese women.** *Am J Clin Nutr* 1993, **57**:470-475.
20. Poon CS, Szumowski J, Plewes DB, Ashby P, Henkelman RM: **Fat/water quantitation and differential relaxation time measurement using chemical shift imaging technique.** *Magn Reson Imaging* 1989, **7**:369-382.
21. Lunati E, Marzola P, Nicolato E, Sbarbati A: **In-vivo quantitative hydrolipidic map of perirenal adipose tissue by chemical shift imaging at 4.7 Tesla.** *Int J Obes Relat Metab Disord* 2001, **25**:457-461.
22. Schick F, Machann J, Brechtel K, Strempfer A, Klumpp B, Stein DT, Jacob S: **MRI of muscular fat.** *Magn Reson Med* 2002, **47**(4):720-727.
23. Huang TY, Chung HW, Wang FN, Ko CW, Chen CY: **Fat and water separation in balanced steady-state free precession using the Dixon method.** *Magn Reson Med* 2004, **51**:243-247.
24. Donnelly LF, O'Brien KJ, Dardzinski BJ, Poe SA, Bean JA, Holland SK, Daniels SR: **Using a phantom to compare MR techniques for determining the ratio of intraabdominal to subcutaneous adipose tissue.** *AJR Am J Roentgenol* 2003, **180**:993-998.
25. Machann J, Thamer C, Schnoedt B, Haap M, Haring HU, Claussen CD, Stumvoll M, Fritzsche A, Schick F: **Standardized assessment of whole body adipose tissue topography by MRI.** *J Magn Reson Imaging* 2005, **21**(4):455-462.
26. Würslin C, Machann J, Rempp H, Claussen C, Yang B, Schick F: **Topography mapping of whole body adipose tissue using a fully automated and standardized procedure.** *J Magn Reson Imaging* 2010, **31**(2):430-439.
27. Stephens MA. Test of fit for the logistic distribution based on the empirical distribution function. *Biometrika* 1979; **66**(3):591-595.
28. D'Agostino RB, Belanger A, D'Agostino RB Jr: **A Suggestion for Using Powerful and Informative Test of Normality.** *The American Statistical Association* 1990, **44**(4):316-321.
29. Yazici B, Yolacan S: **A comparison of various tests of normality.** *J Stat Comput Simul* 2007, **77**(2):175-183.
30. Cohen J: **A power primer.** *Psychol Bull* 1992, **112**(1):155-159.
31. Boettcher M, Machann J, Stefan N, Thamer C, Häring HU, Claussen CD, Fritzsche A, Schick F: **Intermuscular adipose tissue (IMAT): association with other adipose tissue compartments and insulin sensitivity.** *J Magn Reson Imaging* 2009, **29**(6):1340-1345.

32. Gray DS, Fujioka K, Colletti PM, Kim H, Devine W, Cuyegkeng T, Pappas T: **Magnetic-resonance imaging used for determining fat distribution in obesity and diabetes.** *Am J Clin Nutr* 1991, **54**(4):623-627.
33. Bale P, Rowell S, Colley E: **Anthropometric and training characteristics of female marathon runners as determinants of distance running performance.** *J Sports Sci* 1985, **3**(2):115-126.
34. Raschka C, Plath M: **Body fat compartment and its relationship to food intake and clinical chemical parameters during extreme endurance performance.** *Schweiz Z Sportmed* 1992, **40**:13-25.
35. Reynolds RD, Lickteig JA, Deuster PA, Howard MP, Conway JM, Pietersma A, deStoppelaar J, Deurenberg P: **Energy metabolism increases and regional body fat decreases while regional muscle mass is spared in humans climbing Mt. Everest.** *J Nutr* 1999, **129**:1307-1314.
36. Helge JW, Lundby C, Christensen DL, Langfort J, Messonnier L, Zacho M, Andersen JL, Saltin B: **Skiing across the Greenland icecap: divergent effects on limb muscle adaptations and substrate oxidation.** *J Exp Biol* 2003, **206**:1075-1083.
37. Frykman PN, Harman EA, Opstad PK, Hoyt RW, DeLany JP, Friedl KE: **Effects of a 3-month endurance event on physical performance and body composition: the G2 trans-Greenland expedition.** *Wilderness Environ Med* 2003, **14**:240-248.
38. Höchli D, Schneiter T, Ferretti G, Howald H, Claassen H, Moia C, Atchou G, Belleri M, Veicsteinas A, Hoppeler H: **Loss of muscle oxidative capacity after an extreme endurance run: The Paris-Dakar Foot-Race.** *Int J Sports Med* 1995, **16**:343-346.
39. Knechtle B, Knechtle P, Andonie JL, Kohler G: **Influence of anthropometry on race performance in extreme endurance triathletes: World Challenge Deca Iron Triathlon 2006.** *Br J Sports Med* 2007, **41**(10):644-648.
40. Bircher S, Enggist A, Jehle T: **Effects of an extreme endurance race on energy balance and body composition: a case study.** *J Sports Sci Med* 2006, **5**:154-162.
41. Lehmann M, Huonker M, Dimeo F: **Serum amino acid concentrations in nine athletes before and after the 1993 Colmar Ultra Triathlon.** *Int J Sports Med* 1995, **16**:155-159.
42. Knechtle B, Bircher S: **Changes in body composition during an extreme endurance run.** *Praxis* 2005, **94**:371-377.
43. Dressendorfer RH, Wade CE: **Effects of a 15-d race on plasma steroid levels and leg muscle fitness in runners.** *Med Sci Sports Exerc* 1991, **23**:954-958.
44. Nagel D, Seiler D, Franz H, Leitzmann C, Jung K: **Effects of an ultra-long-distance (1000 km) race on lipid metabolism.** *Eur J Appl Physiol* 1989, **59**:16-20.
45. Väänänen II, Vihko V: **Physiological and psychological responses to 100 km crosscountry skiing during 2 days.** *J Sports Med Phys Fitness* 2005, **45**:301-305.
46. Raschka C, Plath M, Cerull R, Bernhard W, Jung K, Leitzmann C: **The body muscle compartment and its relationship to food absorption and blood chemistry during an extreme endurance performance.** *Z Ernährungswiss* 1991, **30**:276-288.
47. Knechtle B, Salas OF, Andonie JL, Kohler G: **Effect of a multistage ultra-endurance triathlon on body composition: World Challenge Deca Iron Triathlon 2006.** *Br J Sports Med* 2008, **42**(2):121-125.
48. Knechtle B, Duff B, Schule I, Kohler G: **A multi-stage ultra-endurance run over 1,200 km leads to a continuous accumulations of total body water.** *J Sports Sci Md*. 2008, **7**:357-364.
49. Martin AD, Daniel MZ, Drinkwater DT, Clarys JP: **Adipose tissue density, estimated adipose lipid fraction and whole body adiposity in male cadavers.** *Int J Obes Relat Metab Disord* 1994, **18**(2):79-83.

50. Mernagh JR, Harrison JE, Krondl A, McNeill KG, Shepard RJ: **Composition of lean tissue in healthy volunteers for nutritional studies in health and disease.** *Nutrition Res* 1986, **6(5)**:499-507.
51. Wells JC, Williams JE, Chomtho S, Darch T, Grijalva-Eternod C, Kennedy K, Haroun D, Wilson C, Cole TJ, Fewtrell MS: **Pediatric reference data for lean tissue properties: density and hydration from age 5 to 20 y.** *Am J Clin Nutr* 2010, **91(3)**:610-8.
52. Schutte JE, Townsend EJ, Hugg J, Shoup RF, Malina RM, Blomqvist CG: **Density of lean body mass is greater in blacks than in whites.** *J Appl Physiol* 1984, **56(6)**:1647-649.
53. Brochu M, Starling RD, Tchernof A, Matthews DE, Garcia-Rubi E, Poehlman ET: **Visceral adipose tissue is an independent correlate of glucose disposal in older obese postmenopausal women.** *J Clin Endocrinol Metab* 2000, **85(7)**:2378-2384.
54. Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000;106:473–481.
55. Wajchenberg BL: **Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome.** *Endocr Rev* 2000, **21**:697–738.
56. Sironi AM, Gastaldelli A, Mari A, Ciociaro D, Postano V, Buzzigoli E, Ghione S, Turchi S, Lomabardi M, Ferrannini E: **Visceral fat in hypertension: influence on insulin resistance and β-cell function.** *Hypertension* 2004, **44**:127–133.
57. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L; INTERHEART Study Investigators: **Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study.** *Lancet* 2004, **364(9438)**:937-952.
58. Montague CT, O'Rahilly S: **The perils of portliness: causes and consequences of visceral adiposity.** *Diabetes* 2000, **49(6)**:883-888.
59. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G: **Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance.** *Am J Physiol Endocrinol Metab* 2001, **280(5)**:E745-751.
60. Marette A: **Molecular mechanisms of inflammation in obesity-linked insulin resistance.** *Int J Obes Relat Metab Disord* 2003, **27 Suppl 3**:S46-48.
61. Sharma AM: **Mediastinal fat, insulin resistance, and hypertension.** *Hypertension* 2004, **44(2)**:117-118.
62. Lee WJ, Fattal G. Mediastinal lipomatosis in simple obesity. *Chest*. 1976; 70: 308–309.
63. Price JE Jr., Rigler LG: **Widening of the mediastinum resulting from fat accumulation.** *Radiology* 1970, **96**:497–500.
64. Stummvoll HK, Wolf A, Pinggera WF, Lobenwein E, Seidl G: **Rare localizations of fat deposition in iatrogenous Cushing's syndrome.** *Munch Med Wochenschr* 1976, **118**: 445–446.
65. Enzi G, Digito M, Marin R, Carraro R, Baritussio A, Manzato E: **Mediastino-abdominal lipomatosis: deep accumulation of fat mimicking a respiratory disease and ascites. Clinical aspects and metabolic studies in vitro.** *Q J Med* 1984, **53**:453–463.
66. Kopelman PG: **The effects of weight loss treatments on upper and lower body fat.** *Int J Obes Relat Metab Disord* 1997, **21(8)**:619-625.
67. Martin AD, Janssens V, Caboor D, Clarys JP, Marfell-Jones MJ: **Relationships between visceral, trunk and whole-body adipose tissue weights by cadaver dissection.** *Ann Hum Biol* 2003, **30(6)**:668-677.
68. Knechtle B, Duff B, Schulze I, Rosemann T, Senn O: **Anthropometry and pre-race experience of finishers and nonfinishers in a multistage ultra-endurance run--Deutschlandlauf 2007.** *Percept Mot Skills* 2009, **109(1)**:105-118.

69. Freund W, Weber F, Billich C, Schütz UH: **The foot in multistage ultra marathon runners: Experience in a cohort study of 22 participants of the Trans Europe Footrace project with mobile MRI.** *BMJ Open* 2012, **2**:2(3).
70. Freund W, Weber F, Billich C, Birklein F, Breimhorst M, Schütz UH: **Ultra marathon runners are different. Investigations into pain tolerance and personality traits of participants of the TransEurope FootRace 2009.** *Pain Pract* 2013, **2** [Epub ahead of print]
71. Millet GY, Banfi JC, Kerherve H, Morin JB, Vincent L, Estrade C, Geyssant A, Feasson L: **Physiological and biological factors associated with a 24 h treadmill ultra-marathon performance.** *Scand J Med Sci Sports* 2011, **21**(1):54-61.
72. Maldonado S, Mujika I, Padilla S: **Influence of body mass and height on the energy cost of running in highly trained middle- and long-distance runners.** *Int J Sports Med* 2002, **23**(4):268-272.
73. Knechtle B, Wirth A, Knechtle P, Zimmermann K, Kohler G: **Personal best marathon performance is associated with performance in a 24-h run and not anthropometry or training volume.** *Br J Sports Med* 2009, **43**(11):836-9.
74. Hoffman MD: **Anthropometric Characteristics of Ultramarathoners.** *Int J Sports Med* 2008, **29**(10):808-811.
75. Arrese AL, Ostáriz ES: **Skinfold thicknesses associated with distance running performance in highly trained runners.** *J Sports Sci* 2006, **24**(1):69-76.
76. Knechtle B, Knechtle P, Rosemann T, Senn O: **What is associated with race performance in male 100-km ultra-marathoners - anthropometry, training or marathon best time?** *J Sports Sci* 2011, **23**:1-7.
77. Knechtle B, Knechtle P, Rosemann T, Lepers R: **Predictor variables for a 100 km race time in female ultra-marathoners.** *Medicina Sportiva* 2010, **14**(4):214-220.
78. Tanaka K, Matsuura Y: **A multivariate analysis of the role of certain anthropometric and physiological attributes in distance running.** *Ann Hum Biol* 1982, **9**(5):473-482.
79. Tomaszewski M, Charchar FJ, Przybycin M, Crawford L, Wallace AM, Gosek K, Lowe GD, Zukowska-Szczechowska E, Grzeszczak W, Sattar N, Dominiczak AF: **Strikingly low circulating CRP concentrations in ultramarathon runners independent of markers of adiposity: how low can you go?** *Arterioscler Thromb Vasc Biol* 2003, **23**: 1640–1644.
80. Morin JB, Samozino P, Millet GY: **Changes in running kinematics, kinetics, and spring-mass behavior over a 24-h run.** *Med Sci Sports Exerc* 2011, **43**(5):829-836.
81. Degache F, Guex K, Fourchet F, Morin JB, Millet GP, Tomazin K, Millet GY: **Changes in running mechanics and spring-mass behaviour induced by a 5-hour hilly running bout.** *J Sports Sci* 2013, **31**(3):299-304.
82. Borrani F, Candau R, Perrey S, Millet GY, Millet GP, Rouillon JD: **Does the mechanical work in running change during the VO₂ slow component?** *Med Sci Sports Exerc* 2003, **35**(1):50-57.
83. Brisswalter J, Legros P, Durand M: **Running economy, preferred step length correlated to body dimensions in elite middle distance runners.** *J Sports Med Phys Fitness* 1996, **36**: 7–15.
84. Svedenhag J, Sjödin B: **Body-mass-modified running economy and step length in elite male middle- and long-distance runners.** *Int J Sports Med* 1994, **15**: 305–310.
85. Candau R, Belli A, Millet GY, Georges D, Barbier B, Rouillon JD: **Energy cost and running mechanics during a treadmill run to voluntary exhaustion in humans.** *Eur J Appl Physiol Occup Physiol* 1998, **77**(6):479-485.

