

1 **Short-termed changes in quantitative ultrasound estimated bone density among young men in an 18-**  
2 **weeks follow-up during their basic training for the Swiss Armed Forces**

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19



20 **Keywords:** fat mass, bone loss, QUS, BUA, basic training



21


22

23 **Abstract**

24 **Background.** Quantitative Ultrasound (QUS) methods have been widely used to assess estimated bone  
25 density. This study aimed to assess changes in estimated bone density in association with changes in body  
26 composition, physical activity, and anthropometry.


27 **Methods.** We examined changes in anthropometry, body composition, and physical activity associated with  
28 changes in estimated bone mineral density (measured using quantitative ultrasound at the heel bone via a  
29 Sonost 3000 device  calculating bone ultrasound attenuation BUA  sound velocity SOS) in a follow-up  
30 sample of n=73 young men at the beginning and again 18 weeks later at the end of basic military training.


31 **Results.** At the end of the basic training, the subjects were on average significantly heavier, slightly taller  
32 and had a higher fat mass and grip strength  significant decrease in mean physical activity and mean  
33 estimated bone density  (calculated with BUA) was observed in the paired t-test. The results of the  
34 multivariable linear regressions (backward selection) show that changes in skeletal muscle mass (delta = 2nd  
35 measurement minus 1st measurement) have negative and body weight (delta) have positive association with  
36 the speed of sound SOS (delta), while fat mass (delta) and physical Activity (delta) had the strongest  
37 negative associations with estimated bone mineral density (delta). In particular, we found a negative  
38 association between fat mass (delta) and estimated bone mineral density (delta, estimated with BUA).

39 **Conclusion.** Our study also suggests that estimated bone density [from the calcaneus](#) can change within a few  
40 months even in young and mostly healthy individuals, depending [upon](#) physical activity levels and other co-  
41 factors. Further studies including other troop types as control groups as well as on women should follow in  
42 order to investigate this public health relevant topic in more depth. To what extent the estimated bone density  
43 measurement with quantitative ultrasound is clinically relevant needs to be investigated in further studies,  
44 since there were no stress fractures in our study group .

45

## 46 1. Introduction

47 Adverse bone health and especially osteoporosis are major public health problems, with an osteoporosis  
48 prevalence of 16% in men and 30% in women in the USA in 2017 <sup>1; 2</sup>. In the European Union, 3.5 million  
49 cases of fragility fractures (defined as a fall from standing height or less, resulting in a fracture) were caused  
50 by osteoporosis and adverse bone health, costing an estimated 2,050 million Swiss Francs per year in  
51 Switzerland in 2010 <sup>3</sup>. Regarding bone health, most research is ~~done~~ conducted on older people. Less  
52 information is available on young and healthy people, although it has been proven that if prevention is  
53 necessary, it best starts at a young age. Bone density determination plays the biggest role in the study of bone  
54 health. Besides the clinical gold standard ~~Dual dual Energy energy X-Ray ray Absorptiometry~~  
55 absorptiometry (DXA), ~~Quantitative quantitative Ultrasound-ultrasound~~ (QUS) is an established alternative  
56 to estimate bone density and predict osteoporotic fractures <sup>4-9</sup> in specific study settings <sup>10; 11</sup>. Systematic  
57 reviews and meta-analyses as well as the International Society for Clinical Densitometry (ISCD) validated  
58 QUS as being a good predictor for hip and other non-vertebral fractures and as reliable as DXA <sup>12-14</sup>. QUS is  
59 an independent predictor of fracture in men and women, even after adjusting for DXA <sup>15</sup>, and showed the  
60 same area under the curve as DXA in calculating the risk for fracture <sup>9; 16</sup>. Direct correlations between QUS  
61 and DXA showed a sensitivity of 70%–85% and a specificity of 44%–70% for detecting osteoporosis, and is  
62 therefore used as an estimated bone mineral density measurement <sup>17; 18</sup>.  the US Preventive Services Task  
63 Force states that QUS at the calcaneus predicts fractures of the femoral neck, hip, and spine as effectively as  
64 DXA <sup>19</sup>.

