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ORIGINAL RESEARCH

Changes in single skinfold thickness in 100 km ultramarathoners

Beat Knechtle[1,2]
Sabrina Baumgartner[1]
Patrizia Knechtle[2]
Christoph Alexander Rüst[1]
Thomas Rosemann[1]
Raúl Bescós[3]

[1]Institute of General Practice and Health Services Research, University of Zurich, Zurich; [2]Gesundheitszentrum St Gallen, St Gallen, Switzerland; [3]National Institute of Physical Education, Barcelona, Spain

Correspondence: Beat Knechtle
Facharzt FMH für Allgemeinmedizin, Gesundheitszentrum St Gallen, Vadianstrasse 26, 9001 St Gallen, Switzerland
Tel +41 07 1226 8282
Fax +41 07 1226 8272
Email beat.knechtle@hispeed.ch

Background: Changes in single skinfold thickness and body fat have been investigated in ultraswimmers and ultracyclists, but not in ultrarunners. The present study investigated the changes in single skinfold thickness during a 100 km ultramarathon.

Methods: Firstly, we investigated associations between prerace preparation and prerace body composition and, secondly, changes in single skinfold thickness during a 100 km ultramarathon in 219 male ultramarathoners. Changes in fat mass and skeletal muscle were estimated using anthropometric methods.

Results: Kilometers run weekly prerace and running speed during training were negatively associated with all skinfold thicknesses ($P < 0.05$) except for the front thigh skinfold. During the race, skinfold thickness at the pectoral (−0.1%), suprailiac (−1.8%), and calf (−0.8%) sites decreased ($P < 0.05$). The subjects lost 1.9 ± 1.4 kg of body mass ($P < 0.001$), 0.7 ± 1.0 kg of estimated skeletal muscle mass ($P < 0.001$), and 0.2 ± 1.3 kg of estimated fat mass ($P < 0.05$). The decrease in body mass was positively related to the decrease in both estimated skeletal muscle mass ($r = 0.21$, $P = 0.0017$) and estimated fat mass ($r = 0.41$, $P < 0.0001$).

Conclusion: Firstly, prerace fat mass and prerace skinfold thickness were associated with both volume and speed in running training. Secondly, during the ultramarathon, skinfold thickness decreased at the pectoral, suprailiac, and calf sites, but not at the thigh site. Percent decreases in skinfold thickness for ultrarunners was lower than the percent decreases in skinfold thickness reported for ultraswimmers and ultracyclists.

Keywords: endurance, athlete, fat mass, muscle mass, adipose subcutaneous tissue

Introduction

A decrease in body mass is a common finding in ultraendurance performance.[1,2] Generally, the decrease in body mass during endurance performance is considered to be due to dehydration.[3–5] However, the decrease in body mass in ultraendurance performance seems not to be due to dehydration[2,6,7] but rather to a decrease in solid mass, ie, fat mass[2,8–13] and skeletal muscle mass.[8,14–17] The type of exercise undertaken seems to affect whether skeletal muscle mass or fat mass would be reduced during endurance performance. Eccentric endurance exercise such as running seems to lead to a decrease in both muscle mass and fat mass,[8,12,13,16,17] whereas concentric endurance exercise such as cycling seems to reduce fat mass.[18,19] Swimming as a non-weight-bearing exercise leads to no changes in body composition in 12-hour ultraswimmers.[20]

During physical exercise, lipolysis of adipose subcutaneous tissue seems to vary between different sites in the exercising body.[21] In a laboratory trial, Boschmann et al[21] showed some differences in metabolism and blood flow in abdominal and femoral

subcutaneous adipose tissue and skeletal muscle between women and men during standardized bicycle exercise. Lipolysis was greater in skeletal muscle tissue than in femoral subcutaneous adipose tissue, and greater in femoral subcutaneous adipose tissue than in abdominal subcutaneous adipose tissue. In skeletal muscle tissue, the increase in lipolysis was greater for women than for men. The authors concluded, firstly, that lipids stored in muscle tissue were used more than lipids stored in adipose tissue for fueling the energy metabolism of muscle during exercise and, secondly, that lipid mobilization during exercise was much greater in women than in men. However, the most important finding with practical application was that lipolysis of subcutaneous adipose tissue was greater in femoral than in abdominal adipose subcutaneous tissue. It seemed that the thickness of adipose subcutaneous tissue over the exercising muscle became reduced.

Changes in single skinfold thickness have been investigated in ultraendurance athletes, such as cyclists[19] and swimmers,[22] but not in runners. In cyclists, skinfold thickness at the pectoral (−14.7%), abdominal (−14.9%), and thigh (−10.2%) sites showed the largest decrease.[19] The decrease in abdominal skinfolds was significantly and negatively related to cycling speed during the race.[19] In male swimmers, skinfold thickness at the abdominal (−8.4%) and suprailiac (−7.5%) sites decreased, whereas in female swimmers, skinfold thickness at the axillary site (−18.8%) was reduced.[22] Running training seemed also to be beneficial for reduction of subcutaneous adipose tissue. In elite runners, intense training resulted in significant increases in performance and significant decreases in the sum of six skinfolds, as well as single skinfolds at the abdominal, front thigh, and medial calf sites.[23]

The aims of the present study were, firstly, to investigate, any potential change in skinfold thickness and body fat during an ultramarathon, and, secondly, to explore potential associations between prerace preparation and prerace body composition. Based upon reported findings in laboratory and field studies, we hypothesized, firstly, that skinfold thickness on the lower body (eg, thigh and calf sites) would decrease to a greater extent than skinfold thickness on the upper body (eg, abdominal and suprailiac sites). Secondly, we hypothesized that volume and speed in prerace training would be related to body fat and skinfold thickness in different ways.