86. Millet GP: **Economy is not sacrificed in ultramarathon runners.** *J Appl Physiol* 2012, **113**(4):686.
87. Millet GY, Hoffman MD, Morin JB: **Sacrificing economy to improve running performance--a reality in the ultramarathon?** *J Appl Physiol.* 2012, **113**(3):507-509.
88. Freund W, Faust S, Birklein F, Gaser C, Wunderlich AP, Mueller M, Billich C, Juchems MS, Schmitz BL, Groen G, Schütz UH: **Substantial and reversible brain gray matter reduction but no acute brain lesions in ultramarathon runners: experience from the TransEurope-FootRace Project.** *BMC Med* 2012, **21**:10:170.

Figures

Figure 1: Semiautomatic separation of adipose bone marrow: selected slices from whole body MRI of a 32 year old male finisher of TEFR09. I: ankles, II: middle of lower legs, III: knees, IV: middle of upper legs, V: hip/pelvis, VI: umbilical level, VII: upper abdomen, VIII: heart/mediastinum, IX: shoulder girth, X: elbows. Left row: before start (t0), green: TLT, red: SAST, yellow: VAT+INF, blue: ABM. Right row: after 4,120km of running (t5), green: TLT, red: SAT (=SAST+ABM), yellow: VAT+INF.

Figure 2: Semiautomatic separation of somatic and visceral volume (right row) and intraluminal nutrition fat (left row): selected slices from whole body MRI of a 32 year old male finisher of TEFR09. V: hip/pelvis, VI: umbilical level, VII: upper abdomen, VIII: heart/mediastinum . Left row: before start (t0), green: SLT, red: TSAT, grey: TVV. Right row: after 4,120km of running (t5), green: TLT, red: SAT (=SAST+ABM), yellow: VAT, blue: INF.

Figure 3: Topography of lean and adipose tissue changes in a 32 year old male finisher.

Figure 4: Comparison of total body volume vs. body mass during TEFR09 (finisher, $n_F=12$)

Figure 5: Adipose and lean volume percentage distribution in finishers at start and end of TEFR09 (finisher, $n_F=12$).

Figure 6: Changes of somatic and visceral percentage volumes during TEFR09 (finisher, $n_F=12$).

Figure 7: Post hoc analysis of significance of paired comparison of total volume measurements at different time intervals (finisher, $n_F=12$)

Figure 8: Post hoc analysis on significance of paired comparison of total fat tissue measurements at different time intervals (finisher, $n_F=12$)

Figure 9: Mean and standard error of relative changes of specific tissue volume during TEFR09 compared to start (total sample, $n=22$)

Figure 10: Absolute changes of specific tissue volume during TEFR09 compared to start (finisher, $n_F=12$)

Figure 11: Absolute volume changes per km compared to start in finisher group (finisher, $n_F=12$).

Figure 12: Changes of adipose and lean tissue percentage volumes during TEFR09 (finisher, $n_F=12$).

Figure 13: Relative changes of visceral adipose volume during TEFR09 compared to start (total sample, n=22)

Figure 14: Relative changes of segmented tissue volume (UE, TR, LE) during TEFR09 compared to start (finisher, n_F=12)

Figure 15: Dropout rate

Figure 16: Difference between F and NF regarding percentage tissue volumes before start of TEFR09 and at MI t1.

Figure 17: Difference between F and NF regarding relative volume changes of tissue compartments at first measurement interval (t1) of TEFR09.

Figure 18: Correlation (one-tailed test) of percentage volumes and prarace-performance.

Figure 19: Correlation of adipose and lean volumes at start with performance at TEFR09.

Tables

Table 1: Abbreviations of compartments after tissue mapping and segmentation with mobile whole body MRI data sets (T2*).

Abbreviation	Description, Definition
Tissue mapping in specific compartments	
ABM	Adipose bone marrow
TV	Total volume of the body (from ankle to wrist), without INF
TVV	Total visceral volume: includes intrathoracic and intraabdominal volume.
TSV	Total somatic volume (TV without TVV).
TLT	Total lean tissue
VLT	Visceral lean tissue: includes lean tissue of intrathoracic and intraabdominal organs.
SLT	Somatic lean tissue: mostly muscles
TAT	Total adipose tissue (without INF)
SAT	Somatic adipose tissue (TAT without VAT)
TAST	Total adipose soft tissue (TAT without ABM)
SAST	Somatic adipose soft tissue (TAT without ABM and VAT)
SCAT	Subcutaneous adipose tissue (SAST without IMAT)
IMAT	Intermuscular adipose tissue (SAST without SCAT)
VAT	Visceral adipose tissue (IAAT + MAT without INF)
IAAT	Intraabdominal adipose tissue: retroperitoneal and intraperitoneal (mesenteric, omental) fat depots (without INF)
MAT	Intrathoracic, mainly mediastinal adipose tissue
INF	(Undigested) intraluminal nutrition fat in the gastrointestinal tract
Tissue mapping of body segments	
TV-LE	Total volume of lower extremities (apex trochanter major to ankle joint)
TV-TR	Total volume of trunk (acromion to apex of trochanter major)
TV-UE	Total volume of upper extremities (wrist to acromion level)
LT-LE	Lean tissue volume of lower extremities (apex trochanter major to ankle joint)
LT-TR	Lean soft tissue volume of trunk (acromion to apex of trochanter major)
LT-UE	Lean soft tissue volume of upper extremities (wrist to acromion level)
AST-LE	Adipose soft tissue volume of lower extremities (apex trochanter major to ankle joint)
AST-TR	Adipose soft tissue volume of trunk (acromion to apex of trochanter major)
AST-UE	Adipose soft tissue volume of upper extremities (wrist to acromion level)

Table 2: Mean volumes [l] of body compartments and segments (all subjects).

Abbreviation	t0: start	t1: 317-789km	t2: 1003-1635 km	t3: 2516-2738 km	t4: 3234-3669 km	t5: 4037-4440 km
Tissue mapping in specific compartments						
TV	57.70	56.63	54.75	53.39	54.16	52.65
TVV	8.74	8.34	7.93	7.64	7.65	7.58
TSV	48.97	48.29	46.82	45.74	46.51	45.08
TLT	44.34	44.61	45.07	45.38	46.20	45.52
VLT	7.02	7.04	7.01	7.10	7.14	7.12
SLT	37.32	37.56	38.06	38.28	39.06	38.40
TAT	13.36	12.02	9.68	8.00	7.96	7.14
SAT	11.65	10.73	8.76	7.46	7.45	6.68
TAST	11.52	10.19	7.76	6.05	6.01	5.19
SAST	9.81	8.89	6.84	5.51	5.50	4.73
VAT	1.71	1.30	0.92	0.54	0.51	0.45
IAAT	1.55	1.15	0.77	0.39	0.36	0.33
MAT	0.17	0.15	0.15	0.15	0.14	0.13
INF	0.05	0.21	0.36	0.57	0.70	0.86
ABM	1.98	1.93	1.92	1.95	1.95	1.95
Tissue mapping of body segments						
TV-LE	23.51	22.49	21.46	21.65	22.02	20.60
TV-TR	28.83	28.63	27.86	26.57	27.03	27.00
TV-UE	5.79	5.89	5.75	5.73	5.64	5.60
TLT-LE	17.51	16.97	16.82	17.45	17.83	16.97
TLT-TR	23.22	23.12	23.46	23.02	23.39	23.39
TLT-UE	4.79	4.62	4.70	4.81	4.74	4.77
SAST-LE	4.84	4.36	3.43	2.96	2.96	2.40
SAST-TR	4.31	3.47	2.12	1.02	0.99	0.81
SAST-UE	0.98	0.95	0.72	0.57	0.54	0.48

Table 3: BM and BMI loss during TEFR09

n			distance run [km]	BM [kg]						BMI [kg/m ²]						
				all			F		NF			all			F	
all	F	NF	mean	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	me
22	12	10	0.00	71.75	11.13	72.07	11.23	71.38	11.60	23.58	2.55	23.36	2.58	23		
15	10	5	116.1	72.43	10.82	71.38	12.48	74.54	7.14	23.33	2.47	22.98	2.75	24		
20	11	9	354.2	70.53	11.67	72.20	11.63	68.49	12.07	23.18	2.68	23.26	2.58	23		
21	12	9	618.5	70.20	11.20	70.51	11.04	68.08	12.22	22.98	2.59	22.84	2.35	22		
20	12	8	893.8	68.73	10.96	70.04	10.60	66.76	11.92	22.68	2.51	22.70	2.23	22		
17	12	5	1168.2	69.52	10.65	69.83	10.78	68.76	11.56	22.72	2.31	22.62	2.23	22		
16	12	4	1456.9	68.18	10.72	68.93	10.70	65.93	12.09	22.20	2.21	22.33	2.25	21		
15	12	3	1754.0	68.39	10.61	68.65	10.45	67.37	13.59	22.26	2.08	22.24	2.12	22		
15	12	3	2026.1	67.64	10.93	67.94	10.57	66.43	14.78	22.01	2.18	22.01	2.19	22		
14	11	3	2294.2	68.01	10.81	68.19	10.75	67.37	13.47	22.11	2.13	22.03	2.18	22		
14	12	2	2594.5	67.05	10.83	67.44	10.30	64.70	18.53	21.73	2.05	21.85	2.18	20		
14	12	2	2852.0	68.88	9.85	67.66	10.14	76.20	2.12	22.17	2.19	21.92	2.09	23		
13	12	1	3134.2	68.45	9.95	67.73	10.04	77.00		21.90	1.95	21.95	2.03	21		
13	12	1	3401.7	68.58	10.07	67.78	10.06	78.30		21.93	1.91	21.95	1.99	21		
12	12		3724.8	67.35	10.16	67.35	10.16			21.82	2.15	21.82	2.15			
12	12		4010.8	67.48	9.91	67.48	9.91			21.87	2.06	21.87	2.06			
12	12		4307.3	66.83	10.50	66.83	10.50			21.64	2.12	21.64	2.12			

Table 3: Significance of topographic tissue volume changes regarding percentage volume [vol. %].

		Mauchly-Test	Univariate ANOVA ^b				Multivariate ANOVA ^c			
[vol. %]			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	-	-	-	-	
TLT ^a		0.000	44.605	0.000	1.000	51.592	0.000	1.000		
SLT ^a		0.000	43.573	0.000	1.000	19.556	0.001	1.000		
VLT ^a		0.005	22.980	0.000	1.000	6.699	0.013	0.884		
TAT ^a		0.000	44.655	0.000	1.000	52.762	0.000	1.000		
SAST ^a		0.000	44.721	0.000	1.000	57.109	0.000	1.000		
VAT ^a		0.000	23.718	0.000	1.000	8.598	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”

Table 4: Significance of topographic tissue volume changes regarding absolute volume measurements [l] ($n_F=12$).

[l]	Mauchly-Test		Univariate ANOVA ^b			Multivariate ANOVA ^c		
	p-value	F value	p value	test power	F value	p value	test power	
TV^a	0.000	20.162	0.000	0.999	5.758	0.020	0.828	
TSV^a	0.001	18.607	0.000	0.999	4.937	0.030	0.762	
TVV^a	0.000	21.516	0.000	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^a	0.000	32.274	0.000	1.000	18.577	0.001	1.000	
SAST^a	0.000	32.692	0.000	1.000	15.624	0.001	0.998	
VAT^a	0.000	21.607	0.000	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”

Table 5: Significance of segmental volume changes regarding repeated absolute volume [l] measurements ($n_F=12$).

