Chapter 7

Skeletal muscle analyses: agreement between non-contrast and contrast CT scan measurements of skeletal muscle area and mean muscle attenuation

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Abstract

Background: Low skeletal muscle area (SMA) and muscle radiation attenuation (MRA) have been associated with poor prognosis in various patient populations. Both non-contrast and contrast CT scans are used to determine SMA and MRA. The effect of the use of a contrast agent on SMA and MRA is unknown. Therefore, we investigated agreement between these two scan options.

Methods: SMA and MRA of 41 healthy individuals were analysed on a paired non-contrast and contrast single CT scan and agreement between paired scan results was assessed with use of Bland Altman plots, intraclass correlation coefficients (ICC), standard error of measurements (SEM) and smallest detectable differences at a 95% confidence level (SDD_{95}). Analyses were stratified by tube voltage.

Results: Difference in SMA between non-contrast and contrast scans made with a different tube voltage was 7.0±7.5 cm²; for scans made with the same tube voltage this was 2.3±1.7 cm². Agreement was excellent for both methods: ICC 0.952, SEM 7.2 cm², SDD_{95} 19.9 cm² and ICC 0.997, SEM 2.0 cm², SDD_{95} 5.6 cm² respectively. MRA of scans made with a different tube voltage differed 1.3±11.3 HU and agreement was poor (ICC 0.207, SEM 7.9 HU, SDD_{95} 21.8 HU). For scans made with the same tube voltage the difference was 6.7±3.2 HU and agreement was good (ICC 0.682, SEM 5.3 HU, SDD_{95} 14.6 HU).

Conclusion: SMA and MRA can be slightly influenced by the use of contrast agent. To minimize measurement error, image acquisition parameters of the scans should be similar.

Keywords: Muscle mass; muscle density; computed tomography; single slice; contrast agent
Introduction

Computerized tomography (CT) scan analysis is a frequently used method to analyse skeletal muscle. With this method both skeletal muscle area (SMA) and mean muscle radiation attenuation (MRA) can be assessed. SMA is an estimate for skeletal muscle mass and is measured on a single slice at the level of the third lumbar vertebra (L3). At this level, SMA is strongly correlated to total body skeletal muscle volume.\(^1\) This method is increasingly used in research to quantify skeletal muscle, a key feature of both sarcopenia and cachexia\(^2\). In addition, CT scan analysis can be used to evaluate MRA, a measure for muscle density which is inversely related to muscle lipid content.\(^3\) Low SMA and MRA have been associated with poorer prognosis in various patient populations, such as oncologic and intensive care patients.\(^3\)-\(^5\)

To analyse abovementioned skeletal muscle parameters on a single CT slice, pre-defined radiation attenuation ranges are used. The attenuation is the decrease in the intensity of the x-ray beam when passing through the body and is expressed in Hounsfield units (HU). It indicates what type of tissue may be present, because each type of tissue has typical attenuation characteristics.\(^6\) For example, the typical attenuation value for skeletal muscle is about +40 HU.\(^6\)

Attenuation on CT scan may be influenced by the use of contrast agents. Intravascular iodinated contrast agents are frequently used for diverse diagnostic purposes.\(^7\) After injection, the intravascular contrast agent mixes with blood and distributes into the interstitial space.\(^8\)\(^,\)\(^9\) Because iodine absorbs radiation to a greater degree than soft tissue or vessels\(^8\) with a typical attenuation of about +130 HU\(^6\), it allows better visualization of soft tissue and vessels and thereby facilitates diagnosis.\(^8\) The attenuation of the contrast agent depends on the number of iodine molecules present in the tissue to be imaged, which in its turn depends on perfusion of the tissue.\(^7\) Presence of the iodinated contrast agent in skeletal muscle – either intravascular or interstitial – may lead to more radiation absorption by the muscle. This may result in an increased muscle radiation attenuation (MRA) and influence SMA.