65 Current clinical calcaneal bone QUS devices measure two parameters after passing the : broadband  
66 ultrasound attenuation (BUA) and speed of sound (SOS). BUA and SOS provide supplementary information  
67 on the mechanical and structural properties of bone, which are distinct from bone mineral density (BMD) <sup>20;</sup>  
68 <sup>21</sup>. SOS is additionally influenced by mechanical factors like elasticity and compressive strength along with  
69 structural factors like the density and architecture of trabeculae <sup>22</sup>. BUA results from a combination of  
70 absorption and scattering, and reflects particularly structural properties such as bone size, bone volume, and  
71 orientation of the trabecular network <sup>23; 24</sup>.

72 Most studies investigating bone density are performed in the elderly, particularly postmenopausal women,  
73 concerning osteoporosis, while studies in young men are few. Specifically in young adults, physical activity  
74 influences bone density: Highhigh-impact sports (e.g. rugby and powerlifting) lead to higher BMD  
75 (measured with DXA) than low-impact or non-weight bearing sports (such as rowing, cycling, and  
76 swimming) <sup>25</sup>. An 18-month follow-up study in gymnasts compared to controls (with 3-monthly QUS  
77 measurements) showed a continuous increase in estimated bone density (calculated with BUA), but with no  
78 change in SOS <sup>26</sup>. Another study with 3-month circuit training showed an increase in SOS and BUA in young  
79 female students <sup>27</sup>. Similar associations have also been observed in military settings, where life circumstances  
80 (exercise, diet, etc.) are equal for most participants. A handful of studies in various military follow-up  
81 settings over a few months have already documented changes in bone density. For example, in young healthy  
82 recruits, a high response of bone density and remodelling microarchitecture (measured with DXA and CT

83 scan) was observed after 8–10-weeks of physical training<sup>27</sup>. The same observation of increasing estimated  
84 bone density (calculated with BUA) was made after ~~6~~six months of military service in Finland, while  
85 physical training had the largest effect<sup>28</sup>. An investigation of a 12-week program of physical military  
86 training in the UK showed an increase in BMD (DXA), estimated bone density (BUA), and bone volume  
87 (particularly in cortical and periosteal volume), but no change in SOS<sup>29</sup>. For the Swiss Armed Forces  
88 context, there is no information yet on changes in bone status during military service. In general, little is  
89 known about short-term changes in bone status of young men in Switzerland, especially about associations  
90 with changes in body composition, anthropometry, and physical activity.

91 This study aimed to investigate the longitudinal use of QUS in a four months' follow-up of a sample of basic  
92 military training recruits to assess estimated bone density in association with co-factors such as body  
93 composition, physical activity, and anthropometry.


94

## 95 2. Methods

96 The first measurement took place in March 2017 in Kloten (Canton Zurich, Switzerland); the follow-up  
97 measurements were taken 18 weeks later in July 2017 in Neunkirch (Canton Schaffhausen, Switzerland).  
98 The precise study protocol has been described elsewhere<sup>30;31</sup>. The voluntary participants were Swiss male air  
99 defence recruits of the Swiss Armed Forces, aged 19–23 years at the beginning and at the end of their basic  
100 military training. Typically, during the first weeks of the basic training, recruits are taught the basic  
101 knowledge for soldiers. Parallel to this, the function-related basic training ~~already starts~~begins. In the third  
102 part of the basic training, the focus is on unit training. During the training weeks, the recruits undergo a  
103 standardized program, which involves similar nutrition and physical activity levels for most recruits of a  
104 specific troop type. The air defence has in comparison to other types of troops (e.g. grenadiers, infantry) a  
105 lower physical requirement.

106 For our study, no selection was made for socioeconomic background, regional origin, or demographic  
107 factors. Because this study was the first with follow-up in the basic training setting of the Swiss Armed  
108 Forces, and measurement times and availability of participants also depended on troop organizational factors,  
109 the sample size was not calculated ~~before the start of the study~~. However, the power of our models was  
110 calculated post-hoc (see below). A total of 104 young men participated at the baseline measurements at the  
111 beginning of the basic training; 73 (70.2%) could be reassessed four months later. Due to splitting and  
112 relocation of subjects from the initial troop or quitting the service, we were not able to re-assess 31 subjects;  
113 thus, they were excluded from the study. The same measurement protocol and devices were used during both  
114 examinations. Participation was voluntary. Written and oral briefings were provided at the start of the study  
115 and shortly before the examination, respectively. The participants signed a detailed informed consent form.  
116 This study was approved by the Ethics Committee of the Canton of Zurich (No. 2016-01625).