[l]	Mauchly-Test		Univariate ANOVA ^b			Multivariate ANOVA ^c		
	p-value	F-value	p value	test power	F-value	p value	test power	
TV_LE	0.003	7.763	0.002	0.946	2.341	0.149	0.423	
TV_TR^a	0.133	6.349	0.003	0.918	-	-	-	
TV_UE^a	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	3.534	0.21	0.769	-	-	-	
SLT_UE	0.700	3.128	0.29	0.734	-	-	-	
SAST_LE^a	0.000	63.294	0.000	1.000	20.644	0.000	1.000	
SAST_TR^a	0.000	17.388	0.000	0.996	13.387	0.002	0.995	
SAST_UE^a	0.000	16.151	0.000	0.987	3.389	0.041	0.584	

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”

TV_LE^a	0.001	27.504	0.000	1.000	13.942	0.002	0.996
TV_TR^a	0.133	6.349	0.003	0.918	-	-	-
TV_UE	0.003	7.763	0.002	0.946	2.341	0.149	0.423

Table 6: Analysis on difference of percentage volume [vol%] between F/NF at start (t0) and MI (t1, t2) for total, lean and adipose tissue compartments.

	t0: n_F=12, n_{NF}=10		t1: n_F=12, n_{NF}=8		t2: n_F=12, n_{NF}=6	
	mean diff. of percentage volume [vol.%]	p value (ITT*)	mean diff. of percentage volume [vol.%]	p value (ITT*)	mean diff. of percentage volume [vol.%]	p value (ITT*)
TSV	1.52	0.005^a	1.35	0.036^a	1.07	0.182
TVV	-1.52	0.005^a	1.35	0.036^a	1.07	0.182
TLT	4.04	0.031^a	6.39	0.015^a	5.05	0.088
SLT	4.16	0.014^a	6.18	0.010^a	4.65	0.058
VLT	0.032	0.953	0.21	0.707	0.39	0.604
TAT	-5.47	0.031^a	-6.39	0.015^a	-5.04	0.088
SAST	-4.21	0.080	-5.27	0.032^a	-3.64	0.184
VAT	-1.60	0.006^a	-1.56	0.021^a	-1.46	0.060

t0: at start of TEFR09, t1: stage 5-12 (317-789 km), t2: stage 15-24 (1,003-1,635 km)

*ITT: t-test for independent samples

a: mean differences between finishers and non-finishers are significant

Table 7: Analysis on difference of relative volume changes [%] at MI t1 and t2 compared to start between F/NF for total, lean and adipose tissue compartments.

	t1 vs. t0: n_F=11, n_{NF}=9		t2 vs. t0: n_F=11, n_{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	0.0332	-0.18	0.913
SLT	1.34	0.319	-0.21	0.917
VLT	0.07	0.923	-0.24	0.545
TAT	-4.86	0.086	-9.52	0.078
SAST	-6.93	0.031^a	-14.27	0.046^a
VAT	-8.92	0.191	-8.88	0.276

t0: at start of TEFR09, t1: stage 5-12 (317-789 km), t2: stage 15-24 (1,003-1,635 km)

*ITT: t-test for independent samples

a: mean differences between finishers and non-finishers are significant

Table 8: Endurance running history of subjects (n=22)

	mean	SD	95%-percentile	range
Years of regular endurance running	17.4	7.6	6.3-31.8	6-32
M finished [n]	123,1	218,2	2-297.8	2-988
UM finished [n]	90,8	68	11.3-248.9	11-255
MSUM finished [n]	6,3	2,9	1.3-13.6	1-14
endurance training extent 2008 (one year before TEFR)				
Annual running distance [km/y]	5468	1720	3000-9000	2580-9152
PRT08 volume [km/week]	105.1	32.4	50.8-175.4	50-176
PRT08 time [h/week]	12.5	3.1	7.1-19.6	7-20
PRT08 intensity [km/h]	8.3	1.5	6.5-10.9	7-11
endurance training extent last 2 months before TEFR				
Total running distance [km]	898	267	500-1260	500-1500
PRT09 volume [km/week]	110.5	33.8	60.5-186	60-190
PRT09 time [h/week]	13.2	3.5	8-21.6	8-22

Table 9: Distribution type and analyses on difference between F/NF regarding pre-race performance indices

	values		mean (SD)	test on normal distribution Shapiro-Wilk [29]	test on difference F/NF				
	valid	missing							
	n	n	[%]	F	NF	statistic	p	test type*	p**
PRY [yrs]	21	1	4.5	16.5	18.7	0.936	0.185 ^a	ITT	0.530
n _F M [n]	19	3	13.6	81.5	194.3	0.481	0.000	MWU	0.211
n _F UM [n]	22	0	0	94.8	86.0	0.858	0.005	MWU	0.895
n _F MSUM [n]	22	0	0	7.0	5.4	0.936	0.165 ^a	ITT	0.146
PRT _{Vol08} [km/week]	21	1	4.5	117.9 (34.3)	88.0 (20.8)	0.959	0.505 ^a	ITT	0.032^b
PRT _{Time08} [h/week]	21	1	4.5	13.0	11.9	0.971	0.761 ^a	ITT	0.427
PRT _{Int08} [km/h]	22	0	0	9.0 (1.47)	7.5 (0.93)	0.909	0.044	MWU	0.040^b
PRT _{Vol09} [km/week]	21	1	4.5	126.1 (35.2)	89.7 (17.4)	0.956	0.435 ^a	ITT	0.010^b
PRT _{Time09} [h/week]	21	1	4.5	14.2	12.0	0.947	0.303 ^a	ITT	0.159
PRR _M [h]	15	7	31.8	3.1	3.1	0.851	0.018	MWU	0.676
PRR _{50km} [h]	9	13	59.1	4.3 (0.57)	5.1 (0.26)	0.914	0.343 ^a	ITT	0.026^b
PRR _{100km} [h]	17	5	22.7	9.5	10.4	0.933	0.248 ^a	ITT	0.241
PRR _{6hr} [km]	13	9	40.9	67.9	59.7	0.918	0.238 ^a	ITT	0.218
PRR _{12hr} [km]	10	12	54.5	99.9	87.3	0.940	0.548 ^a	ITT	0.558
PRR _{24hr} [km]	16	6	27.3	199.8 (22.5)	168.5 (26.0)	0.961	0.672 ^a	ITT	0.036^b

*ITT: independent t-test; MWU: Mann-Whitney-U test; ** bilateral (asymptotic) test

a: significance not shown ($p > 0.05$): normal distribution accepted; b: mean differences between finishers and non-finishers are significant



II



III



IV



V



VI



VII



VIII



IX



X

Figure 1



V



VI

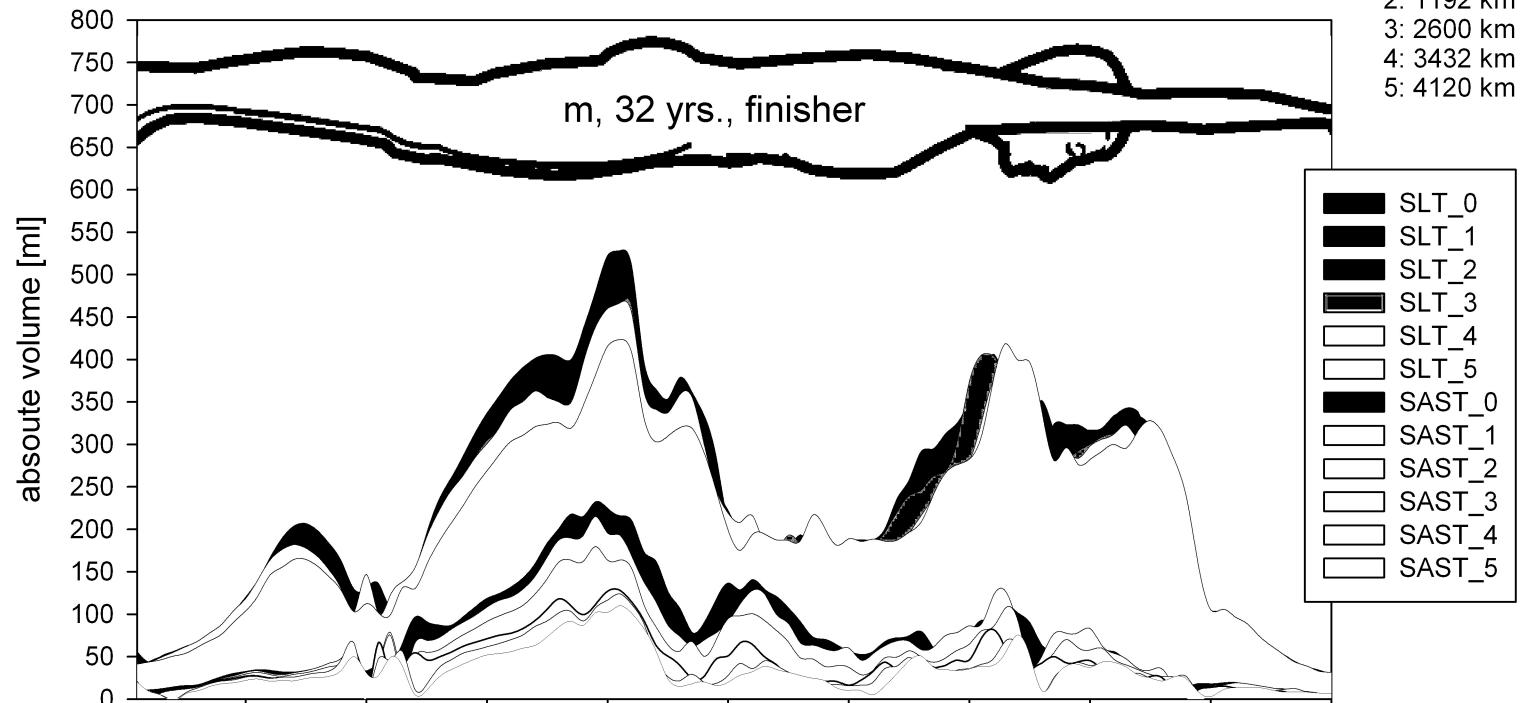
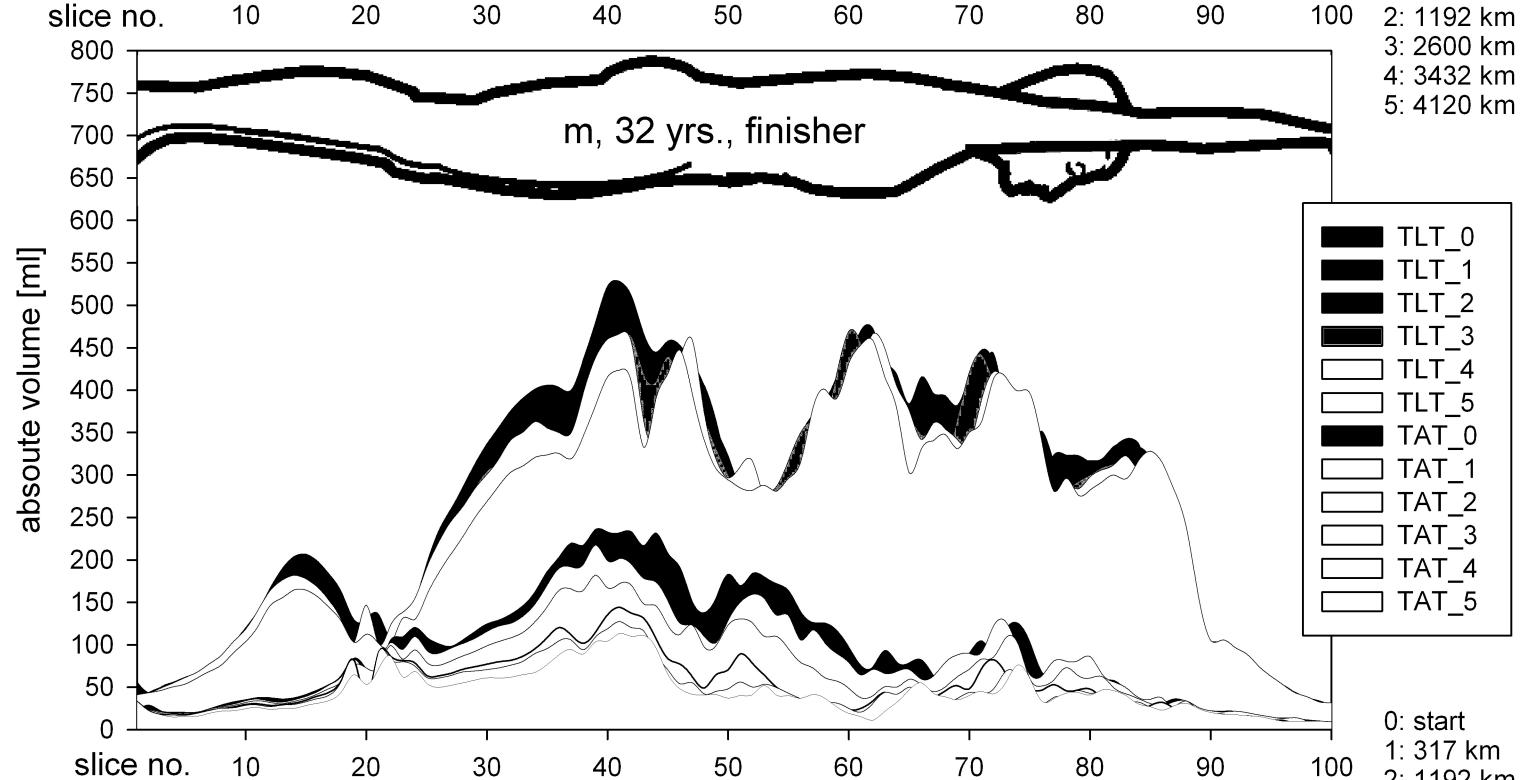
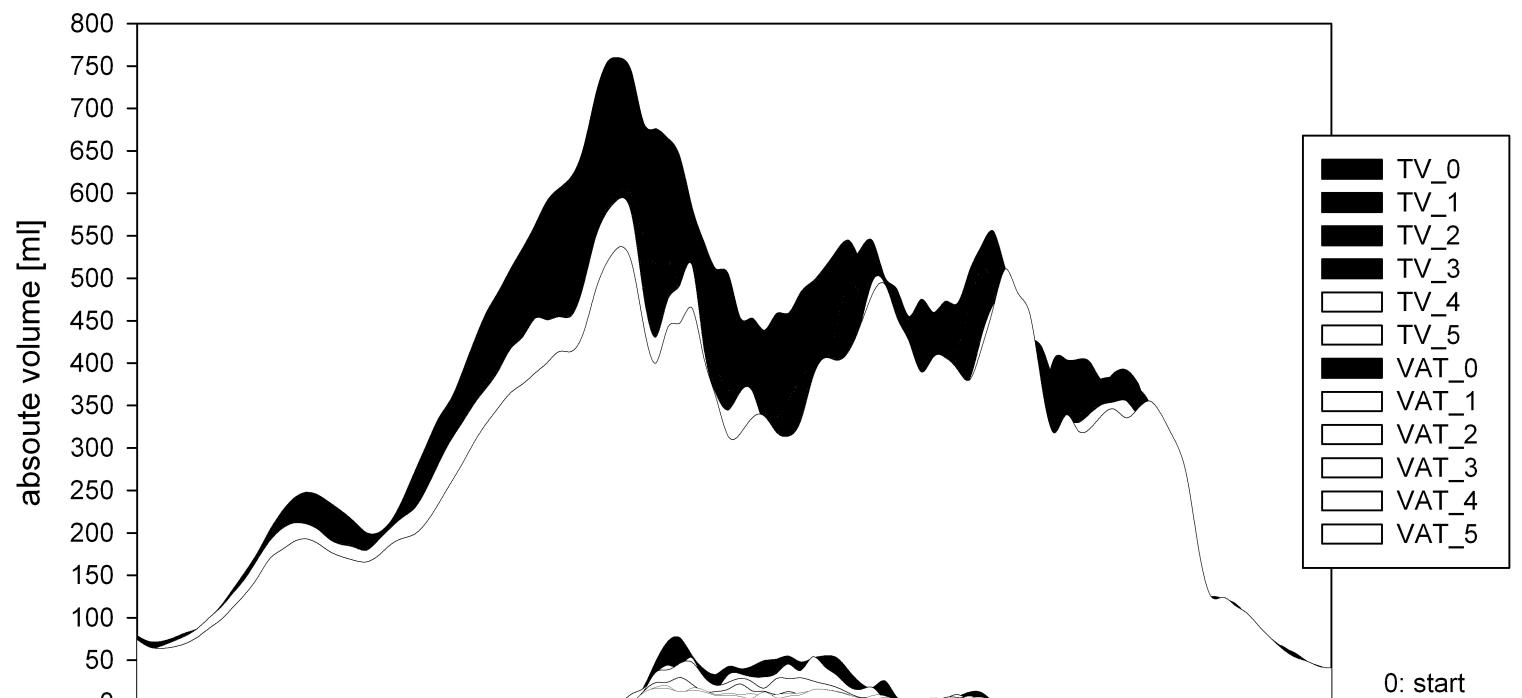