For skeletal muscle measurements both non-contrast and contrast CT scans are used. Until now, it remains unknown to what extent these measurements are influenced by the use of a contrast agent. Therefore, we compared the results of skeletal muscle measurements (SMA and MRA) on a non-contrast and a contrast CT scan in the same individual, with the aim to determine agreement between measurements.
Materials and methods

Study design

This study was performed as an observational study which examined the influence of iodine based intravenous contrast on SMA and MRA, measured by single slice CT scan analysis. The scans were performed at the VU University Medical Centre (VUmc) or the Amsterdam Medical Centre (AMC), Amsterdam, The Netherlands. All data have been acquired as part of standard practice. The Medical Research Involving Human Subjects Act (WMO) does not apply to this study, as confirmed by The Medical Ethics Review Committee of VUmc.

Participants and parameters

The study population consisted of healthy subjects who were screened for kidney transplantation in the period between December 2006 and May 2015. The screening was done by the nephrologist and included medical history and physical examination. Demographical and anthropometrical data were obtained from the medical record. Furthermore, as part of the transplantation screening, healthy subjects underwent two axial abdominal CT scans.

CT scan protocol

Scans were made according to the local CT kidney donor protocol, which differed slightly between the two participating hospitals. The CT scan with the largest slice thickness (3-5 mm) was selected to reduce image noise and when not available, the 1.5 mm reconstruction was selected. Scans were made at a tube voltage of 100 or 120 kilovolts (depending on the standard procedures according to the local protocol) before injection and 120 kVp after injection of the contrast agent. Other scanning parameters were as follows: 64-row CT scanner (Sensation 64, Siemens, Forchheim, Germany (VUmc) or CT Brilliance 64, Philips, Eindhoven, Netherlands (AMC)); rotation time 0.5s; pitch value 0.8 (VUmc) or 0.992 (AMC); collimation 64 x 0.6 mm; effective mAs 70 (VUmc) or 125 (AMC) for non-contrast and 180 (VUmc) or 250 (AMC) for contrast scans; reconstruction algorithms were similar for non-contrast and contrast scans (kernel B30f (VUmc) and filter B (AMC)); scanners were calibrated (tolerance ±4.00 HU) every three months using air-water phantoms.

The CT scans were made in supine position. Subjects had to breathe deeply just before a CT scan was made and to hold their breath during the scan period. First a non-contrast scan was made. Subsequently participants
received 140-145 ml of an iodinated contrast agent with a concentration of 300 mg iodine/ml and 30 ml of saline flush. The contrast agent was given intravenously in three phases, following standard protocols. Between the first and second phase the participant walked or turned around or coughed for good distribution of contrast in urethra and bladder. The arterial phase images were used for analysis of contrast CT scan analyses. The time interval between the non-contrast and contrast CT scan was 420 (VUmc) or 500 seconds (AMC).

Skeletal muscle analyses

On each CT scan, the slice at the level of L3 most clearly displaying both vertebral transverse processes was identified. The non-contrast and contrast slice of the same individual were selected at the same time using a split screen, to maximize similarity in location between both slices. Subsequently the two selected slices of each individual were compared by two researchers (AW and IMD) independently of each other, to qualify whether both selected L3 slices were made at exactly the same location. This was done to minimize variation caused by different slice selection.\textsuperscript{10,11} The judgment was based on similarity of the anatomy (in particular of the L3 vertebra) on the selected slices. Slices were only included if both researchers fully agreed about the similarity of the slices. Two examples of slice pairs are shown in figure 1. The analysed muscles include the psoas muscles, paraspinal muscles (erector spinae, quadratus lumborum) and the abdominal wall muscles (rectus abdominis, transversus abdominis, external oblique and internal oblique). These muscles were identified and analysed as muscle when radiation attenuation fell within the range of -29 to +150 HU, which corresponds to the density of skeletal muscle tissue.\textsuperscript{3,12} The non-contrast and contrast slices of the same individual were analysed by the same researcher at the same time using a split screen. SMA was computed by summing up the area of the selected muscle pixels and MRA was determined by averaging the radiation attenuation of these pixels. Both researchers had completed the Computed Tomography Analysis Training of the Cross Cancer Institute (Edmonton, Alberta, Canada) to perform CT scan analysis using sliceOmatic software (version 5.0; Tomovision, Magog, Quebec, Canada).
Figure 1 Examples of CT slices of the same individual at the level of the third lumbar vertebra of scans made with a different tube voltage (100 kVp non-contrast (A1) and 120 kVp (A2) contrast scan) and with the same tube voltage (120 kVp non-contrast (B1) and 120 kVp (B2) contrast scan).