117 *Outcome variables: estimated bone density via QUS*

118 We used a calcaneal site QUS device (Sonost-3000, medical ECONET, Oberhausen, Germany) which  
119 measured the velocity of sound waves as the speed of sound (SOS in m/s) and the attenuation after passing  
120 the bone as bone ultrasound attenuation (BUA in dB/MHz). In the range of the ultrasound measurements  
121 (between 0.3 and 0.65 MHz), theoretical calculations illustrated a linear function of attenuation dependent on  
122 frequency, and a linear positive correlation between estimated bone density (calculated with BUA) and BMD  
123 (measured with DXA) <sup>17</sup>. This correlation was also observed in experimental studies <sup>18; 32; 33</sup>. The  
124 manufacturer states that the Coefficient of Variation (CV%) for SOS is 0.2 and for BUA 1.5.  In addition,  
125 QUS devices calculate the bone quality index (BQI) from SOS and BUA using the manufacturer's equation.  
126 Because of the manufacturer's custom equation which is based on SOS and BUA, there is no comparability  
127 between different devices. Therefore, we excluded the BQI from further investigations.

128

### 129 *Co-factors: Body composition, anthropometric measurements, and physical activity*

130 For the bioelectrical impedance analysis (BIA), we used a medical 8-point body composition analyser (Seca  
131 mBCA 515, Reinach, Switzerland). We measured body fat mass (%), visceral fat mass (l), skeletal muscle  
132 mass (kg), body weight (kg), and total energy expenditure (kcal/day). Participants stood barefoot on footpads  
133 (each side with two electrodes) and held their hands on handpads (each side with two electrodes). Compared  
134 to the four-compartment measurements for determining body composition, the Seca body composition  
135 analyser correlates with 98%, which suggests an equivalent quality <sup>34</sup>.

136 During the BIA measurements,  we performed manual measurements of the waist circumference according to  
137 the WHO protocol; we used a handheld tape (Seca 201, Reinach, Switzerland) with stretch resistant quality  
138 and automatic retraction, at the midpoint between the lowest point of the ribcage and the highest point of the  
139 pelvis bone, while the participant stood in a relaxed upright position breathing normally <sup>35</sup>. A standard  
140 stadiometer (Seca 274, Reinach, Switzerland) was used to determine body height, with the participants  
141 standing barefoot in a straight-up position, feet together. Body mass index (BMI, kg/m<sup>2</sup>) was calculated from  
142 height and weight. BMI values were classified as underweight (< 18.5 kg/m<sup>2</sup>), normal weight (≥18.5–<24.9  
143 kg/m<sup>2</sup>) and overweight (≥ 25.0 kg/m<sup>2</sup>), according to WHO guidelines. Grip strength was measured using a  
144 hand dynamometer (SH5001, Changwon-si, Korea).

145 The Global Physical Activity Questionnaire (GPAQ) was used to evaluate physical activity at the beginning  
146 of the study (to assess physical activity before starting the basic training) and at follow-up (to assess physical  
147 activity during the basic training) <sup>36</sup>. The questionnaire contained items about low-, mid-, and high-intensity  
148 activities during work (e.g. for *low-intensity*: businessman, merchandiser; for *mid-intensity*: housekeeper,  
149 gardener, farmer; for *high-intensity*: lumberjack, construction worker, bricklayer, roofer, fitness instructor)  
150 and leisure (e.g. for *low-intensity*: sedentary activities, fishing; for *mid-intensity*: casual cycling/swimming,  
151 dancing, riding, yoga, strength training, climbing; for *high-intensity*: soccer, football, athletics, aerobic,  
152 ballet, jogging, boxing, intense cycling/swimming) <sup>36</sup>. The GPAQ allows to calculate the metabolic  
153 equivalents (MET) per week, which are commonly used to express the intensity of physical activities. MET  
154 is the resting metabolic rate and defined as the energy consumption of sitting quietly. One MET is equivalent

155 to a caloric consumption of 1 kcal/kg/hour. During moderate activity, the caloric consumption is four times  
156 as high as the resting metabolic rate and during vigorous activity eight times as high. To calculate the  
157 physical activity as overall energy expenditure, the sum of MET of moderate activities (MET multiplied by  
158 4), and of MET of vigorous activities (MET multiplied by 8), was used <sup>36</sup>.