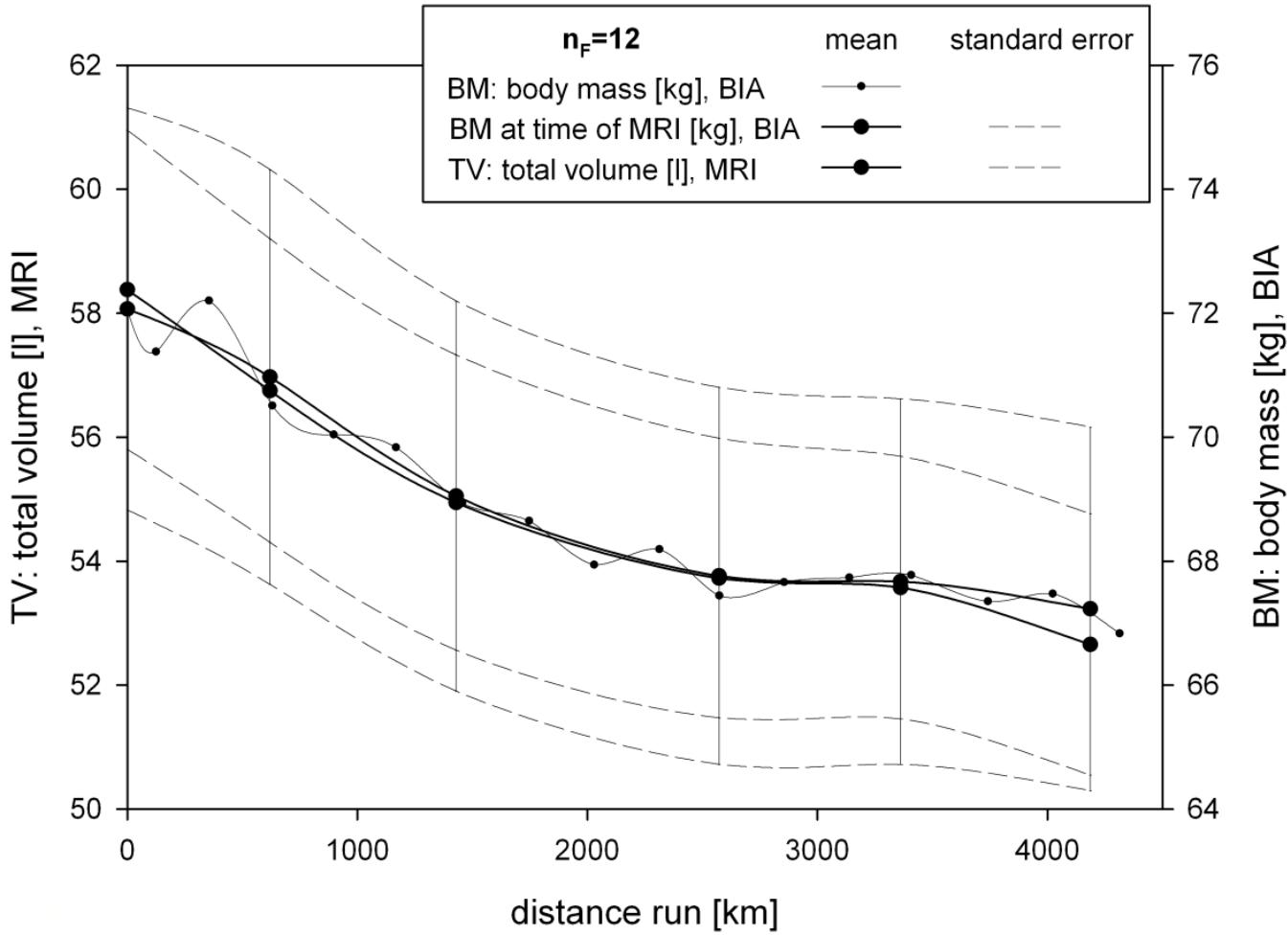
VII



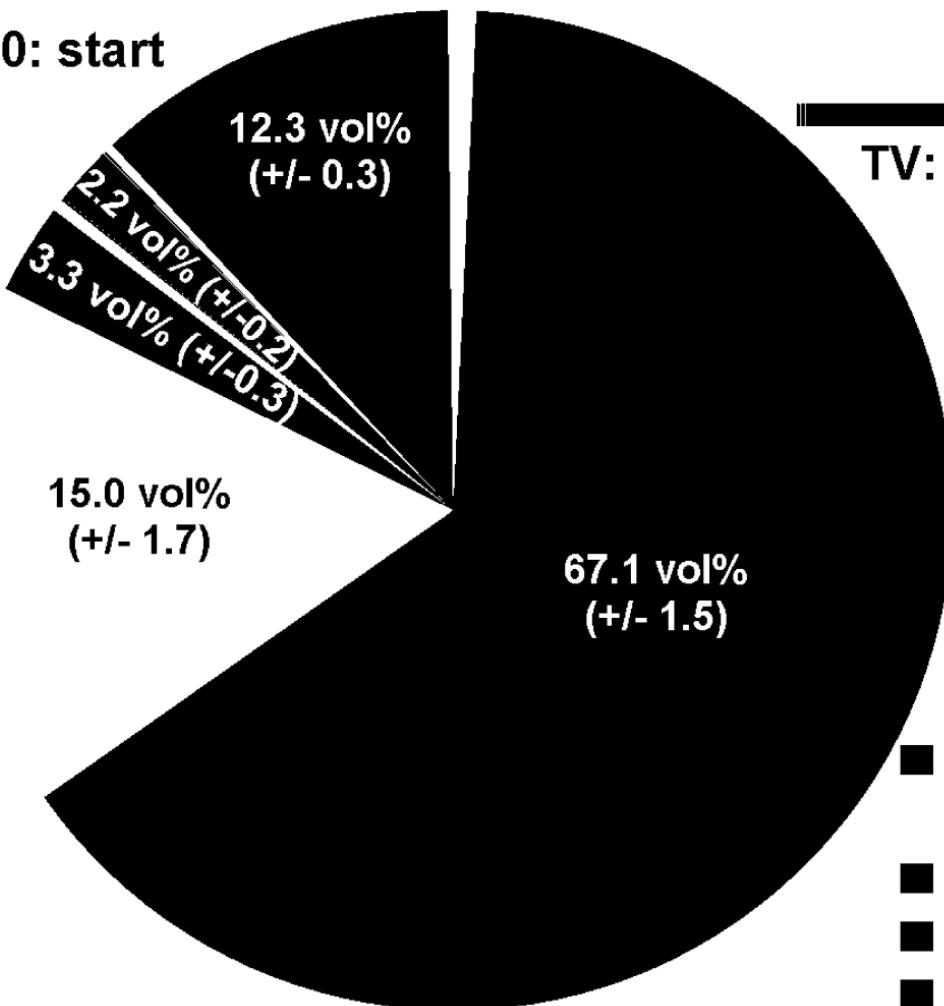
VIII

Figure 2

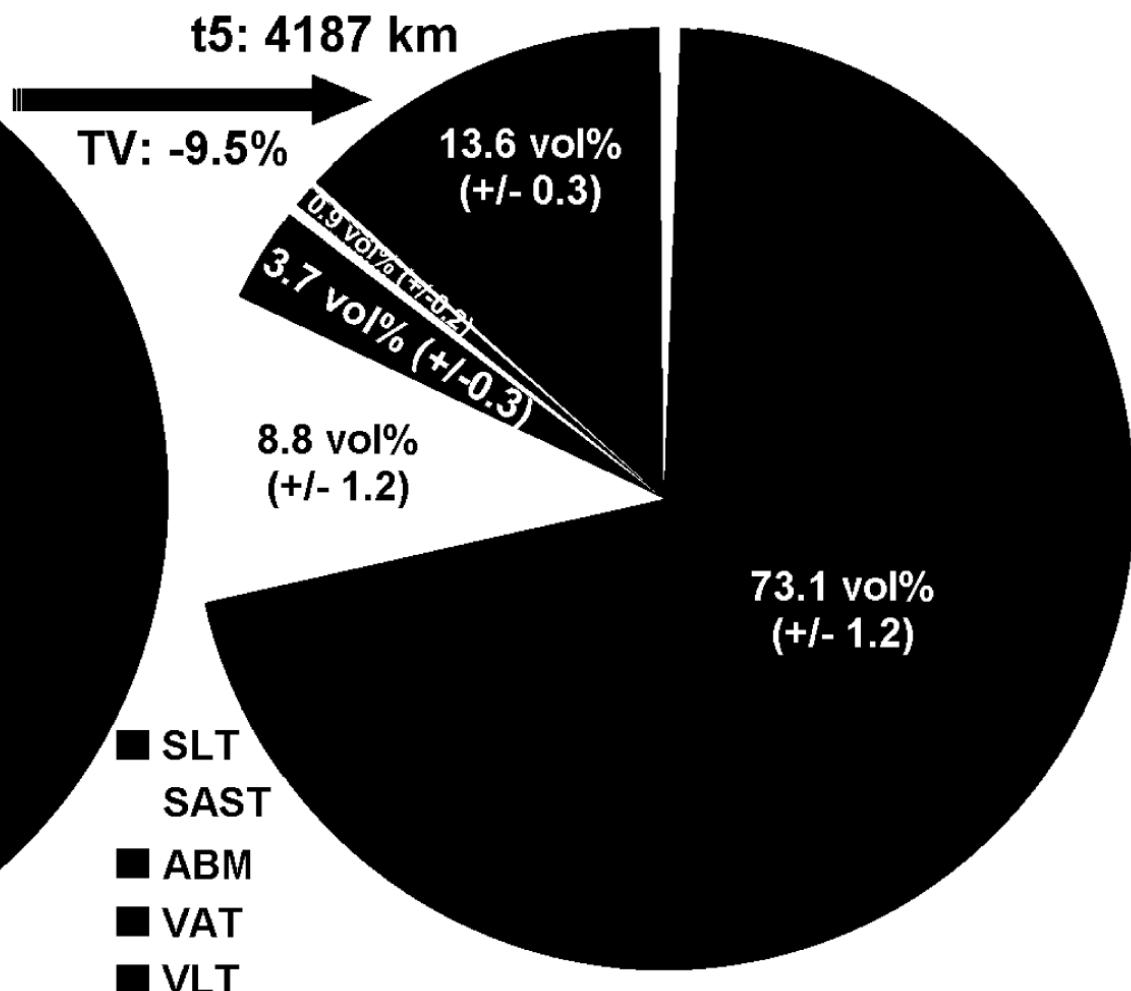




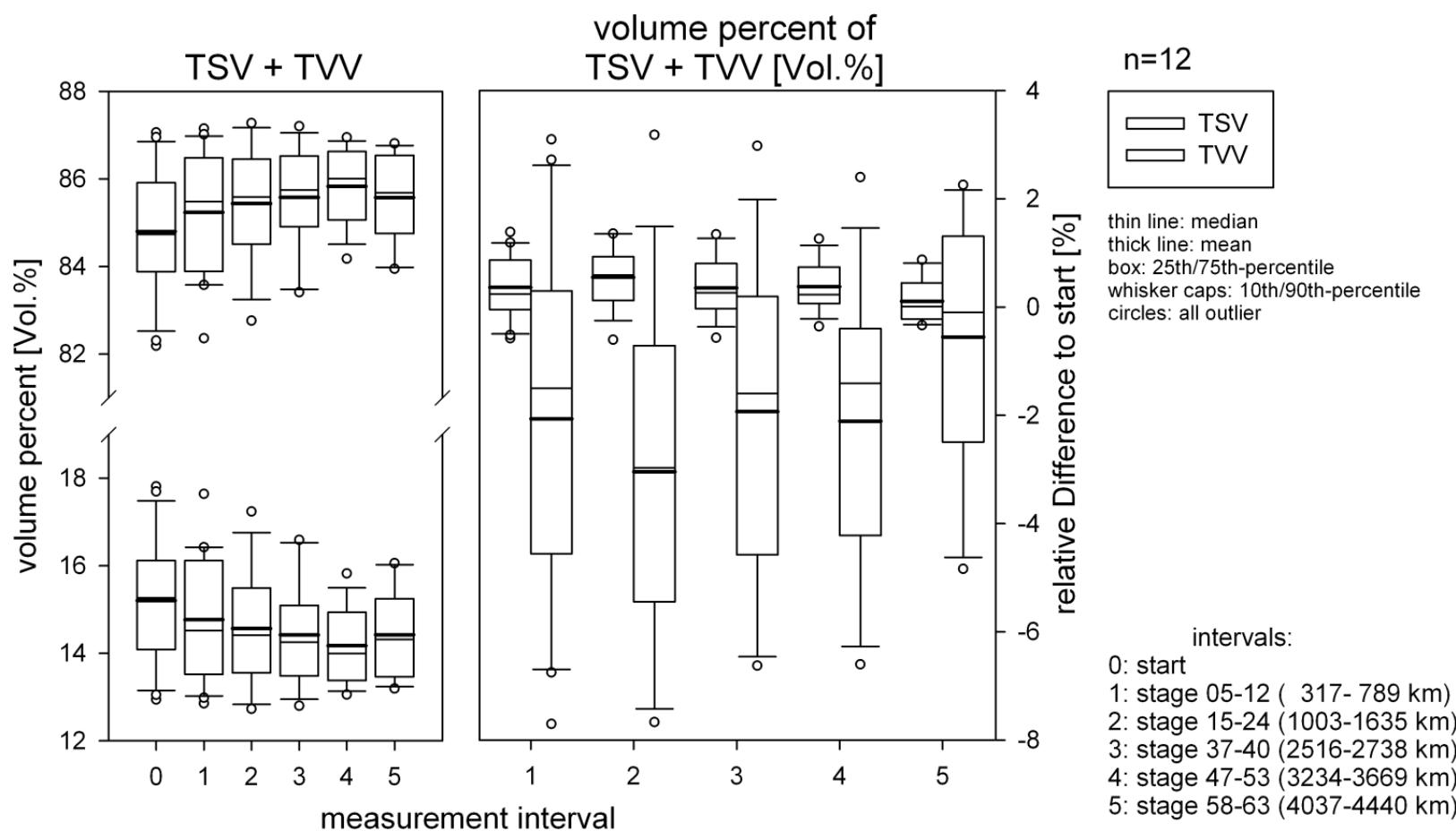
t0: start

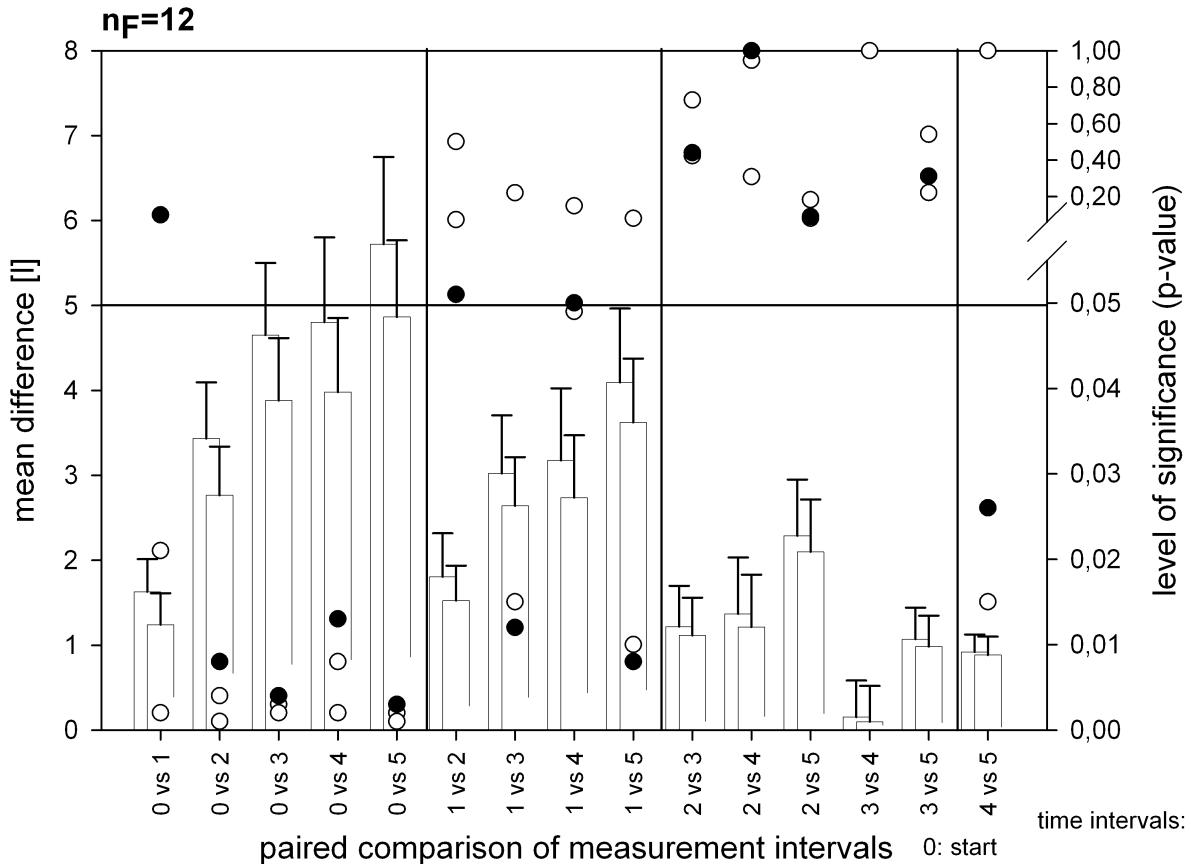


t5: 4187 km



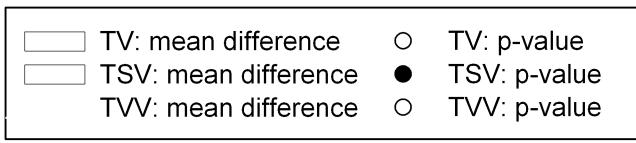
- SLT