Materials and methods
Subjects
In order to increase the sample size, data were collected from an annual 100 km ultramarathon, ie, the "100 km Lauf Biel"

in Biel, Switzerland, over 5 consecutive years. Each year, approximately 1300 male ultrarunners finish this race.[24] The race director contacted all participants in the years 2007–2011 via a newsletter sent 3 months prerace, in which the participants were asked to participate in the study. A total of 239 recreational male ultrarunners volunteered to participate in our investigation. We focused on recreational athletes, defined as athletes pursuing a regular occupation, performing sport during leisure time, having no sponsors, and not earning their livelihood through sponsorship or prize money. The athletes were informed of the procedures and gave their informed written consent. No athlete competing several times in the race was included more than once in the study. The study was approved by the institutional review board for use of human subjects of St Gallen, Switzerland.

Race
The "100 km Lauf Biel" generally takes place during the night of the first weekend in June. The athletes start the 100 km run on Friday at 10 pm, and need to climb a total altitude of 645 meters. During the 100 km, the organizer provides a total of 17 aid stations, offering an abundant variety of food and beverages, including hypotonic sports drinks, tea, soup, caffeinated drinks, water, bananas, oranges, energy bars, cakes, and bread. The athletes are allowed to be supported by a cyclist in order to have additional food and clothing, if necessary. In all 5 years, the general weather conditions were comparable, with the temperature at the start being 15°C–18°C, with night lows of 8°C–10°C, and daily highs of 25°C–28°C the following day. There was no rain or wind from 2007 to 2010. In 2011, there were rain showers during the night and the temperature rose to 18°C on the second day.

Measurements and calculations
Upon recruitment into the investigation 3 months prerace, the participants were instructed to keep a comprehensive training diary until the start of the race. All running training units were recorded, showing distance in kilometers and duration. Within 6 hours of starting the race and immediately upon arrival at the finish line, anthropometric characteristics, including body mass, limb circumference, and thickness of skinfolds at eight sites (ie, pectoralis, mid axillary, triceps, subscapular, abdomen, suprailiac, front thigh, and medial calf) were measured. With these measurements, skeletal muscle mass and fat mass were estimated using anthropometric equations. During the race, the athletes recorded their fluid intake on a sheet of paper which they carried with them

during the run. At each aid station, they marked the number of cups consumed. In addition, all supplemental fluid intake provided by the support crew was recorded. Fluid intake was estimated according to reports from the athletes.

Anthropometric measurements

Body mass was measured after voiding of the urinary bladder using a commercial scale (Beurer BF 15, Beurer GmbH, Ulm, Germany) and recorded to the nearest 0.1 kg. Body height was determined using a stadiometer (Tanita HR 001 portable height measure, Tanita Europe, Amsterdam, The Netherlands) and recorded to the nearest 0.01 m. The circumferences of the right upper arm, right thigh, and right calf were measured using a nonelastic tape measure (KaWe CE, Kirchner und Wilhelm, Asperg, Germany) and recorded to the nearest 0.01 cm. The right upper arm circumference was measured at the mid upper arm, the right thigh circumference at the mid thigh, and the right calf circumference at the mid calf. Skinfold data were obtained using a skinfold caliper (GPM-Hautfaltenmessgerät, Siber and Hegner, Zurich, Switzerland) and recorded to the nearest 0.2 mm. The skinfold caliper measures with a pressure of 0.1 MPa ± 5% over the whole measuring range. One trained investigator took all the measurements. Skinfold thicknesses were determined on the right side of the body in all athletes, and were measured at the pectoral (anterior axillary line), mid axilla (vertical), triceps (in the middle of the upper arm), subscapular (at the inferior angle of the scapula), abdominal (vertical, right to the navel), suprailiac (at anterior axillary), front thigh (mid thigh) and medial calf (maximum girth) sites. The investigator identified the correct anatomical site using orientation with fingerbreadth and handbreadth from prominent anatomical sites, such as a prominent protuberance or insertion of a tendon. The procedure was performed three times and the mean of the three measures was used for the analyses. The skinfold measurements were standardized to ensure reliability, and readings were performed 4 seconds after applying the caliper, according to Becque et al.[25] An intratester reliability check was conducted on 27 male runners prior to testing.[26] Intraclass correlation (ICC) within the two raters was excellent for all anatomical measurement sites and for various summary measurements of skinfold thickness. Agreement tended to be higher within than between raters, but still had excellent reliability (ICC $>$ 0.9) for the summary measurements of skinfold thickness. ICC for measurer 1 versus measurer 1 and for measurer 2 versus measurer 2 for the single skinfold thicknesses were between 0.98 and 0.99, respectively; for the sum of seven and eight skinfolds,

respectively, ICC was 0.99. For the sum of eight skinfolds for measurer 1, bias (average difference between measure 1 and 2) was −0.51 mm, the standard deviation of the average difference was 1.49 mm, and 95% limits of agreement were between −3.44 mm and +2.41 mm. Skeletal muscle mass was estimated using the anthropometric equation devised by Lee et al, with skeletal muscle mass = Ht $\times$ (0.00744 $\times$ CAG^2 + 0.00088 $\times$ CTG^2 + 0.00441 $\times$ CCG^2) + 2.4 $\times$ gender − 0.048 $\times$ age + race + 7.8, where Ht is height, CAG is skinfold-corrected upper arm girth, CTG is skinfold-corrected thigh girth, CCG is skinfold-corrected calf girth, gender = 1 for male, and race = 0 for white.[27] This anthropometric method was evaluated using 189 nonobese subjects and crossvalidated using magnetic resonance imaging (MRI). The equation was validated MRI to determine skeletal muscle mass. There was a high correlation between the predicted skeletal muscle mass and the MRI-measured skeletal muscle mass ($r^2 = 0.83$, $P < 0.0001$, standard error of the estimate = 2.9 kg). The correlation between the measured and predicted skeletal muscle mass difference was highly significant ($r^2 = 0.90$, $P = 0.009$). Fat mass was estimated using the anthropometric method of Stewart and Hannan, with fat mass (g) = 331.5 (abdominal) + 356.2 (thigh) + 111.9 m − 9108, where abdominal is the abdominal skinfold thickness in mm, thigh is the thigh skinfold thickness in mm, and m is body mass in kg.[28] The coefficient of determination was 0.96 and the standard error of the estimate was 1738 g ($P < 0.001$).