159 The participants were also asked about their smoking and sports habits. Accordingly, answers were  
160 categorized into groups, athletes vs. non-athletes (regular sportive activities in leisure time vs. no sportive  
161 activities in leisure time), and smokers vs non-smokers (number of cigarettes per day > 0 vs. number of  
162 cigarettes per day = 0).

163

### 164 *Statistics*

165 Outcome variables (BUA, SOS) and co-factors (body composition measures, anthropometrics, and physical  
166 activity levels) at baseline and follow-up were reported as mean values  $\pm$  standard deviation (SD) for the full  
167 sample as well as for athletes vs. non-athlete and smokers vs. non-smokers. Differences between means were  
168 tested using paired t-tests. The symmetry of the main variable distributions of each parameter was visually  
169 assessed using histograms.

170 The individual longitudinal changes in the outcome variables and co-factors were calculated as deltas (delta  
171 = 2nd measurement minus 1st measurement). First, Pearson correlation coefficients were calculated between  
172 the individual deltas for the outcome variables BUA and SOS on the one hand and all cofactors on the other.  
173 Second, to see which co-factors delta were most associated with delta in the outcome variables (BUA and  
174 SOS), we used stepwise backward selection and calculated multi-variable linear regression models which  
175 included anthropometrics, body composition, physical activity, and smoking status as independent variables.  
176 We also calculated post-hoc power of our models using the *pwr* package.

177 All statistical analyses were performed using R Version 4.1.2.

178

### 179 **3. Results**

180 The descriptive statistics of the outcome variables and co-factors are given in Table 1. At baseline, the age of  
181 the participants ranged from 18.8 years to 23.9 years (mean 20.5, SD 1.0), body height ranged from 1.66 m  
182 to 1.94 m (mean 1.78, SD 0.07), weight ranged from 50.6 kg to 104.7 kg (mean 73.2, SD 12.4), and BMI  
183 ranged from 16.4 kg/m<sup>2</sup> to 30.1 kg/m<sup>2</sup> (mean 23.0, SD 3.3). Eighteen subjects (24.7%) were overweight  
184 (BMI  $\geq$  25.0 kg/m<sup>2</sup>), and three subjects (4.1%) were underweight (BMI < 18.5 kg/m<sup>2</sup>). At follow-up 18  
185 weeks later, 20 of 73 subjects (27.4%) were overweight, while the prevalence of underweight was  
186 unchanged. All variables appeared to be symmetrically distributed. There were 43 subjects in the athletes  
187 group (58.9%) and 30 subjects in the non-athletes group (41.1%). In the smoker group (n=34, 46.6%), two  
188 stopped smoking during the study period. In the non-smoker group, (n=39, 53.4%), two started smoking.

189 The comparison of mean values between baseline and follow-up is shown in Table 1. The means for weight,  
190 height, fat mass, and grip strength significantly increased during basic training, while mean BUA and  
191 physical activity significantly decreased during the same period. No significant changes were observed in  
192 mean BMI, fat-free mass, skeletal muscle mass, waist circumference, visceral adipose tissue, and SOS.  
193 When comparing the athletes vs. non-athletes groups as well as the smoker vs. non-smoker groups, the same  
194 patterns were observed (Appendix Tables 1 and 2).

