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a novel CT-based tissue segmentation methodology

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\textbf{18F-FDG-PET/CT in measuring volume and global metabolic activity of thigh muscles: A novel CT-based tissue segmentation methodology}

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\textbf{Conflict of Interest} None
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Abstract

Purpose We present a novel computed tomography CT-based tissue segmentation methodology for determining volume and global uptake of FDG of the thigh muscles and correlate these parameters with age.

Materials and Methods A total of 71 subjects from a prospective clinical trial [NCT01724749] were included. PET/CT scans were acquired 180 min after intravenous injection of FDG. A 3D growing region algorithm with neighborhood Hounsfield unit (HU) threshold between 1 and 150 was used to highlight the muscle. FDG uptake was expressed as the average mean standardized uptake value (SUVmean) normalized for lean body mass (average SULmean). Femur volume was used to normalize thigh muscle volume to calculate normalized volume and correlate with age.

Results We found a significant negative correlation between normalized volume and age (left side r=-0.262, p=0.02; right side r=-0.286, p=0.01). No statistically significant difference was found between SUL and age (left side r=0.10, p=0.41; right side r=0.14, p=0.24) or between SUL and BMI (left side r=0.03, p=0.78; right side r=0.02, p=0.90). There was no statistically significant difference in muscle volume on the two sides (left 3606.5±1195.6 cm$^3$, right 3633.5±1215.7 cm$^3$, p=0.28). Statistically significant difference was noted in the global metabolic activity (SUL) between the two sides (left 0.39±0.06, right 0.42±0.08, p<0.001), with 56/71 (78.8%) subjects having higher uptake on the right side.
**Conclusion** FDG-PET/CT using CT-based segmentation is a novel imaging modality assessing the volume and global metabolic activity of the thigh muscles. It could be possible to utilize this methodology for the research and understanding of lower limb muscle pathophysiology.

**Keywords** FDG, PET/CT, thigh muscle volume, sarcopenia, aging, footedness
Introduction

Skeletal muscle accounts for approximately 30-40% of total body weight and is affected by alterations in the pathophysiology and cellular proteins as a result of adaptations to advancing age [1-3]. The normal reduction in muscle mass that occurs primarily due to aging is called sarcopenia [4]. It is one of the main causes of functional impairment and physical disability, particularly in older women [5]. Reduced muscle mass is associated with critical illness and severe trauma and recovery is prolonged in such patients and, hence, loss of muscle mass holds clinical importance [6]. Along with reduction in muscle mass with aging, there is increased accumulation of adipose tissue around the muscle [7]. Similarly, there is altered skeletal muscle composition with increased fat infiltration in obese individuals [8].

Imaging as a tool to assess muscle and its function is important in clinical practice and several evaluation techniques are available. Computed tomography (CT) and magnetic resonance imaging (MRI) are considered gold standard imaging modalities [9,10]. Other imaging techniques to assess muscle mass are dual energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), anthropometry and ultrasonography (US) [11].

The glucose transporter (GLUT) 4 is present in skeletal muscle and its glucose uptake is primarily insulin dependent [12,13]. Similar to skeletal muscle glucose uptake, $^{18}$F-fluorodeoxyglucose (FDG) enters the cells via the GLUTs. FDG-PET/CT has assumed an important role in muscle disorder evaluation [14-16], and shown superiority over CT alone and MRI in assessing some muscle disorders [14].

The purposes of our study were twofold: (1) to introduce a new CT-based tissue segmentation technique to quantify volume and metabolic activity of the thigh muscles and (2) to evaluate the effects of age on muscle volume and metabolic activity using FDG-PET/CT.
Subjects and Methods

This retrospective study utilized FDG scans from the “Cardiovascular Molecular Calcification Assessed by $^{18}$F-NaF PET/CT” (CAMONA) study. CAMONA was a prospective study approved by the Danish National Committee on Health Research Ethics (NCT01724749), and conducted from 2012 to 2016 in accordance with the Declaration of Helsinki [17]. A detailed description of this prospective study was previously published by Blomberg BA et al. [17].