Laboratory measurements

Capillary blood and urine samples were collected at the same time as the anthropometric measurements. Capillary blood samples were taken from the fingertip, and both plasma sodium concentration ([Na^+]) and hematocrit were analyzed using the i-STAT® 1 system (Abbott Laboratories, Abbott Park, IL). Standardization of posture prior to blood collection was adhered to because postural changes can influence blood volume, and therefore hemoglobin concentration and hematocrit. The percent change in plasma volume was calculated from prerace and post-race levels of hematocrit following the Beaumont equation.[29] Urine specific gravity was analyzed using a Clinitek Atlas® automated urine chemistry analyzer (Siemens Healthcare Diagnostics, Deerfield, IL).

Statistical analysis

The data are presented as the mean ± standard deviation. Prerace and post-race results are compared using the paired *t*-test. Pearson's correlation was used to investigate any potential association between anthropometric

and training characteristics. In order to investigate whether changes in both skinfold thickness and limb circumference used to estimate changes in both skeletal muss and fat mass during the race would be influenced by running speed, fluid intake, or changes in hydration status,[4,5] we performed correlation analyses between changes in both skinfold thickness and limb circumference with markers of hydration status, including changes in body mass, changes in hematocrit, changes in plasma [Na$^+$], changes in urine specific gravity, as well as running speed during the race and fluid intake. For the strength of correlation, $r > 0.70$ indicated a very strong, $r = 0.40–0.69$ a strong, $r = 0.30–0.39$ a moderate, $r = 0.20–0.29$ a weak, and $r = 0.01–0.19$ a negligible relationship, respectively. Pearson's correlation was again applied to investigate for a potential association between anthropometric and training characteristics and race time. An alpha level of 0.05 was used to indicate significance for all statistical tests.

Results

Of the 239 subjects who started the race, 20 dropped out due to overuse injuries of the lower limbs. Table 1 shows the age along with anthropometric and training characteristics for the 219 successful subjects. The subjects completed the 100 km ultramarathon within 713 ± 123 minutes, running at an average speed of 8.6 ± 1.4 km/hour.

Changes in anthropometric characteristics during run

During the run, the subjects lost 1.9 ± 1.4 kg of body mass ($P < 0.001$), 0.7 ± 1.0 kg of estimated skeletal muscle mass ($P < 0.001$), and 0.2 ± 1.3 kg of estimated fat mass ($P < 0.05$, Table 2). Expressed as a percentage, the subjects lost 2.5% ± 1.8% in body mass, 1.8% ± 2.5% in estimated skeletal muscle mass, and 1.4% ± 8.1% in estimated fat mass. The loss

Table 1 Age, anthropometric measurements, and training characteristics of the subjects (n = 219)

Variable	Mean ± SD
Age (years)	46.2 ± 9.3
Body mass (kg)	75.0 ± 9.4
Body height (m)	1.78 ± 0.06
Body mass index (kg/m²)	23.4 ± 2.2
Weekly running hours (hours)	8.2 ± 8.1
Weekly running kilometers (km)	69.6 ± 27.6
Running speed during training sessions (km/hour)	10.3 ± 2.1
Completed marathons (n = 214)	34.6 ± 69.4
Personal best marathon time (minutes)	208 ± 31
Completed 100 km ultramarathons (n = 148)	8.9 ± 6.5
Personal best 100 km ultra marathon time (minutes)	648 ± 166

of 0.7 ± 1.0 kg of estimated skeletal muscle mass was significantly higher compared with the loss of 0.2 ± 1.3 kg of estimated fat mass ($P < 0.0001$). The change in body mass was weakly positively related to the change in estimated skeletal muscle mass (Figure 1) and strongly positively related to the change in estimated fat mass (Figure 2). Additionally, the change in body mass was negligibly related to running speed during the race (Figure 3). However, neither the change in estimated fat mass nor the change in estimated skeletal muscle mass correlated with running speed ($P > 0.05$). Skinfold thickness at the pectoral, suprailiac, and calf sites decreased significantly (Table 2). All limb circumferences also decreased significantly. The decrease in upper arm and thigh circumference was not related to running speed during the race ($P > 0.05$). However, the decrease in calf circumference was negligibly negatively associated with running speed (Figure 4).

Changes in laboratory results during run

Table 3 summarizes the laboratory results. Urine specific gravity increased ($P < 0.0001$), and was not related to the change in body mass or running speed. Hematocrit decreased ($P < 0.0001$), plasma volume increased by 7.0% ± 15.6% ($P < 0.05$), and plasma [Na$^+$] increased ($P < 0.0001$). The increase in plasma volume was negligibly associated with the change in calf skinfold thickness ($r = 0.16$, $P = 0.0189$), but not with the change in thigh skinfold thickness ($r = 0.06$, $P > 0.05$).