195 When the individual changes (delta) in selected outcome variables and co-factors are plotted against their  
196 baseline value at baseline (Appendix Figure 1), it becomes apparent that most variables (except height)  
197 showed regression to the mean-like behaviour with an approximation to the mean: High values at the  
198 beginning dropped and low values at baseline increased during basic training. Pearson correlation  
199 coefficients between deltas in the outcome variables (SOS and BUA) on the one hand and deltas in the co-  
200 factors on the other hand are reported in Table 2. For individual changes in SOS, a weak positive correlation  
201 was observed with grip strength ( $r=0.21$ ), whereas weak negative correlations were found with skeletal muscle  
202 mass ( $r=-0.18$ ), waist circumference ( $r=-0.15$ ). For individual changes in BUA, there were weak negative correlations  
203 with fat mass ( $r=-0.12$ ), physical activity ( $r=-0.11$ ).

204 Results from the multivariable linear regressions (backwards selection) are reported in Table 3. For the  
205 model with delta of SOS as dependent variable, delta in skeletal muscle mass ( $\beta$ -coefficient -6.01,  $p<0.001$ )  
206 and delta in body weight ( $\beta$ -coefficient +0.93,  $p=0.086$ ) were the remaining co-factors, the model explained  
207 nearly one-third of the variation in delta of SOS ( $R^2=0.32$ ). For the model with delta in BUA as dependent  
208 variable, delta in fat mass ( $\beta$ -coefficient -1.74,  $p<0.001$ ) and delta in physical activity ( $\beta$ -coefficient -0.0005,  
209  $p=0.006$ ) were the remaining co-factors, the model explained nearly a quarter of the variation in delta of  
210 BUA ( $R^2=0.22$ ). The very low  $\beta$ -coefficient of physical activity was due to higher absolute values (mean at  
211 start=7547.9) than fat mass (mean at start=13.7). The post-hoc calculated power of the two models was  
212 0.998.

213

#### 214 4. Discussion

215 | In this study, we analysed changes in the estimated bone density at the calcaneus (calculated with BUA) and  
216 their associations with the co-factors like anthropometry, body composition, and physical activity in a short-  
217 term follow-up setting (18 weeks) during basic military training in a sample of 73 young Swiss men. At the  
218 end of their basic training, the participants were by average heavier, slightly taller, and had higher fat mass  
219 and grip strength. A significant decrease in mean physical activity and estimated bone density (calculated  
220 with BUA) was observed. Generally, a regression to the mean-like change of individual differences was  
221 visible, except for height. Weakly negative associations were found between deltas (delta = 2nd  
222 measurement minus 1st measurement) in estimated bone density and deltas in fat mass as well as physical  
223 activity. As has been proven in various studies (see introduction), the QUS measurement can also show a  
224 change in the estimated bone density over a short period of 3 months<sup>26 27</sup>.

225 Several studies have investigated the associations of anthropometry and QUS in children, young and elderly  
226 adults, particularly women <sup>37-39</sup>. In children and men, age, weight, height, BMI, and fat-free mass were  
227 positively correlated with SOS and BUA, while fat mass and waist circumference were correlated with BUA.  
228 Vigorous physical activity correlated positively with SOS and BUA, but dietary factors showed no  
229 association <sup>37-39</sup>. In our study, an increase in height during basic training was visible due to growth. Physical  
230 growth can be observed until the age of 24 <sup>40</sup>. The general increase in grip strength at the end of basic  
231 training could be explained by increased physical load caused by manual work (e.g., repeated packing,  
232 carrying, or putting on and taking off the heavy backpacks).

233 At the end of basic training, an increase in weight and fat mass and a decrease in physical activity were  
234 observed. No changes in fat-free mass or skeletal muscle mass were observed; thus, it can be assumed that  
235 the increase in weight is due to increased fat mass. Maybe, recruits had less physical activity during the basic  
236 training than before, which can explain the gain in weight and fat mass. Furthermore, changes in eating  
237 habits (e.g. regular breaks for subsistence) and/or a higher physical/mental stress level could have negative  
238 effects on weight and fat mass <sup>41</sup>.