- SAST
- ABM
- VAT
- VLT



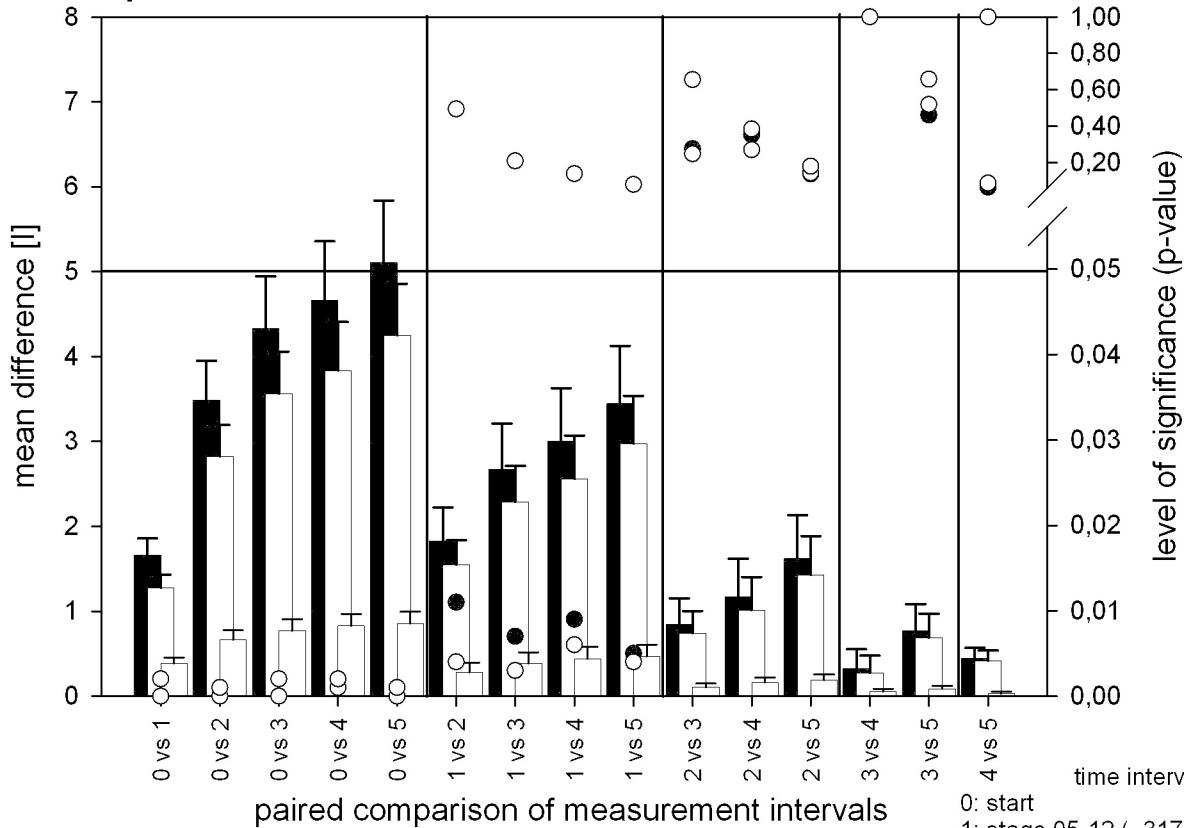


paired comparison of measurement intervals

- time intervals:
- 0: start
 - 1: stage 05-12 (317- 789 km)
 - 2: stage 15-24 (1003-1635 km)
 - 3: stage 37-40 (2516-2738 km)
 - 4: stage 47-53 (3234-3669 km)
 - 5: stage 58-63 (4037-4440 km)