Fluid intake and changes in anthropometry and laboratory results

While running, the athletes consumed 7.6 ± 2.6 L of fluids, equivalent to 0.65 ± 0.27 L/hour. Fluid intake was moderately negatively related to running speed ($r = −0.39$, $P < 0.0001$). Fluid intake was weakly positively associated with the change in body mass ($r = 0.22$, $P = 0.0012$), but not to post-race plasma [Na$^+$] nor to change in plasma [Na$^+$] ($P > 0.05$). The change in body mass was moderately negatively related to the change in plasma [Na$^+$] ($r = −0.38$, $P < 0.0001$). Fluid intake was negligibly associated with change in thigh skinfold thickness ($r = 0.14$, $P = 0.0332$) but not with change in calf skinfold thickness ($r = 0.06$, $P > 0.05$). The change in calf circumference was negligibly related to fluid intake ($r = 0.17$, $P = 0.0113$), but not to change in thigh circumference ($r = 0.05$, $P > 0.05$).

Prerace training and prerace anthropometry

Skinfold thickness, estimated fat mass, and estimated skeletal muscle mass were not related to hours run per week (Table 4).

Table 2 Changes in anthropometric characteristics (n = 219)

	Prerace	Post-race	Absolute change	Percent change	Significance
Body mass (kg)	75.0 ± 9.4	73.1 ± 9.3	−1.9 ± 1.4	−2.5 ± 1.8	***
Skinfold pectoral (mm)	8.0 ± 4.2	7.8 ± 4.1	−0.2 ± 1.7	−0.1 ± 22.4	*
Skinfold axillary (mm)	9.2 ± 4.2	9.2 ± 3.7	−0.0 ± 1.8	−0.0 ± 17.3	
Skinfold triceps (mm)	8.0 ± 3.0	8.2 ± 2.8	+0.2 ± 1.5	+4.8 ± 19.7	
Skinfold subscapular (mm)	11.1 ± 4.6	11.1 ± 4.6	−0.0 ± 1.7	−0.9 ± 13.9	
Skinfold abdominal (mm)	16.3 ± 8.1	16.3 ± 8.2	−0.0 ± 2.2	−0.9 ± 14.4	
Skinfold suprailiac (mm)	16.5 ± 7.4	16.0 ± 7.8	−0.6 ± 3.6	−1.8 ± 22.7	*
Skinfold front thigh (mm)	11.6 ± 5.6	11.5 ± 5.0	−0.1 ± 3.1	−2.8 ± 22.0	
Skinfold medial calf (mm)	5.9 ± 2.6	5.6 ± 2.5	−0.3 ± 1.6	−0.8 ± 27.4	*
Sum of skinfolds (mm)	86.7 ± 32.7	85.8 ± 32.8	−0.9 ± 7.7	−0.9 ± 7.6	
Circumference upper arm (cm)	29.5 ± 2.0	29.3 ± 1.9	−0.2 ± 0.8	−0.4 ± 2.6	*
Circumference thigh (cm)	54.9 ± 3.1	54.3 ± 3.0	−0.6 ± 1.4	−0.9 ± 2.5	***
Circumference calf (cm)	38.6 ± 2.3	37.6 ± 2.3	−1.0 ± 0.8	−2.4 ± 2.0	***
Skeletal muscle mass (kg)	38.9 ± 3.8	38.2 ± 3.7	−0.7 ± 1.0	−1.8 ± 2.5	***
Fat mass (kg)	8.8 ± 4.7	8.6 ± 4.2	−0.2 ± 1.3	−1.4 ± 8.1	*

Notes: Results are presented as the mean ± standard deviation. *$P < 0.05$; ***$P < 0.001$.

Number of kilometers run per week was negligibly to weakly related to all skinfold thicknesses. Running speed during training was negligibly to weakly associated with all skinfold thicknesses except for the front thigh skinfold. All skinfold thicknesses (weakly to strongly), upper arm and thigh circumferences (negligibly to weakly), and body mass (moderately) were related to race time (Table 5). Upper arm ($r = 0.24$, $P = 0.0004$) and thigh ($r = 0.16$, $P = 0.021$) circumferences were negligibly to weakly related to race time. The upper arm circumference was weakly negatively associated with kilometers run per week ($r = −0.20$, $P = 0.0026$) and negligibly negatively associated with running speed during training sessions ($r = −0.17$, $P = 0.011$).

Changes in anthropometry and markers of hydration status

The decrease in calf circumference was negligibly related to both running speed and fluid intake (Table 6), weakly related to change in hematocrit, moderately to change in body mass, and moderately to change in plasma [Na$^+$]. The decrease in upper arm circumference was negligibly related to change in body mass, and the decrease in thigh circumference was weakly related to change in body mass. The decrease in medial calf skinfold thickness was negligibly related to fluid intake. The nonsignificant change in front thigh skinfold thickness was negligibly related to change in body mass. The nonsignificant change in axillary
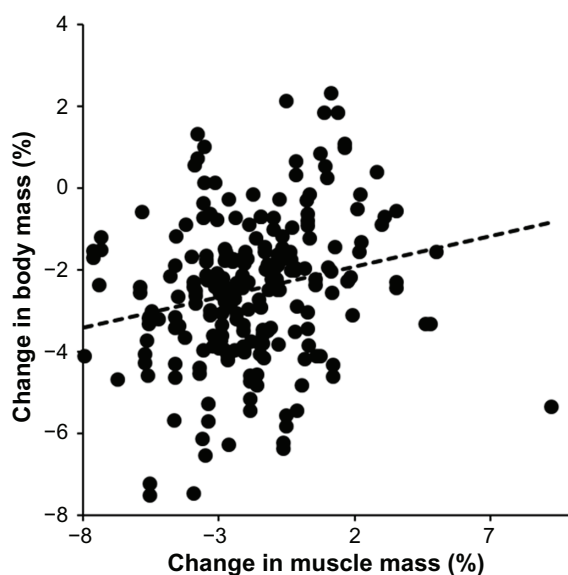