Subject selection

The CAMONA study included 139 subjects. Eighty-nine were healthy volunteers who did not have a history of cardiovascular disease, oncologic disease, autoimmune disease, immunodeficiency syndromes, alcohol use, illicit drug use, or any prescription medication [17]. The other 50 were patients with a history of chest pain who did not have any history of major cardiovascular events, malignancy, chronic inflammatory disease, illicit drug use, or renal insufficiency. Healthy subjects were recruited from the general population by local advertisement or from the blood bank of Odense University Hospital, Denmark. Subjects who were non-smokers with negative cardiovascular disease, total serum cholesterol below 6.2 mmolL$^{-1}$ and glycated hemoglobin (HbA1c) below 48 mmolmol$^{-1}$ were included. The angina pectoris patients were from the Department of Cardiology. Further details appear from an article by Blomberg BA et al. [18].

Out of the total 139 CAMONA subjects, we included 71 subjects that fell into the age groups of 20-40 years (N= 33) and 60 years and above (N= 38). This was done to compare the effects of age on thigh muscle volume and metabolic uptake. Of these 71, 45 were healthy volunteers and 26 had angina pectoris and 35 were females. Forty subjects were excluded as they did not fall into either age group. Twenty-eight subjects were excluded due to incomplete or unavailable PET/CT
scans in our database. Eleven subjects were on lipid-lowering drugs and none of the subjects had either type of diabetes mellitus (no subjects had HbA1c above 49 mmol/mol).

**Study Design**

The imaging protocol was previously published by Blomberg *et al.* [18]. In summary, all subjects were told to stay calm, warm and not perform any level of physical activity 24 hours before the PET/CT examination. After an overnight fast of at least 8 hours and a confirmed blood glucose concentration of below 8mmol/L, 4.0 MBq/kg FDG was given intravenously. Before imaging, the subject rested in a warm and quiet room with an instruction to stay calm and relaxed. FDG-PET/CT imaging was performed on hybrid PET/CT systems (GE Discovery STE, VCT, RX, and 690/710 systems) 180 minutes after. This time point was selected because the original CAMONA study was performed to assess for delayed time point imaging, and the study included complete scans for the most subjects at 180 minutes. PET images were corrected for scatter, attenuation, random coincidences, and scanner dead time. Low-dose CT imaging (140kV, 30-110mA, noise index 25, 0.8 second/rotation, slice thickness 3.75mm) was performed for attenuation correction and anatomical orientation.

**Image Analysis**

For the present study, all images were analyzed using OsiriX MD v.9.5 (DICOM viewer and image-analysis program, Pixmeo SARL; Bernex, Switzerland). Subject names were anonymized prior to image analysis. On the 3D maximum intensity projection (MIP) view of the CT images, we set upper and lower boundaries 5 cm below the greater trochanter and 5 cm above the intercondylar notch, respectively to acquire a desired anatomical region of interest (ROI), *fig. 1*. A 3D growing region algorithm with a neighborhood Hounsfield unit (HU) of 1 and 150 was then used to highlight the thigh muscle on the fused PET/CT images, *fig. 2*. 
Mean standardized uptake value (SUVmean) and ROIvolume were measured for each transaxial slice and exported to a Comma-Separated Values (CSV) file by Osirix. The tracer uptake in each slice was calculated by multiplying the slice SUVmean by the slice ROIvolume. The tracer uptake of all slices was summed up to get the total metabolic activity:

\[
Total \text{ metabolic activity} = \sum (SUV_{\text{mean}} \times ROI_{\text{volume}})
\]

Average SUVmean was used for the semi-quantification of FDG according to the following equation [19]:

\[
Average \ SUV_{\text{mean}} = \frac{Total \ Metabolic \ Activity}{\sum (ROI_{\text{volume}})}
\]

Volumes (cm\(^3\)) of the left and right muscle groups were independently measured, determined as summation of the cross-sectional areas (CSA) in each slice multiplied slice thickness which was 0.35 cm.

The Janmahasatian formula was used to normalize SUV by lean body mass (LBM) SUV using LBM as mass estimate is referred to as SUL [20].

Femur length was obtained by using a length tool on the 3D MIP view to draw a straight line from the proximal point of greater trochanter to distal point of lateral condyle, fig 3, [21]. We measured the CSA (cm\(^2\)) at 60% of the length of femur (from proximal end of femur), assuming the CSA in this region is relatively constant and multiplied it with femur length (cm) to obtain femur volume (cm\(^3\)) [21]:

\[
Femur \ volume \ (cm^3) = cross - sectional \ area \ (CSA) \ (cm^2) \times femur \ length \ (cm)
\]

We used femur volume to normalize thigh muscle volume [21].

\[
Normalized \ volume = thigh \ muscle \ volume \div femur \ volume
\]

Statistical analyses
Descriptive statistics were given as mean ± standard deviation (SD) and frequencies with corresponding percentages in case of continuous and categorical variables, respectively. Correlations between SUL and normalized volume and age were statistically analyzed using linear regression analysis and Pearson’s correlation coefficients. Paired t-test was used to analyze the significance of the difference between the left and right thigh and two-sample t-test was used to analyze statistical differences between men and women and young and older subjects. Statistical analysis was conducted using IBM SPSS Statistics version 25.0 (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 25.0. Armonk, NY: IBM Corp).