$n_F=12$

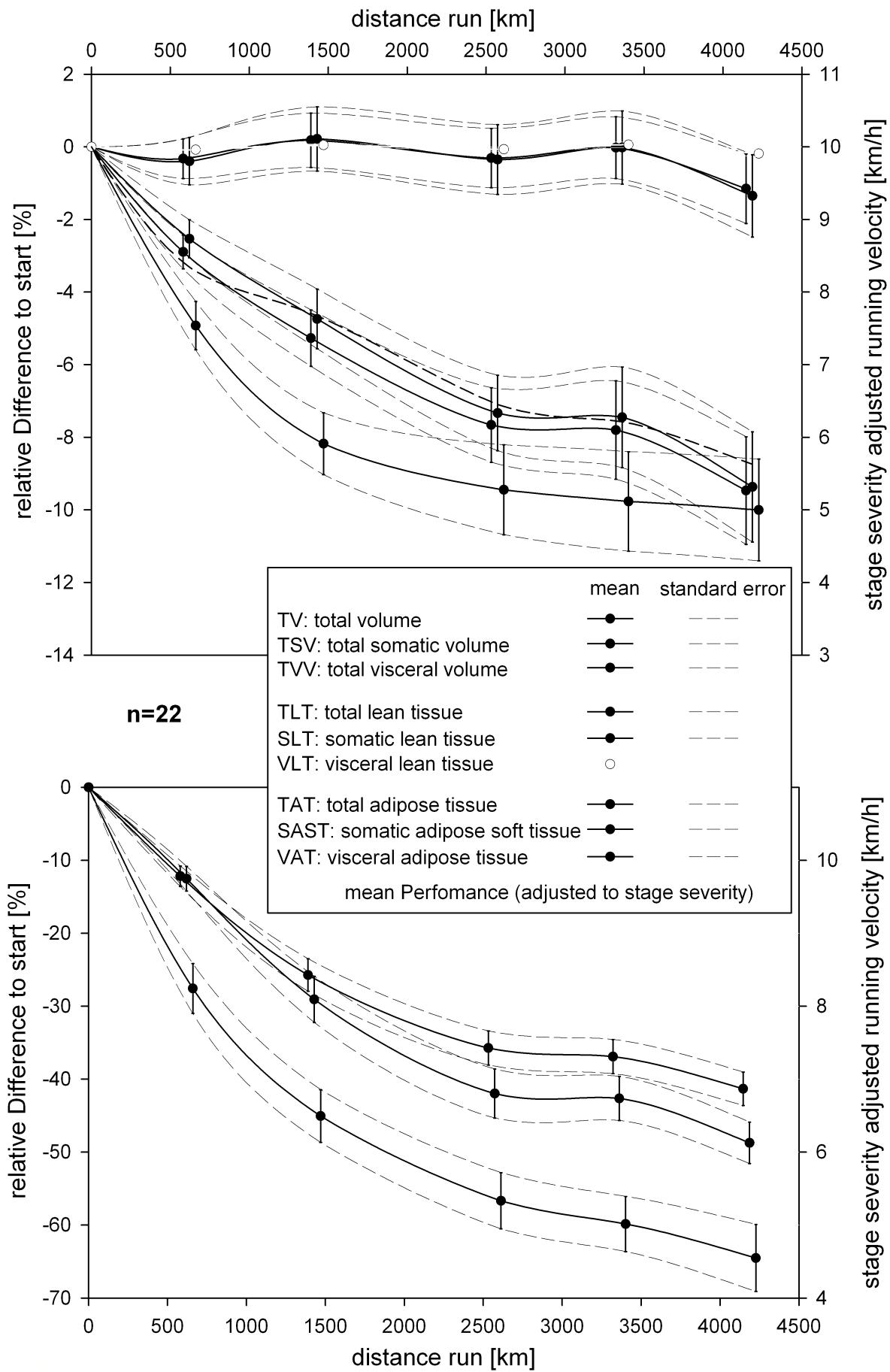


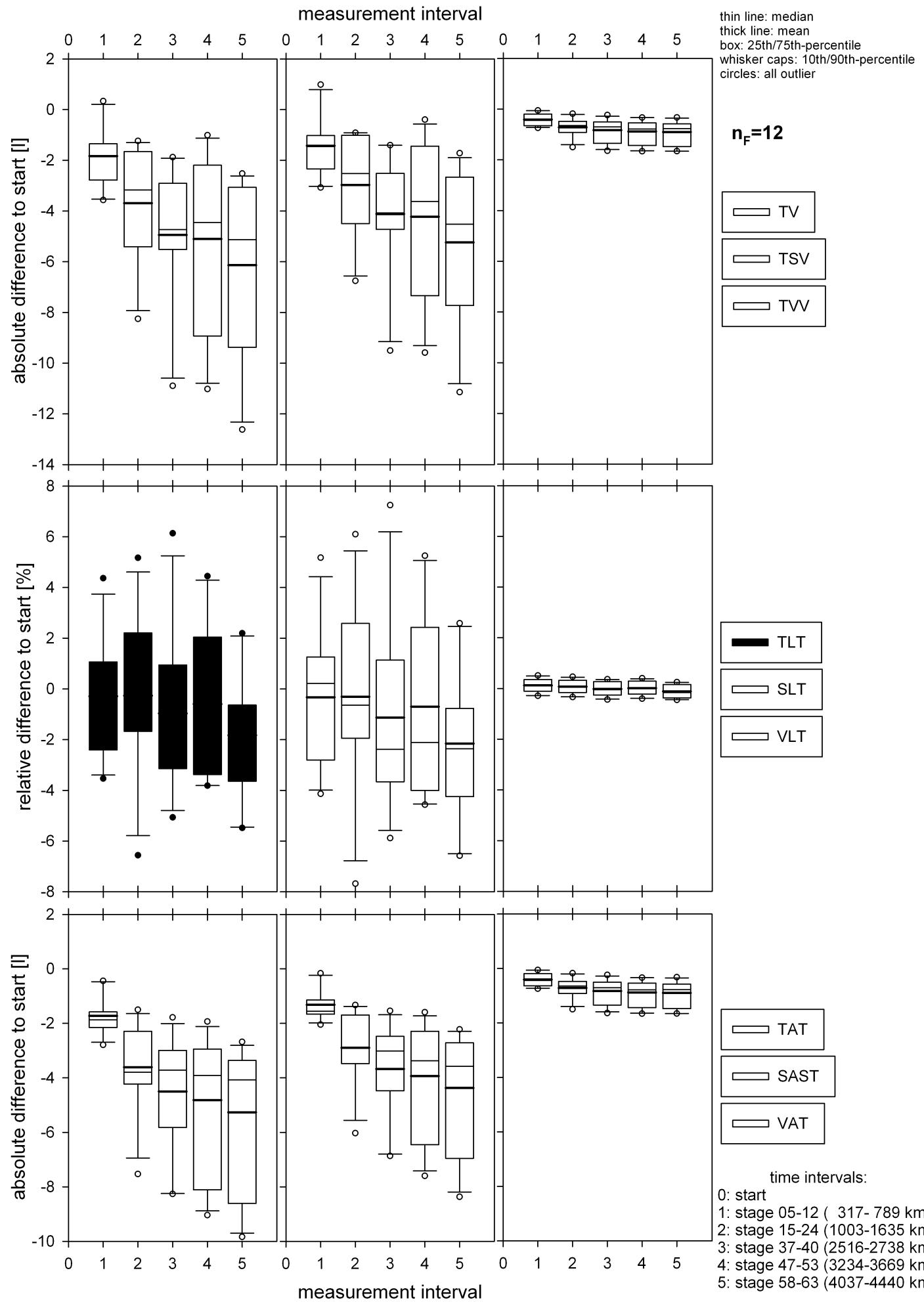
paired comparison of measurement intervals

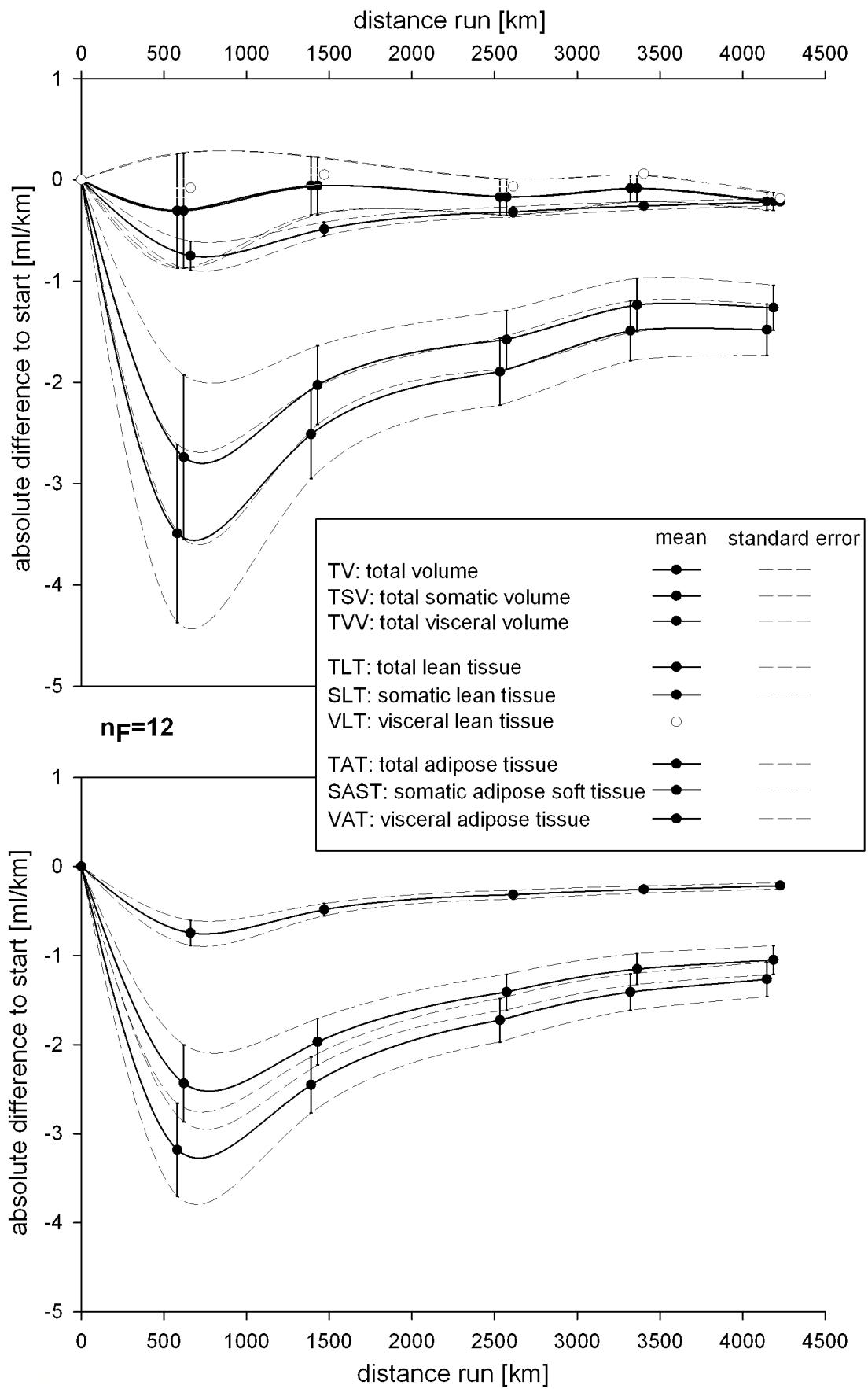
- | | |
|--|--|
| TAT: mean difference | ● TAT: p-value |
| SAST: mean difference | ○ SAST: p-value |
| VAT: mean difference | ○ VAT: p-value |

time intervals:

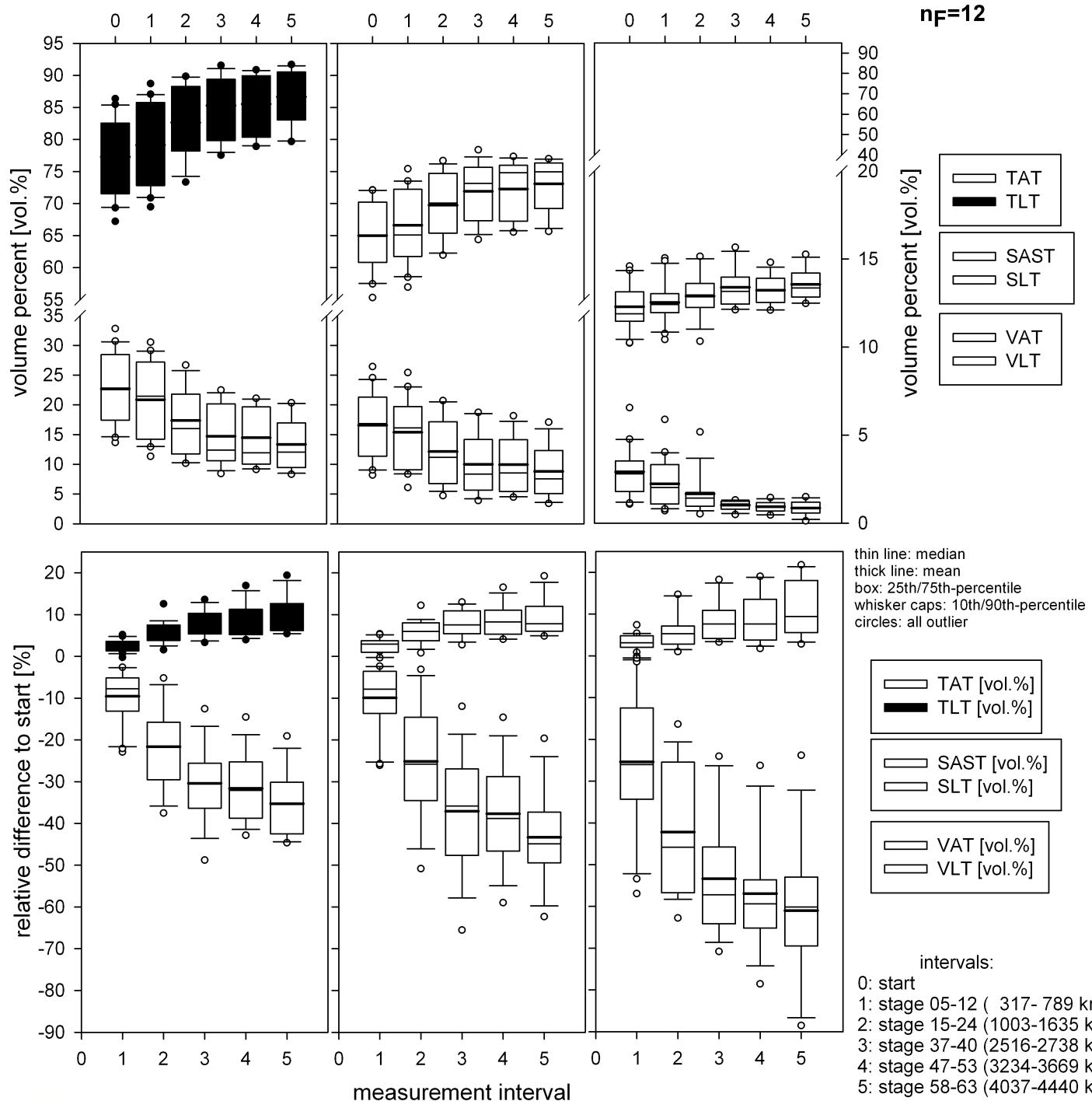
- 0: start
- 1: stage 05-12 (317- 789 km)
- 2: stage 15-24 (1003-1635 km)
- 3: stage 37-40 (2516-2738 km)
- 4: stage 47-53 (3234-3669 km)
- 5: stage 58-63 (4037-4440 km)