239 After the 18-week follow-up, a change in the estimated bone density is visible with a significant decrease in  
240 the attenuation of the ultrasound (BUA). Interestingly, the regressions revealed a weak association between  
241 changes in BUA and changes in fat mass, which could indicate that increase in fat mass – alongside with  
242 decreased physical activity – during the 18 weeks of basic training already harmed the estimated bone  
243 density. The negative influence of fat mass on bone metabolism has already been documented several times  
244 <sup>42-45</sup>. It is known that greater mechanical stress (with weight gain) on the bone can lead to an adaptation and  
245 increase in bone mass, as it has been shown for high-impact sports but less so for walking alone <sup>25; 46-51</sup>.  
246 However, it is also known that visceral and subcutaneous adipose tissue negatively affects bone remodelling  
247 via inflammatory factors such as the upregulation of the nuclear factor  $\kappa$ B ligand, which leads to a  
248 stimulation of the osteoclast activity and thus to bone resorption <sup>44; 45; 52-54</sup>. Our results imply that the weight  
249 gain, which is mainly due to the increase in fat mass, already has a weak negative association with estimated  
250 bone density (calculated with BUA) in the short period of 18 weeks.

251 Our study had several strengths and limitations. The strengths include the homogeneous sample of young  
252 men, the well-controlled environment, and the uniform amount and type of physical activity and nutrition  
253 during basic training. The main limitation of the study is the lack of a control group. It is known that air  
254 defence recruits have less demand for activities (marches, runs, inactivity per day) than other units in the  
255 Swiss Armed Forces <sup>55</sup>. To better understand the circumstances of these highlighted changes in estimated  
256 bone density and associations with co-factors on the group and individual levels, a larger study design with  
257 one or more control groups (e.g., other troop types) would be desirable. Further limitations include the  
258 shortcoming of distinguishing between high-and low-impact activities/sports in the GPAQ. A questionnaire  
259 may be answered subjectively; therefore, it has limited reliability. The examination battery did only include  
260 grip strength as measurement of physical fitness, and a comprehensive assessment of nutrition and diet in a  
261 longer and thus more time-consuming questionnaire could not be performed in the current setting.



262 Information about alcohol consumption should also be included in future studies, as lifestyle behaviour can  
263 change during basic training and there is a known negative association with bone density. Furthermore, BIA  
264 is not the gold standard for measuring body composition. However, due to time constraints within the army  
265 setting, we were not able to perform more time-consuming (e.g., multiple quantitative ultrasound  
266 measurements of the same subject) and invasive measurements. Similarly, waist circumference could be  
267 measured only once per measurement slot because of time constraints. By appointing the same experienced  
268 researcher, we excluded inter-observer bias; however, an intra-observer bias could not be excluded. Our  
269 sample size was limited owing to the setting reasons. As the sample was homogeneous, the internal validity  
270 of the study was given, but the external validity was limited. Other quality control measures, such as  
271 determination of heel thickness and repeated measurements for subjects, could not be collected due to time  
272 constraints in our study setting. The measurement of the existing temperature during the examinations was  
273 also not recorded. To verify the results from our study, a prospective randomized study comparing low and  
274 high impact activities as well as a detailed survey of eating and nutritional habits (particularly alcohol intake)  
275 during basic training should be carried out. More studies including more age groups and both sexes should  
276 be performed. To what extent the estimated bone density measurement with quantitative ultrasound is  
277 clinically relevant needs to be investigated in further studies, since there were no stress fractures in our  
278 cohort group.

279

## 280 **5. Conclusion**

281 The loss of physical activity of the recruits during the basic training suggests a low level of physical and  
282 athletic request in this type of troop, which presumably led to weight gain. The weight gain, which is mainly  
283 due to the increase in fat mass, already shown a negative association with estimated bone density (calculated  
284 with BUA) in the short period of 18 weeks. As shown in other studies, the force exerted on the bones while  
285 walking is insufficient to strengthen the bone. For troop types with a low or medium load pattern, we  
286 recommend additional physical exercise during basic military training, such as a daily standard jump  
287 program to strengthen the bone.

288

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290

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296

297 **Ethics approval and consent to participate:**

298 Participation in this study was voluntary. The participants signed a detailed informed consent form after oral  
299 and written briefings. This study was approved by the Ethics Committee of the Canton of Zurich (No. 2016-  
300 01625).

301

302 **Patient consent for publication:**

303 Not required.

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