**Results**

The length, volume and CSA at 60% length of femur were higher in men compared to women. Similarly, the total volume of the thigh muscles was higher in men. Young women had significantly higher total thigh muscle volume than older women. Although not significant, the total volume of the thigh muscles in young men was higher than in older men, highlighted in Table 1.

Normalized volume was significantly higher in the younger population and the SUL was insignificantly higher in the older population (Table 2).

Normalized volume was significantly and inversely correlated with age (left side r=-0.262, p=0.02; right side r=-0.286, p=0.01), **fig. 4**. SUL was not significantly associated with BMI (left side r=0.03, p=0.78; right side r=0.02, p=0.90) or age (left side r=0.10, p=0.41; right side r=0.14, p=0.24).

A statistically significant difference was noted in global metabolic activity (SUL) between the two sides (left 0.39±0.06, right 0.42±0.08, p<0.001), **fig. 5 A**, with 56/71 (78.8%) subjects
having higher uptake on their right sides. There were no significant differences in muscle volume on the two sides (left 3606.5±1195.6 cm$^3$, right 3633.5±1215.7 cm$^3$, p=0.28), \textbf{fig. 5 B}, (\textbf{Table 3}).

\textbf{Discussion}

Previous studies have quantified metabolic activity of the muscles [22,23]. However, to our knowledge, this is the first study to investigate the role of FDG-PET/CT in measuring volume and global metabolic uptake of thigh muscles and comparing these measures with age. We found a significant inverse correlation between volume and age, but no statistically significant association between SUL or BMI and age.

PET/CT has the advantage of evaluating molecular as well as structural information in muscular disorders, and quantification of muscle activity is possible only with PET/CT. Chronic low-grade inflammation has been implicated in various age-related diseases including but not limited to joint diseases, cardiovascular, and endocrine disorders. Sarcopenia is an outcome of multiple factors; including physical inactivity and other processes taking place at a molecular level, such as inflammation. Although the relationship between inflammation and sarcopenia is not clearly defined, the role of inflammation as an important factor in the pathogenesis of sarcopenia has been demonstrated in some studies [24]. They implicate the role of cytokines such as C-reactive protein (CRP), interleukin-6, 10, 15 (IL-6, 10, 15), tumor necrosis factor-a (TNF-a), growth hormone (GH) as the inflammatory triggers of sarcopenia [25,26]. This concept was tested in a study by Schaap \textit{et al.} who measured the mid-thigh muscle CSA on CT images over a period of 5 years and showed that age related decline in thigh muscle CSA and strength was positively associated with TNF-a (p=0.02) [27]. Similarly, our study showed a statistically significant higher total normalized volume in the younger population (p<0.01). The SUL was
higher, but insignificantly so, in the older population. One potential reason for this trend might be the adipose tissue infiltration seen in older subjects.

Other potential causes of sarcopenia with aging include a decline in the anabolic hormones like estrogen, testosterone and its analogs [28,29]. It has also been observed that there is an imbalance between protein synthesis and degradation, with rates of protein degradation being higher with advancing age [30]. According to one study, the thigh muscles are a preferred site to assess age related changes as compared to the whole body and in that the anterior compartment undergoes preferential age-related skeletal muscle decline [31]. A study by Frontera et al. found a 0.5% per annum decline in the total thigh muscle area in patients who were in their eighth decade of life, and the decline from baseline over the 8.9 year study was predominantly in the anterior compartment (5.7%) as compared to the posterior compartment (3.2%) [32]. Moreover, in their twelve-year follow up of males (baseline age 65.4 years), Frontera et al. found a decline of 12.5% in the total mid-thigh CSA on CT images and one of 14.7% in the thigh muscle CSA [3]. Mitchell et al. reviewed studies that assessed lean body mass between years 1972 and 2011 and found that the decline starts to become prominent at age 45 but is only statistically different from a young adult at the age of 50 [33]. Our study showed a significantly higher total thigh muscle volume in the young compared to the older women (p-value <0.01). Even though not significant, young men were found to have higher total volume than older men.