Figure 1 Change in body mass was significantly and positively related to change in skeletal muscle mass (n = 219, $r = 0.21$, $P = 0.0017$).
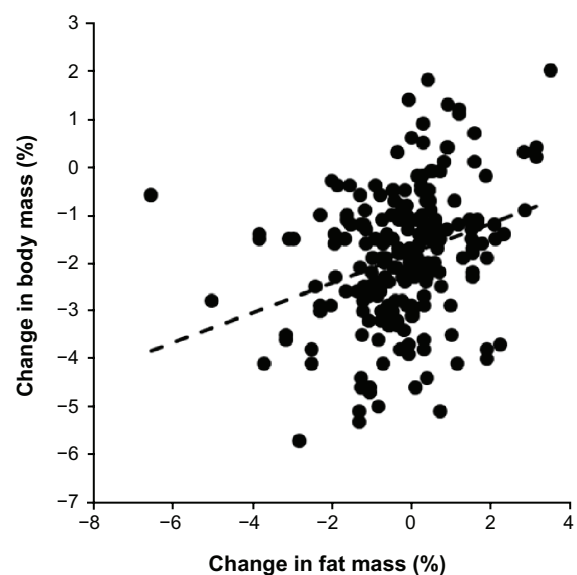

Figure 2 Change in body mass was significantly and positively related to change in fat mass (n = 219, $r = 0.41$, $P < 0.0001$).
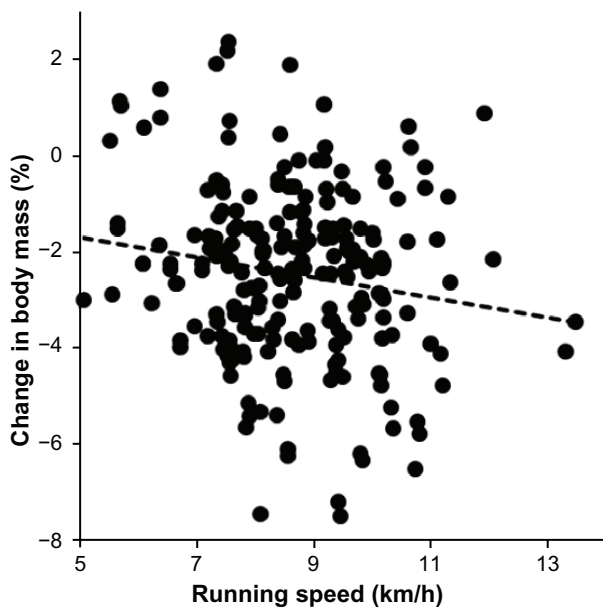
Figure 3 Change in body mass related to running speed (n = 219, r = −0.16, P < 0.05).

skinfold thickness was weakly related to change in body mass, and negligibly to change in plasma [Na$^+$] and change in urine specific gravity. The decrease in pectoral skinfold thickness was negligibly related to change in body mass. The change in calf circumference explained 32% of the variance in change in skeletal muscle mass, 12% of change in thigh circumference, and 42% of change in upper arm circumference.
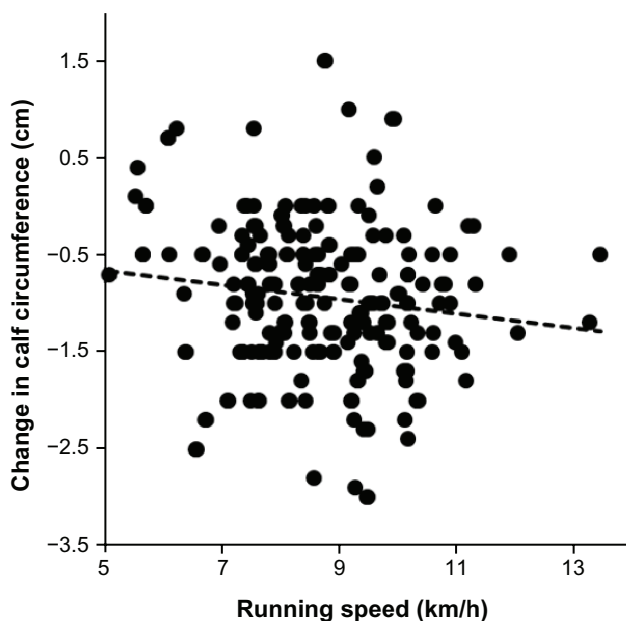

Figure 4 Change in calf circumference is significantly and negatively associated with running speed during the race (n = 219, r = −0.13, P = 0.0479).

Discussion

We intended to investigate, firstly, potential associations between prerace preparation and prerace body composition and, secondly, any potential change in skinfold thickness during a 100 km ultramarathon. The changes in skinfold thickness and limb circumference may be indicative of changes in fat mass and skeletal muscle mass. The main findings were that, firstly, both weekly running hours and running speed during training sessions were negligibly to weakly related to prerace skinfold thickness and estimated prerace fat mass and, secondly, during the ultramarathon, skinfold thicknesses decreased at the pectoral, suprailiac, and calf sites, but not at the thigh site. The percent decreases in skinfold thickness in these ultrarunners were lower compared with the percent decreases in skinfolds reported for ultraswimmers and ultracyclists.