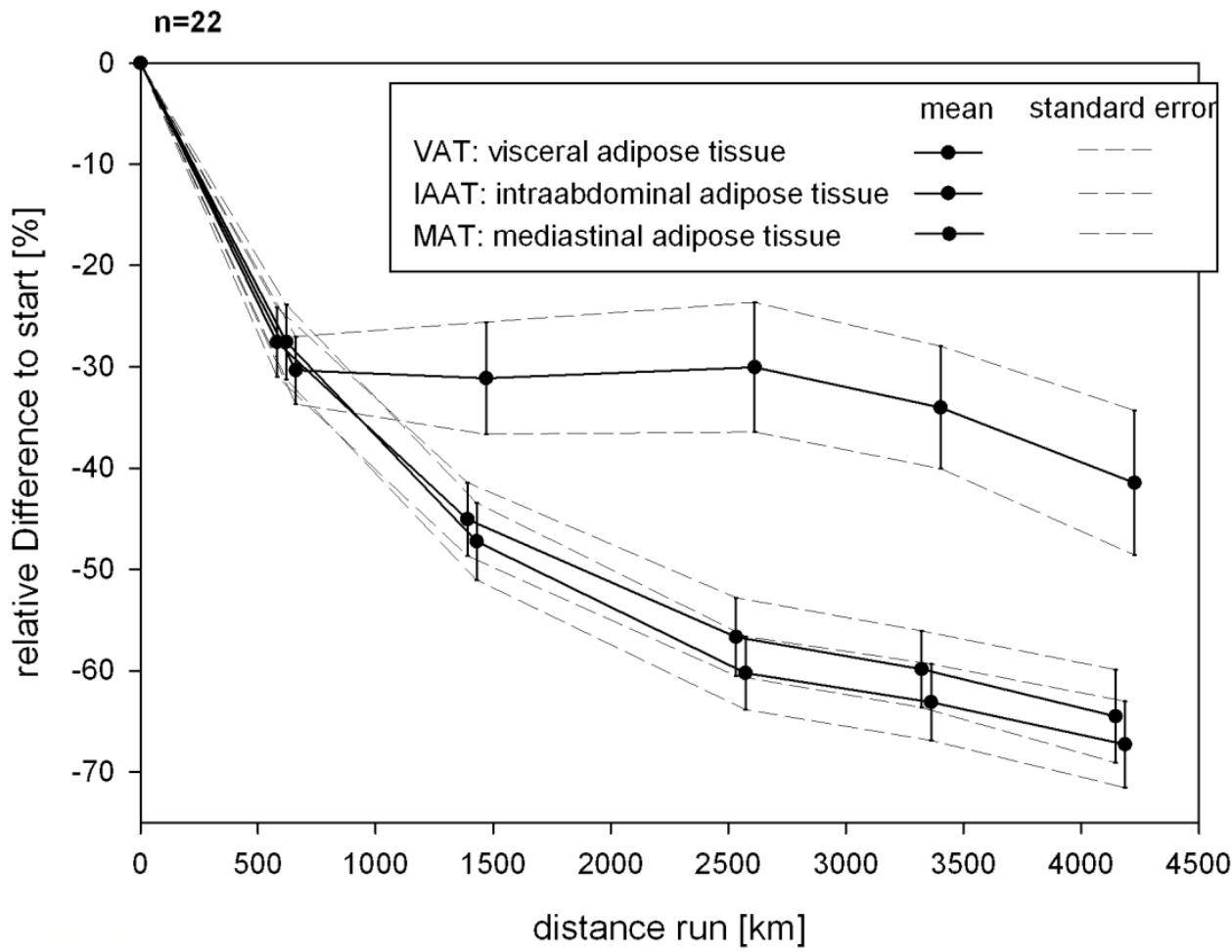


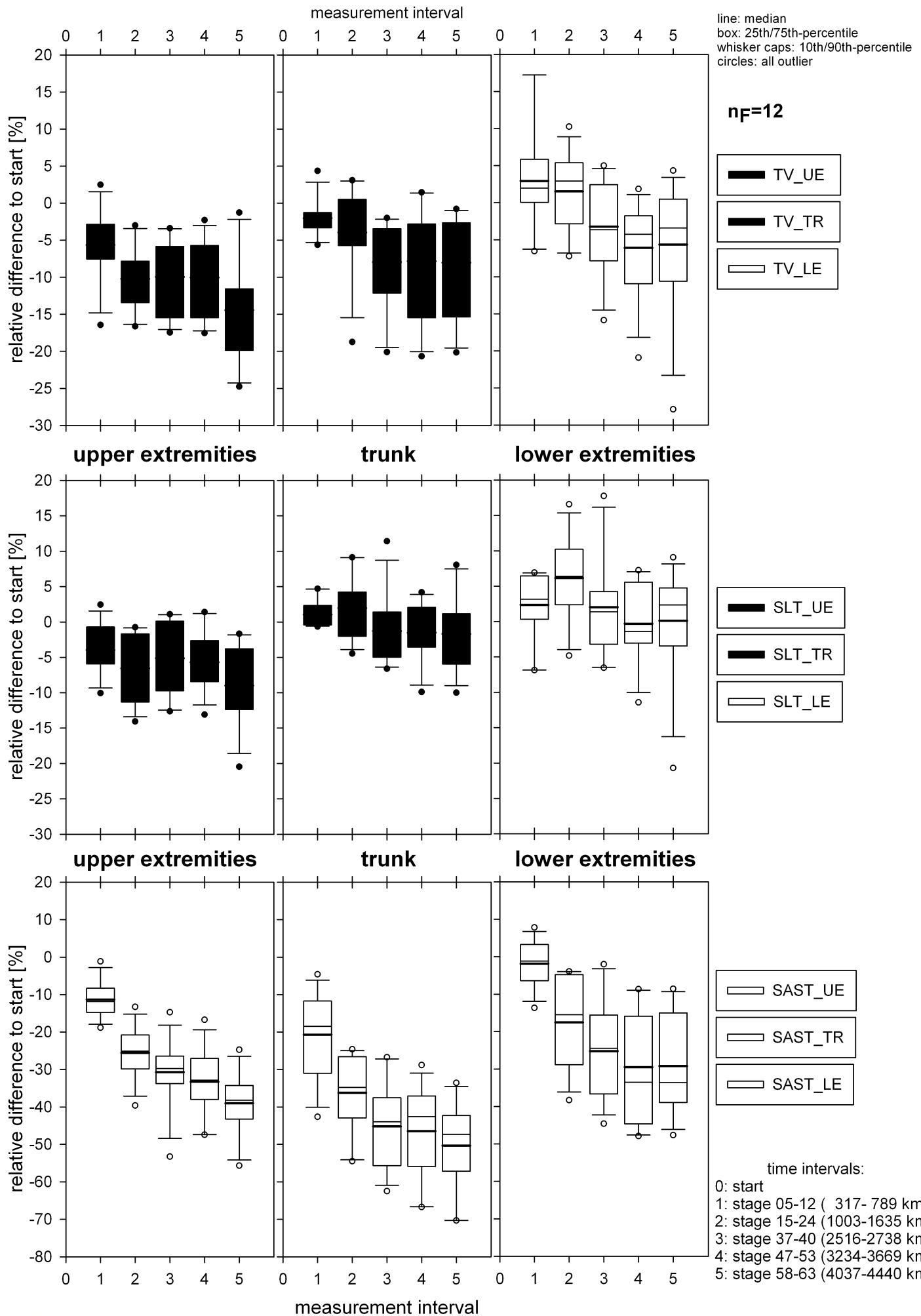


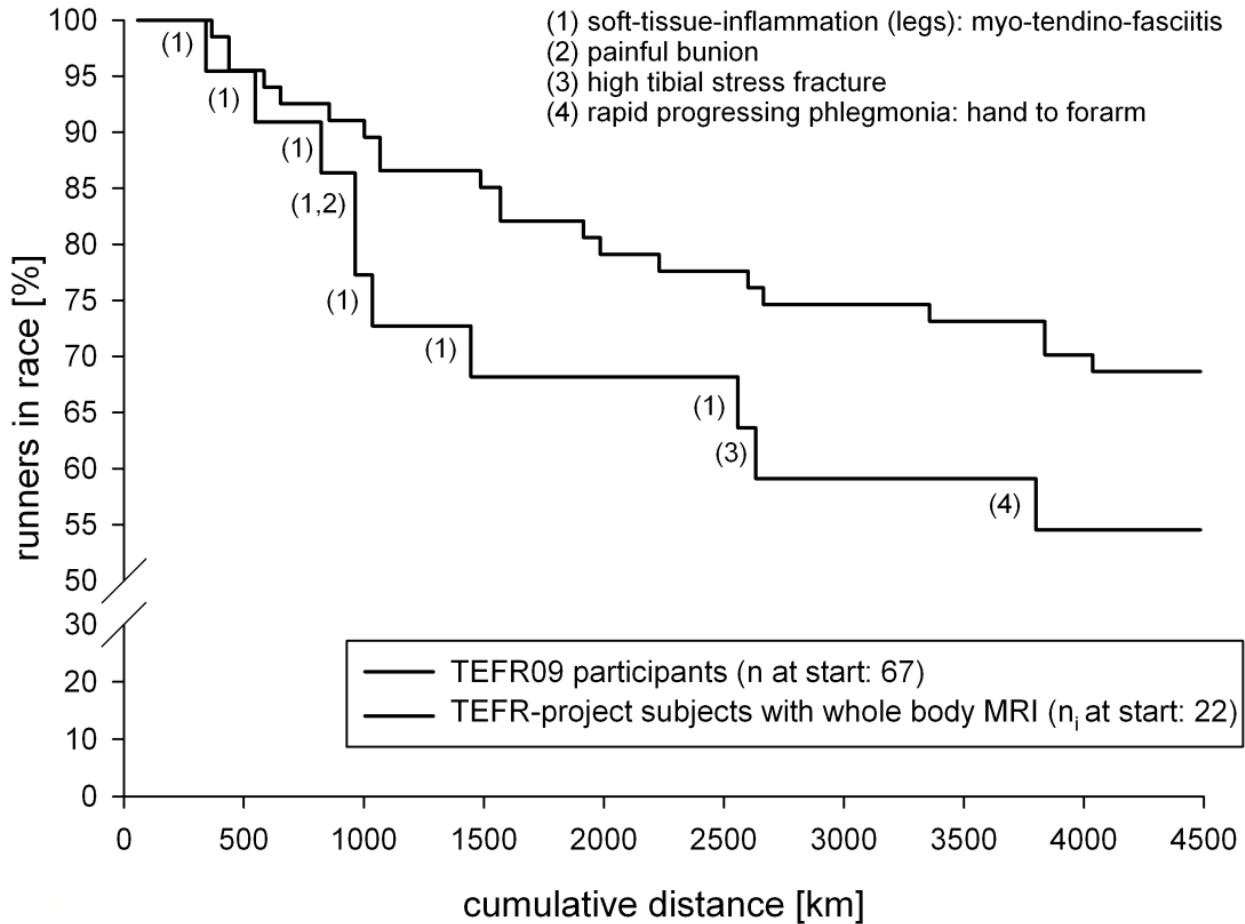


measurement interval

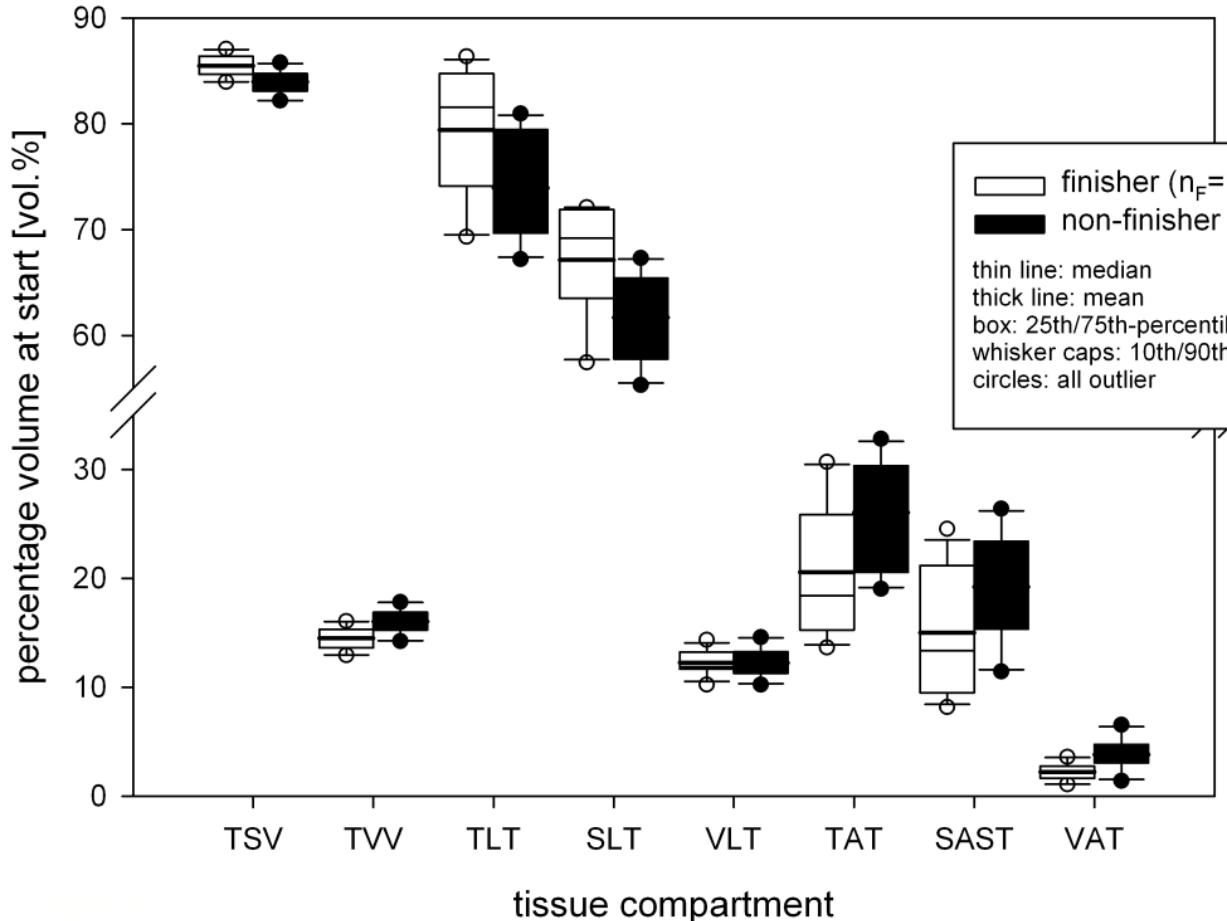




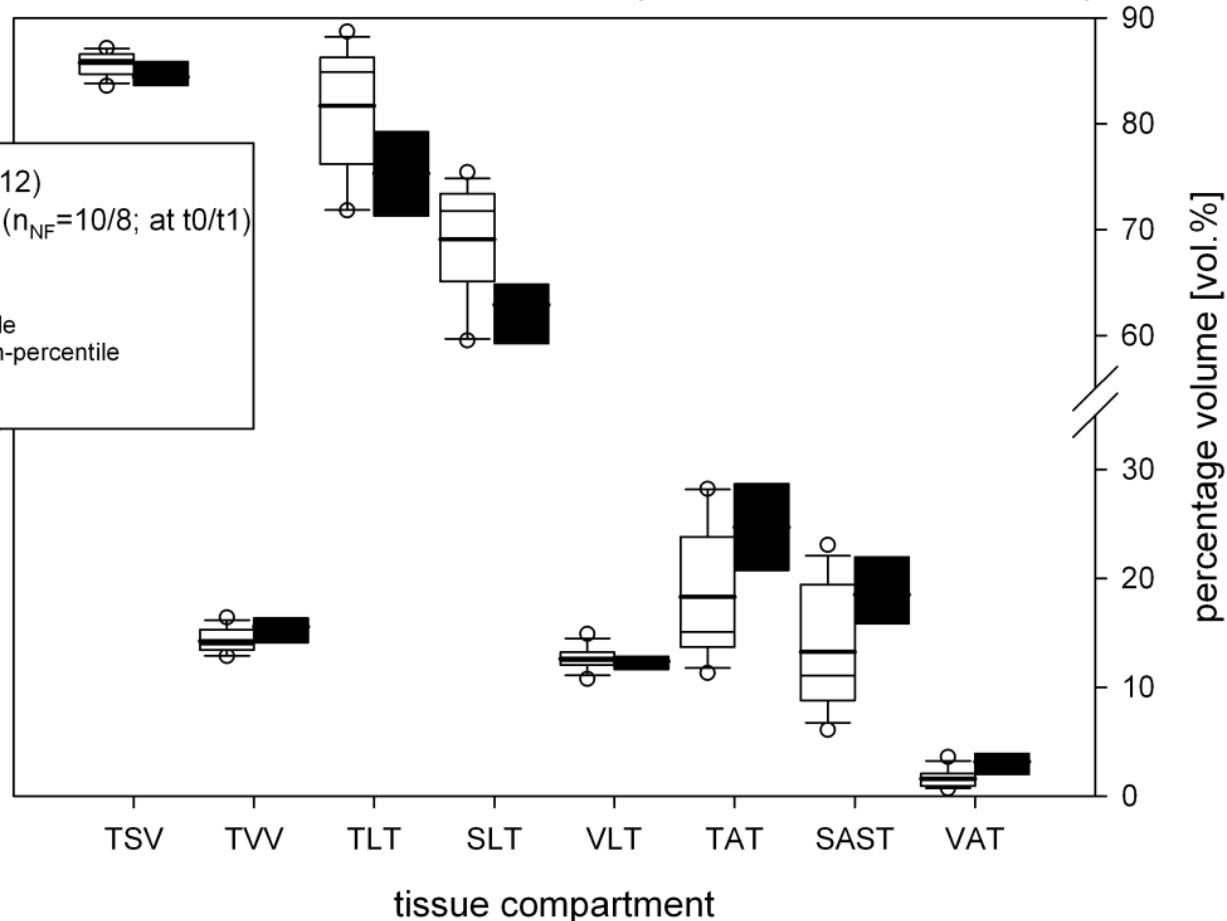


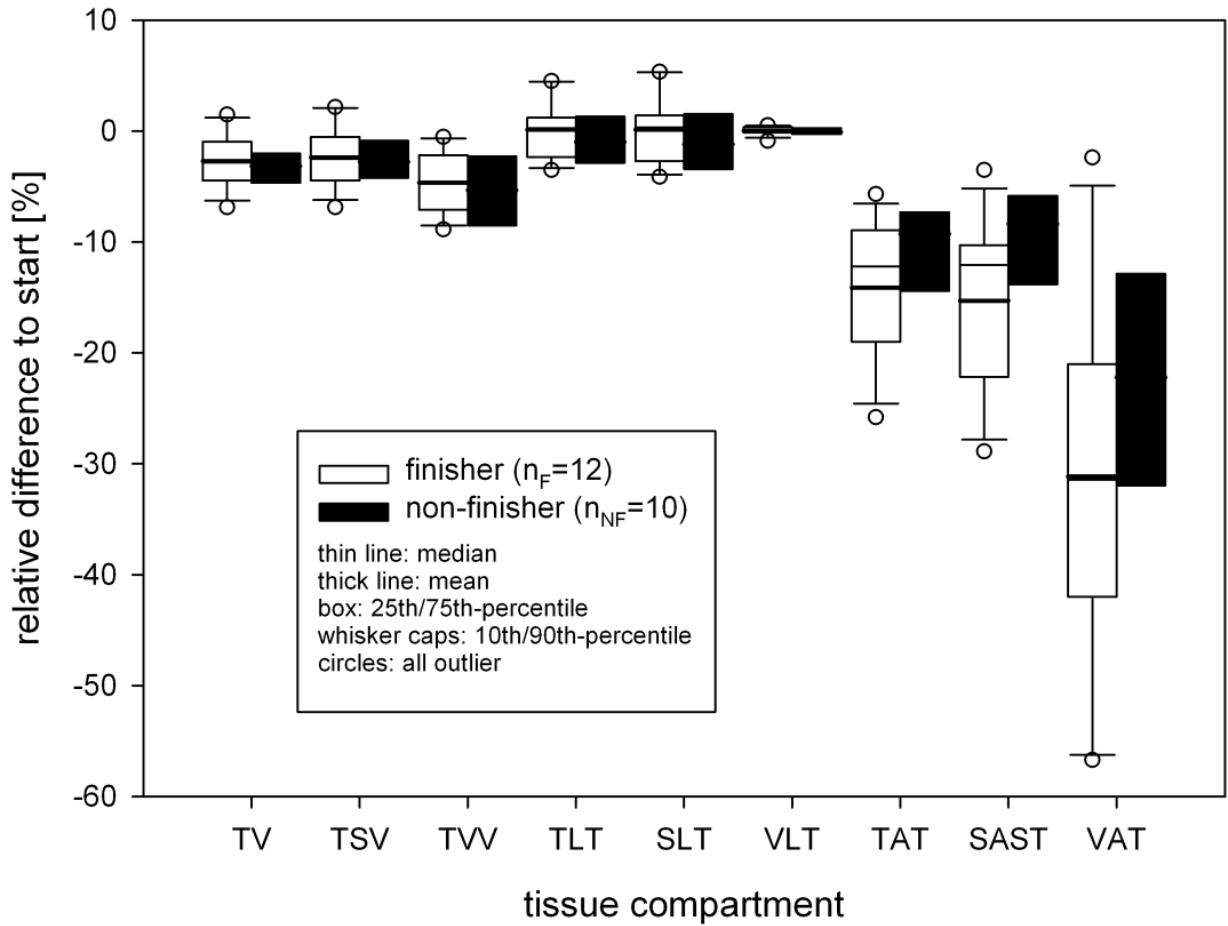


at t0: before start of the race

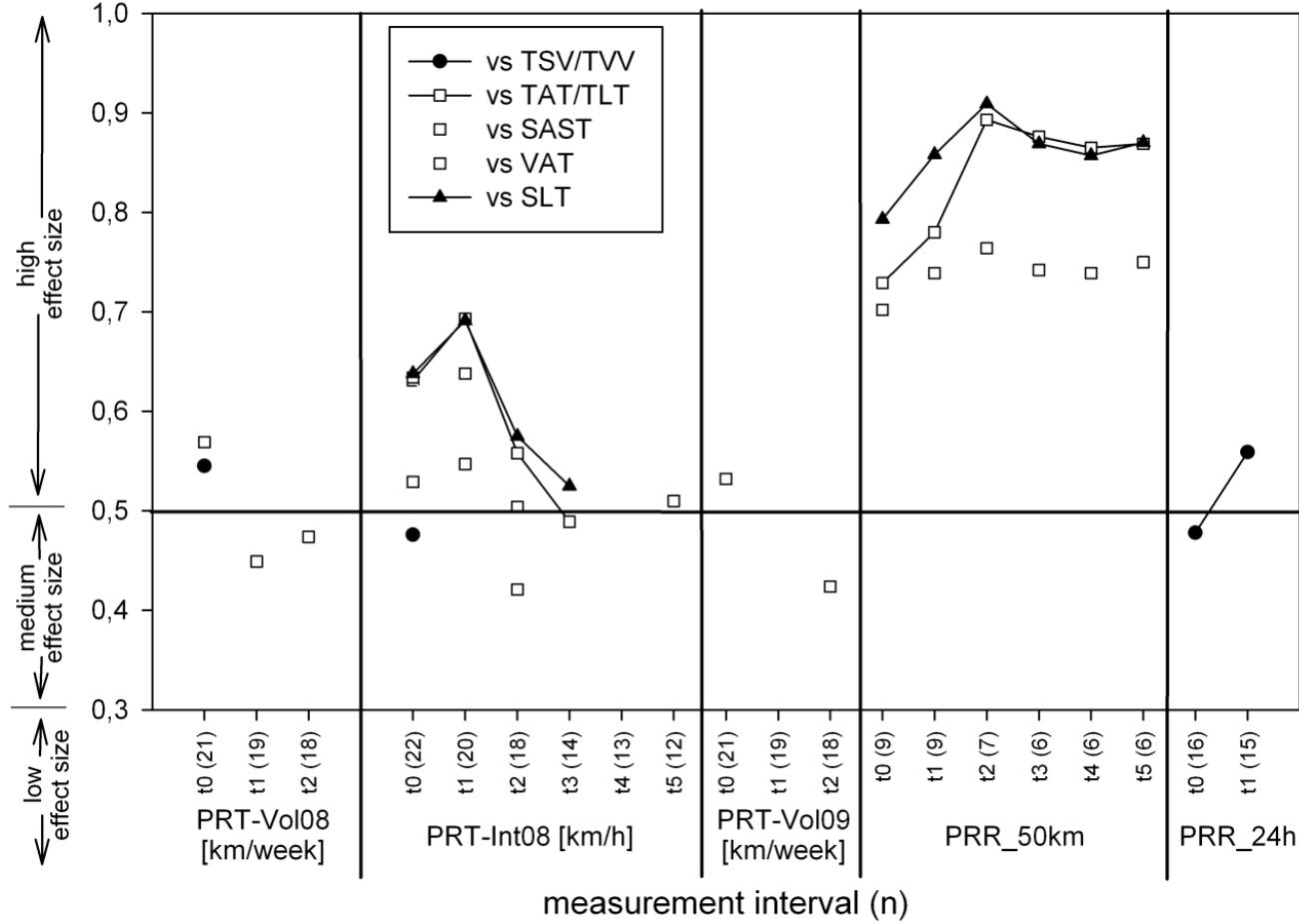


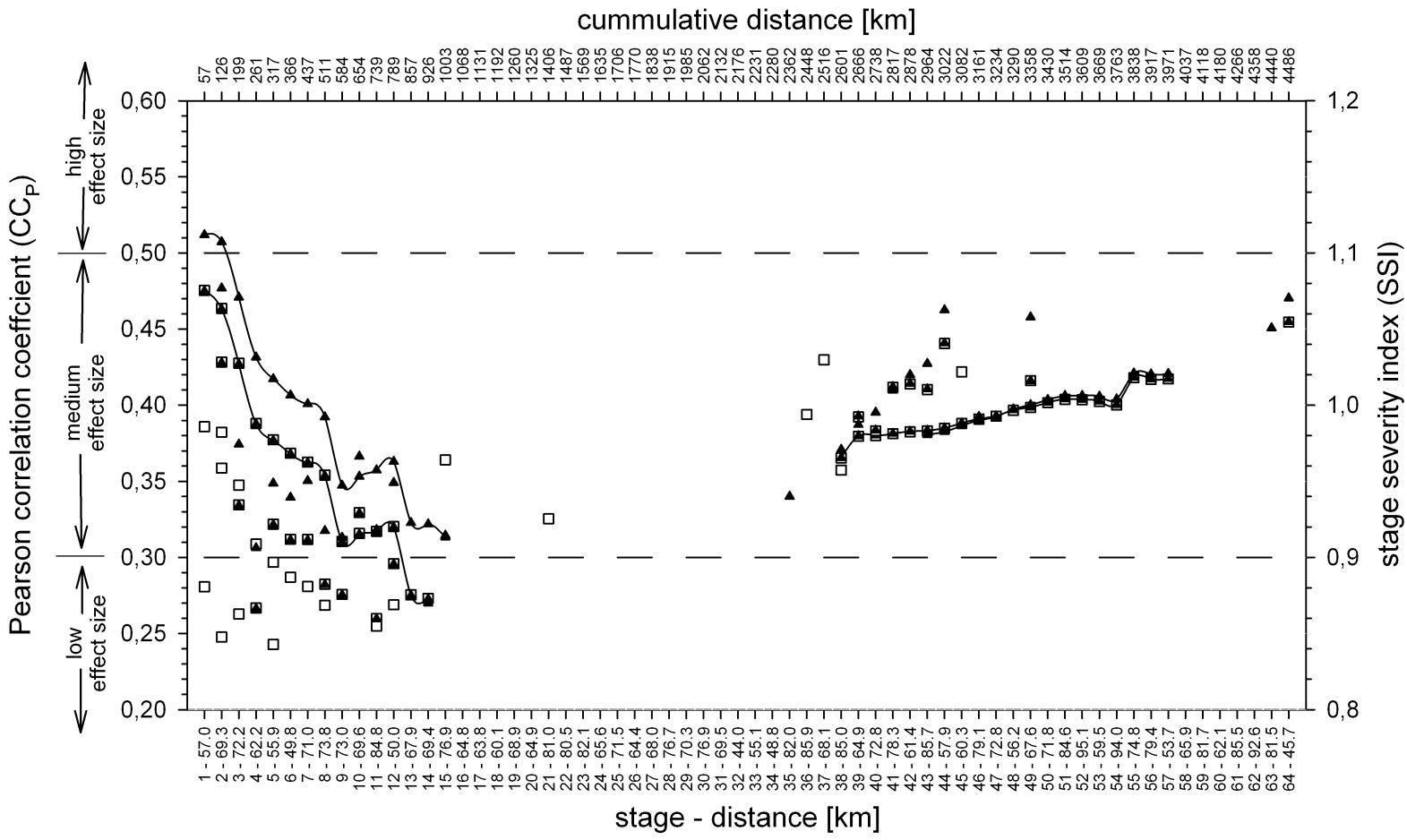
at t1: first measurement intervall (distance run: 317-789 km)





Pearson CC_p (for parametric distribution)
 Spearman-Rho CC_s (for non-parametric distribution)





Individual stage performance at TEFR09 [km/h] vs.

- TAT at start [vol.%]
- SAST at start [vol.%]
- VAT at start [vol.%]
- ▲ TLT at start [vol.%]
- ▲ SLT at start [vol.%]

stage severity index (SSI)

Cumulative performance at TEFR09 [km/h] vs.

- TAT at start [vol.%]
- SAST at start [vol.%]
- VAT at start [vol.%]
- ▲ TLT at start [vol.%]
- ▲ SLT at start [vol.%]

— borders of correlation effect size