Body weight may influence the SUV uptake and different formulations to normalize SUV (SUL) by lean body mass (LBM) have been suggested to give better precision and intercompatibility of the semi-quantitative/uptake measurements [20]. We used the Janmahasatian formula in the present study as this formula has proven to best cancel the bias caused by body weight on the SUV uptake in females and reduces it in males [20]. The volume
of muscles may vary among people of the same age and of different ages due differences in body structure (a large person may have more muscle mass than a small of similar or different age or gender) and in the ageing process (which vary vastly from one to another individual). For these and other reasons, normalization is called for. Thus, Maden et al. [21] found that using femur volume to normalize muscle volume is superior to dividing muscle mass by body height squared for measuring muscle loss with ageing. Using this methodology, we found a significant negative correlation between volume and age (left side $r=0.262$, $p=0.02$; right side $r=0.286$, $p=0.01$). In addition, we found a slightly higher volume and FDG uptake on the right side. Footedness becomes stable only in adulthood with the majority of the population being right-footed [34]. During normal locomotion using the bi-pedal gait, the dominant leg is used to initiate movement and the contralateral limb or the non-dominant leg stabilizes the body while maintaining posture. Lateralization with preference for the right foot is seen during activities like walking, running, jumping, cycling and kicking a ball [35]. Carey et al. studied footedness of 236 soccer players playing in the 1998 World Cup and found that 79% of these were right-footed [36]. Similarly, our study found that 58 out of 71 (81.6%) studied subjects had higher metabolic uptake in the right thigh than the left femoral muscles indicating higher work load on the right side, suggesting, even in the absence of information on footedness and on leg muscle disorders of the study subjects, that the majority people have a right-sided preponderance.

This study was not without limitations. We did not have relevant clinical information regarding footedness and history of any musculoskeletal injuries and disorders. FDG uptake in the muscles is easily influenced by physical activity and although the study followed standard protocol for patient preparation, which includes no physical activity the last 24 hours before the PET examination, there could be possibility of the findings being confounded by example, lack of
compliance. Additionally, 180-minute imaging may increase incidental uptake due to limitations in having subjects sit quietly. An HbA1c of 48mmol/mol (6.5%) is recommended as the cut off point for diagnosing diabetes. As a general guide, HbA1c levels of less than or equal to 40 mmol/mol is normal. 41 to 49 mmol/mol is prediabetes or 'impaired fasting glucose' 50 mmol/mol and above suggests diabetes if symptomatic. No subjects had HbA1c above 49 mmol/mol, and 6 subjects in the study group had HbA1c of 41 to 49 mmol/mol. Due to the low number of subjects with HbA1c values within the prediabetic range and the fact that only one of the patients had a HbA1c value in the upper threshold level, we believe that the effect on our result is minor. Finally, these results need to be interpreted in the confines of the study. Surgery, chemotherapy, radiotherapy, and other modalities may all alter systemic FDG uptake. Nonetheless, FDG-PET/CT could also have important clinical implications in including evaluating the adverse responses to these therapies and other musculoskeletal pathologies.

In conclusion, our methodology could be used in the evaluation of muscles and understanding effects of aging on muscles. It also addresses the need for structural as well as metabolic assessment of muscles with possible applications of PET/CT as a sensitive tool in diagnosis, assessment and monitoring of muscular disorders and other system diseases that affect the musculoskeletal system. Additional studies regarding this interesting application of FDG-PET/CT would be helpful in furthering research and understanding role of FDG-PET/CT in muscular disorders.

References


Figure 1. 3D maximum intensity projection (3D MIP) view of the computer tomography (CT) image showing methodology to obtain a desired anatomical region of interest (ROI). Using the scissors editing tool, two parallel lines (parallel green lines in the figure above) corresponding to 5 cm above the intercondylar notch and 5 cm below the greater trochanter were drawn to acquire a set anatomical section of the thigh. The green box represents our anatomical region of interest.
Figure 2. A growing region of interest (ROI) algorithm with lower and upper threshold between 1 and 150 Hounsfield unit (HU) was used to highlight the muscle.
**Figure 3.** A length tool was used to measure the distance between the proximal end of greater trochanter and distal end of lateral condyle on 3D maximum intensity projection (3D MIP) view of the CT image to obtain femur length. Femur length was utilized to calculate femur volume.
Figure 4. The volume of the thigh muscles decreased with age. We found a significant negative correlation between age and volume bilaterally (left side $r=-0.262$, $p=0.02$; right side $r=-0.286$, $p=0.01$).
Figure 5. The metabolic uptake on the right side was significantly higher (left 0.39±0.06, right 0.42±0.08, p<0.001) than the left side (A). Although not significant, we found higher volume on the right side (B). 58/71 (81.6%) subjects had higher uptake on the right side.
Table 1. Comparison of femur characteristics and radiotracer uptake between men and women.