Change in anthropometric characteristics during the race

In these ultramarathoners, skinfold thickness decreased at the pectoral (−0.1%), suprailiac (−1.8%), and calf sites (−0.8%). The percent decreases were considerably lower compared with the decreases reported for ultracyclists[19] and ultraswimmers.[22] In ultracyclists, skinfold thickness at the pectoral (−14.7%), abdominal (−14.9%), and thigh (−10.2%) sites showed the largest percent decrease.[19] In male ultraswimmers, the skinfold thickness at the abdominal (−8.4%) and suprailiac (−7.5%) sites decreased, whereas in female swimmers, skinfold thickness decreased at the axillary site (−18.8%).[22] Duration of performance might not explain these differences. While the present runners competed for 713 ± 123 minutes, the cyclists competed for 1580 ± 296 minutes[19] the male swimmers competed for 538 ± 100 minutes, and female swimmers competed for 603 ± 141 minutes.[22] The eccentric component in running[33] likely led to a decrease in skeletal muscle mass rather than to a decrease in fat mass.

The runners lost 1.9 kg of body mass, with approximately 37% of the loss being due to loss of skeletal muscle mass and about 11% due to loss in fat mass. Skeletal muscle mass and fat mass were estimated using anthropometric equations for skinfold thickness and limb circumference. Because all the limb circumferences decreased, but skinfold thickness decreased at only three of eight sites, anthropometric changes may explain the higher decrease in estimated skeletal muscle mass (−0.7 kg) compared with estimated fat mass (−0.2 kg). Fat mass was estimated using the anthropometric method of Stewart and Hannan[28] which includes skinfold

Table 3 Changes in laboratory results (n = 219)

	Prerace	Post-race	Absolute change	Percent change	Significance
Hematocrit	45.6 ± 4.1	44.1 ± 3.4	−1.5 ± 3.3	−2.9 ± 6.7	****
Plasma [Na+] (mmol/L)	138.1 ± 2.1	138.7 ± 2.8	+0.6 ± 3.4	+0.4 ± 2.5	****
Urine specific gravity (g/mL)	1.014 ± 0.008	1.023 ± 0.007	+0.009 ± 0.008	+0.9 ± 0.8	****

Notes: Results are presented as mean ± standard deviation. ****$P < 0.0001$.

thickness at the abdominal and thigh sites and body mass. Because skinfold thickness decreased only at the pectoral, suprailiac, and medial calf sites and not at the abdominal or thigh sites, these minor changes in skinfold thickness might explain the small decrease in estimated fat mass. The eccentric component of running might lead to skeletal muscle damage,[33] which might impair fat oxidation, thus leading to a smaller decrease in skinfold thickness compared with that induced by more concentric exercise, such as swimming and cycling.[19,22]

Adipose subcutaneous tissue is stored energy, and one might hypothesize that the thickest skinfolds might show the largest decrease during prolonged endurance exercise, as has been shown for ultracyclists.[19] We found the largest absolute decrease (−0.6 mm) at the suprailiac skinfold, which was also the thickest skinfold, with 16.5 mm (Table 2). Boschmann et al investigated the metabolic response to exercise in subcutaneous adipose tissue.[21] When their subjects cycled under laboratory conditions, lipolysis was higher in femoral subcutaneous adipose tissue than in abdominal adipose subcutaneous tissue. In contrast with that laboratory study, our ultramarathoners showed no decrease in front thigh skinfold thickness, but did show a decrease in medial calf skinfold thickness. This difference might be explained by the different kind of exercise performed. In running, the strain on the calf might be greater than that on the thigh. Also, prerace kilometers run per week and running speed during training were negatively associated with all skinfold thicknesses, except for that at the front thigh site.

The loss in skeletal muscle mass was approximately 0.5 kg higher than the loss in fat mass. Because the anthropometric equation for estimation of skeletal muscle mass uses limb circumference, the decrease in limb circumference would explain the decrease in estimated skeletal muscle mass. However, using this method, we are not able to differentiate between a decrease in contractile fibers or a depletion of energy stored within the muscle fibers such as glycogen and intracellular fat.[30] In addition, this method is not able to determine whether the decrease is due to a loss of water. Boschmann et al reported that lipids stored in muscle are used rather than lipids stored in adipose tissue for fueling the energy metabolism of muscle during exercise.[21] They showed that the increase in lipolysis was greater in muscle than in femoral subcutaneous adipose tissue. Intracellular triglyceride stores in muscle form an important substrate source of fuel during moderate intensity exercise in endurance-trained male athletes.[31]

Table 4 Association of training variables with prerace skinfold thickness and body fat (n = 219)

	Weekly running hours		Kilometers run per week		Running speed during training	
	r	P	r	P	r	P
Skinfold pectoral	−0.07	−	−0.23	0.0006	−0.19	0.0052
Skinfold triceps	−0.04	−	−0.26	−0.0001	−0.22	0.0008
Skinfold subscapular	−0.05	−	−0.18	0.0062	−0.27	<0.0001
Skinfold abdominal	−0.12	−	−0.25	0.0002	−0.28	<0.0001
Skinfold suprailiac	−0.09	−	−0.26	<0.0001	−0.25	0.0002
Skinfold front thigh	−0.08	−	−0.19	0.0039	−0.08	
Skinfold medial calf	−0.04	−	−0.14	0.038	−0.14	0.042
Sum of skinfolds	−0.09	−	−0.27	<0.001	−0.26	<0.0001
Fat mass	−0.12	−	−0.24	0.0004	−0.21	0.0016
Circumference upper arm	0.00	−	−0.20	0.0026	−0.17	0.011
Circumference thigh	0.05	−	−0.10		−0.02	
Circumference calf	−0.01	−	0.11		0.06	
Skeletal muscle mass	−0.04	−	0.05		0.09	

Note: P value is inserted for statistically significant associations.

Table 5 Association of skinfold thickness, body fat, and muscle mass with race time (n = 219)

	r	P
Skinfold pectoral	0.45	<0.0001
Skinfold axillary	0.44	<0.0001
Skinfold triceps	0.32	<0.0001
Skinfold subscapular	0.36	<0.0001
Skinfold abdominal	0.41	<0.0001
Skinfold suprailiac	0.37	<0.0001
Skinfold front thigh	0.30	<0.0001
Skinfold medial calf	0.28	<0.0001
Sum of skinfolds	0.45	<0.0001
Fat mass	0.43	<0.0001
Circumference upper arm	0.24	0.0004
Circumference thigh	0.16	0.021
Circumference calf	0.04	
Skeletal muscle mass	−0.02	
Body mass	0.30	<0.0001

Note: P values are inserted for statistically significant associations.

Differences between thigh and calf circumference

The decrease in calf circumference was significantly ($P < 0.0001$) higher (−2.4%±2.0%) than the decrease in thigh circumference (−0.9%±2.5%). Further, the decrease in calf circumference was associated with running speed, but the decrease in thigh circumference was not. The strain of running might be higher at the calf than at the thigh. We assume that these athletes primarily used their calf muscles as energy stores. Krssak et al showed that glycogen depletion was two-fold greater at the calf than at the thigh when subjects ran on a treadmill at submaximal intensity to exhaustion.[32] In addition, intracellular lipids were significantly reduced in the soleus muscle. An alternative explanation might be that while running the activation of the muscle pump pressed fluid out of muscle, interstitium and dermis. However, the eccentric component of running may

also lead to skeletal muscle damage,[33,34] leading to a decrease in skeletal muscle mass.[8,12,13,16,17] This assumption is supported by the greater decrease in calf circumference compared with the decrease in thigh circumference. In addition, the decrease in calf circumference was related to running speed but not to thigh circumference. We need also to consider that circulation during exercise might have an effect on limb volume. Stick et al showed that calf volume decreased during cycling, while thigh volume remained unchanged.[35] These authors concluded that lowered venous pressure, increased lymphatic flow, and increased tissue pressure in the contracting muscle may lead to reduction in calf volume during exercise. Thermal stress might also have an effect on calf volume. Yamazaki et al demonstrated that the changes in calf volume during orthostatic stress were modulated according to changes in venous distensibility and blood flow to the skin during thermal loading.[36]

Changes in anthropometry and markers of hydration status

Skinfold thickness on the legs might be influenced by the type of exercise such as running, changes in plasma volume, changes in hydration status, compression of the tissue, limb swelling, and fluid intake. Milledge et al assumed that fluid accumulation during prolonged walking was associated with an increase in leg volume.[37] Bracher et al showed that fluid intake was related to changes in leg volume in 100 km ultramarathoners.[38] Cejka et al demonstrated an association between fluid intake and foot volume in 100 km ultramarathoners.[39] Slight changes in water distribution of the body also influence thickness of the dermis, changes in which are pronounced in the lower leg.[40] Additionally, changes in plasma volume lead to changes in tissue thickness.[41] For both thigh and calf skinfolds, we found a negligible association between fluid intake and change in

Table 6 Correlation of changes (Δ) in skinfold thickness and limb circumference with running speed, fluid intake, and parameters of hydration status

	Running speed	Fluid intake	ΔBody mass	ΔHematocrit	ΔPlasma [Na⁺]	ΔUrine specific gravity
Δ Skinfold pectoral	0.00	0.09	0.18**	0.02	−0.05	−0.06
Δ Skinfold axillary	−0.06	0.07	0.24**	−0.17*	−0.12	−0.19**
Δ Skinfold triceps	−0.05	0.03	0.13	−0.04	0.03	0.02
Δ Skinfold subscapular	−0.02	0.05	0.07	−0.07	−0.03	−0.10
Δ Skinfold abdominal	−0.08	0.02	0.08	0.03	−0.10	0.07
Δ Skinfold suprailiac	−0.06	−0.06	−0.03	0.02	−0.02	0.07
Δ Skinfold front thigh	0.03	0.06	0.16*	−0.13	0.01	0.01
Δ Skinfold medial calf	0.07	0.14*	0.06	−0.08	−0.04	−0.03
Δ Circumference upper arm	0.02	0.00	0.16*	0.04	−0.13	−0.02
Δ Circumference thigh	−0.02	0.05	0.25**	−0.05	−0.09	0.04
Δ Circumference calf	−0.13*	0.17*	0.34***	−0.23***	−0.30***	−0.08

Notes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

body mass. Fluid intake was negligibly associated with change in skinfold thickness at the calf but not at the thigh. The increase in plasma volume was negligibly associated with change in skinfold thickness at the calf, but not with change in skinfold thickness at the thigh. For calf circumference, we found a negligible association with running speed and fluid intake, a weak association with change in hematocrit, and a moderate association with change in both body mass and plasma sodium. Changes in skinfold thickness in the lower limbs do not affect the Stewart and Hannan equation used to estimate fat mass.[28] However, the change in calf circumference affects the estimation of skeletal muscle mass, given that the equation of Lee et al uses the skinfold-corrected circumferences of the upper arm, thigh, and calf to estimate skeletal muscle mass.[27] The decrease in calf circumference explained 32% of the decrease in skeletal muscle mass, which is considerably more than the decrease in thigh circumference (12%) but less than the decrease in upper arm circumference (42%).