<table>
<thead>
<tr>
<th></th>
<th>Men (mean±SD)</th>
<th>Women (mean±SD)</th>
<th>p-value (men vs women)</th>
<th>p-value (young vs older)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young men</td>
<td>Older men</td>
<td>Young women</td>
<td>Older women</td>
</tr>
<tr>
<td></td>
<td>(20-40 years, N=21)</td>
<td>(60 years and above, N=15)</td>
<td>(20-40 years, N=12)</td>
<td>(60 years and above, N=23)</td>
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<tr>
<td>Femur length, cm</td>
<td>42.3±4.1</td>
<td>43.4±4.8</td>
<td>0.48</td>
<td>40.8±3.3</td>
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<tr>
<td>Femur volume, cm³</td>
<td>277.3±64.9</td>
<td>273.2±62.7</td>
<td>0.85</td>
<td>220.2±64.3</td>
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<td>Femur CSA at 60% length, cm²</td>
<td>6.4±1.0</td>
<td>6.2±0.9</td>
<td>0.49</td>
<td>5.3±0.7</td>
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<tr>
<td>Normalized volume R</td>
<td>18.2±5.4</td>
<td>16.4±4.5</td>
<td>0.31</td>
<td>14.3±2.6</td>
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<tr>
<td>Normalized volume L</td>
<td>18.1±5.1</td>
<td>16.3±4.5</td>
<td>0.31</td>
<td>13.7±2.4</td>
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<tr>
<td>SUL (R)</td>
<td>0.42±0.08</td>
<td>0.46±0.08</td>
<td>0.13</td>
<td>0.39±0.06</td>
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<tr>
<td>SUL (L)</td>
<td>0.41±0.09</td>
<td>0.44±0.07</td>
<td>0.33</td>
<td>0.37±0.06</td>
</tr>
<tr>
<td></td>
<td>Young, 20–40 years (mean±SD)</td>
<td>Older, &gt;60 years (mean±SD)</td>
<td>p-value</td>
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<tr>
<td>----------------------</td>
<td>------------------------------</td>
<td>-----------------------------</td>
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<td></td>
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<tr>
<td><strong>Age</strong></td>
<td>29±5.4</td>
<td>64.4±4.0</td>
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<tr>
<td><strong>Normalized volume (R)</strong></td>
<td>16.8±4.9</td>
<td>13.4±4.6</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td><strong>Normalized volume (L)</strong></td>
<td>16.4±4.6</td>
<td>13.5±4.5</td>
<td>&lt;0.01</td>
<td></td>
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<tr>
<td><strong>Total normalized muscle volume</strong></td>
<td>33.3±9.4</td>
<td>27±9.2</td>
<td>&lt;0.01</td>
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<tr>
<td><strong>SUL (R)</strong></td>
<td>0.41±0.08</td>
<td>0.43±0.09</td>
<td>0.37</td>
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<tr>
<td><strong>SUL (L)</strong></td>
<td>0.40±0.08</td>
<td>0.41±0.08</td>
<td>0.62</td>
<td></td>
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<tr>
<td><strong>Total SUL</strong></td>
<td>0.40±0.08</td>
<td>0.42±0.08</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

CSA = cross-sectional area; (R)= Right side; (L)= Left side; N= number of subject; SUL= SUV normalized to lean body mass

Table 2. Comparison of femur characteristics and radiotracer uptake between young and old age groups.
Table 3. Side differences in SUL and volume of the thigh.

<table>
<thead>
<tr>
<th></th>
<th>Right</th>
<th>Left</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>SUL (mean±SD)</td>
<td>0.42±0.08</td>
<td>0.39±0.06</td>
<td>&lt;0.001</td>
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<tr>
<td>Volume, cm³ (mean±SD)</td>
<td>3633.5±1215.7</td>
<td>3606.5±1195.6</td>
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