Prerace training and prerace anthropometry

We were also interested to find associations between training variables and skinfold thickness, because Legaz and Eston have shown that running training results in a significant increase in performance and a decrease in the sum of six skinfolds, as well as the single skinfold thickness at the abdominal, front thigh, and medial calf sites.[23] Legaz Arrese et al reported that body fat was only associated with marathoners, probably due to the fact that these runners are engaged in higher training volumes and that fat metabolism prevails in both training and competition only in marathon running.[42] Legaz and Eston determined differences in skinfold thickness after the training period whereas we, in contrast, performed correlation analyses with cross-sectional data.[23] Although we found negligible to weak correlations between kilometers run weekly, running speed during training, and skinfold thickness, correlation analyses do not demonstrate a cause and effect relationship. Intense running may lead to lower body fat. However, dieting may also lead to lower body fat. Furthermore, a decrease in body fat may lead to a decrease in body mass, thus increasing running speeds.

Limitations and implications for future research

We must acknowledge that the present study has limitations relating to the methods used to estimate skeletal muscle mass and fat mass. For instance, changes in muscle circumference during prolonged exercise may have been affected by other mechanisms, such as dehydration and overhydration, which

are different from energy depletion. For this reason, we performed additional analyses in order to estimate the reliability of the equation of Lee et al for prerace and post-race measures.[27] More specifically, we analyzed how a change of 0.5 cm in limb circumference – which is a great change related with other factors different than loss of muscle mass – affected our results for skeletal muscle mass. We found that such a reduction in limb circumference corresponded to a decrease of 0.4 ± 0.2 kg in skeletal muscle mass. In addition, a 0.5 cm reduction in thigh and arm circumference corresponded to a decrease of 0.1 ± 0.1 kg and 0.2 ± 0.2 kg in skeletal muscle mass, respectively. Considering these differences, we found that the change in skeletal muscle mass remained statistically significant between prerace and post-race measurements ($P < 0.05$). In addition, another measure which can alter the anthropometric equation established by Lee et al is body height.[27] From this viewpoint, it is known that running induces an acute increase in load on the spine and may reduce overall body height by 0.4–0.8 cm in subjects after exercise.[43,44] We also analyzed how this change in body height could modify the results obtained using the equation of Lee et al.[27] We found that a height reduction of 1 cm overestimated skeletal muscle mass by 0.2 ± 0.1 kg. However, differences between prerace and post-race measurements of skeletal muscle mass were still statistically significant ($P < 0.05$). In addition, we also analyzed the reliability of the equation of Stewart and Hannan when used to estimate fat mass.[28] We hypothesized that a 50% change in our results could be due to reasons other than loss of fat mass. It was estimated that 0.1 ± 0.1 kg could be related with this fact. However, as shown in Table 2, skinfold thickness at the abdominal and thigh sites did not change significantly during the race. Thus, almost all body fat reduction estimated by the equation used in this study could be explained by a loss of body weight. In relationship with this point, the average results for urine specific gravity were below 1.030 g/mL, indicating adequate physiological hydration status. Anthropometric methods have been shown to be a reliable method of demonstrating changes in fat mass. Cisar et al investigated the validity of anthropometric equations in determining changes of body composition in adult males during training, and reported that anthropometric measures, including skinfolds, were a stable predictor of body density during training.[45] Application of anthropometric methods may depend upon the sporting discipline involved. For elite male judo athletes before a competition, anthropometric models using seven or three skinfolds were not valid when relative fat mass, absolute fat mass, and fat-free mass were

evaluated at baseline and before a competition.[46] However, for body builders, comparison of underwater weighing, deuterium dilution, a three-compartment model incorporating total body water, a three-compartment model incorporating bone mineral content, and descriptive methods, namely dual-energy x-ray absorptiometry, predictive equations based on body mass index, skinfold measurement, and bioimpedance analyses during strength training, gave acceptable group mean values.[47] Anthropometric methods also seem to be reliable when used to measure changes in lean mass. Slater et al showed that, for elite rugby players, determination of lean mass index using a skinfold-based index for tracking proportional changes was a reliable method when compared with hydrodensitometry, deuterium dilution, and dual-energy v-ray absorptiometry.[48] Therefore, overall, despite the fact that the anthropometric methods used in this study had limitations in the estimation of both skeletal muscle mass and fat mass, we believe that anthropometric methods are reliable for analyzing changes in large samples of subjects.

This study is also limited by the lack of consideration of food intake during the race. Energy intake during the run might have influenced both performance and changes in body mass. Body mass changes result from substrate use (with water production), water loss (urine, sweat, and breath), water intake, and energy intake. Furthermore, prerace diet was not considered. Some athletes may have followed a diet either to increase body mass or to decrease body mass, depending on their specific aim for the race. In some subjects, both skeletal muscle mass and fat mass increased. Estimation of skeletal muscle mass using an anthropometric method with limb circumference and skinfold thickness may be limited because peripheral edema can occur in ultramarathoners, which might affect anthropometric measurements.[38] Intensity during both training and the race was not estimated. Therefore, comparisons using laboratory derived intensities expressed in $\%VO_{2max}$ are difficult. The decrease in both skeletal muscle mass and fat mass requires future confirmation using dual-emission x-ray absorptiometry. Changes in extracellular and intracellular volumes due to running might be detected using segmental and wrist-ankle bioimpedance spectroscopy.[49]

Conclusion

To summarize, prerace fat mass and prerace skinfold thickness were negligibly to weakly associated with both volume and speed in running training. Further, during the ultramarathon, skinfold thickness at the pectoral, suprailiac, and calf sites decreased, but not at the thigh site. The percent decreases in skinfold thickness for ultrarunners are lower than the decreases in skinfold thickness reported for ultraswimmers and ultracyclists. To verify the observed changes in both estimated skeletal muscle mass and estimated fat mass, changes in body composition should be investigated using dual-emission X-ray absorptiometry or MRI (magnetic resonance imaging). Changes in extracellular and intracellular volume variations due to running might be detected using segmental and wrist-ankle bioimpedance spectroscopy.

Disclosure

The authors report no conflicts of interest in this work.

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