Statistics

Scan characteristics were described using median and interquartile range (IQR) or mean ± standard deviation (SD) according to the distribution of the characteristic. Because the non-contrast CT scans had been made with two tube voltages and different tube voltages may have led to significant change of HU values\textsuperscript{13,14}, it was first tested whether differences in SMA and MRA were related to tube voltage. This was done by performing independent samples t-tests for both the difference in SMA and MRA with tube voltage as grouping variable (i.e. the subgroup with non-contrast and contrast CT scan at a different tube voltage (respectively 100 kVp and 120 kVp) versus the subgroup in which both scans were made with 120 kVp). A p-value <0.05 was considered significant and reason to stratify analyses by tube voltage subgroups. Subgroup characteristics were compared with a 2-sided Fishers exact test (categorical data) or independent t-test (normally distributed continuous data). Agreement of non-contrast and contrast scan measurements was assessed by a Bland Altman plots, which represent the difference between the two measurement results against their mean. By plotting the within subject difference in result between the two measurements on the y-axis, the remaining variation is due to
measurement difference.\textsuperscript{15} Intraclass correlation coefficients (ICC) were used to assess reliability.\textsuperscript{15,16} This coefficient can range from 0 to 1, corresponding to no agreement and complete agreement between measurements, respectively.\textsuperscript{17} Agreement is considered ‘excellent’ when the ICC exceeds 0.75, ‘good’ from 0.60 to 0.74, ‘moderate’ from 0.40 to 0.59 and ‘poor’ when less than 0.40.\textsuperscript{18} In addition, the standard error of measurement (SEM) and smallest detectable difference at a 95\% confidence interval (SDD\textsubscript{95}) were calculated with the following equations:

\[
\text{ICC} = \frac{\text{variance between patients}}{\text{total variance}} \quad \text{19}
\]
\[
\text{SEM} = \sqrt{\text{error variance}} \quad \text{20}
\]
\[
\text{SDD}_{95} = 1.96 \times \sqrt{2} \times \text{SEM} \quad \text{21}
\]

The SDD\textsubscript{95} depends on the measurement error and refers to the magnitude of difference between two measurements that is required to detect a real change. To be reasonably certain (at a 95\% confidence interval) that there is a true difference between two measurements, the difference should exceed the SDD\textsubscript{95}.\textsuperscript{21}

\textbf{Results}

Non-contrast and contrast CT scans were both available in 135 individuals. After screening for similarity in slice location, 41 paired slices were included. Subject characteristics are shown in table 1. Slice thickness was the same for the non-contrast and contrast scan in 36 scan pairs and differed in 5 scan pairs (all 5 scan pairs of the same tube voltage). In 20 scan-pairs, the used tube voltage was 100 kVp for the non-contrast and 120 kVp for the contrast scan (different tube voltage), in the other 21 scan-pairs both scans were made with 120 kVp (same tube voltage). As both difference in SMA and difference in MRA between non-contrast and contrast scans was different for the tube voltage subgroups (p=0.012 for SMA and p=0.042 for MRA), the results were stratified by voltage group. Subject characteristics are described separately for the tube voltage groups (table 1), showing there were no differences between the groups regarding age, height, weight and body mass index. There was a tendency towards a higher percentage of male subjects in the different tube voltage group compared to the same voltage group (60\% vs. 29\%, p=0.062).
The median SMA was 127.5 (IQR 114.3-162.7) cm$^2$ on non-contrast scans and 131.1 (IQR 116.0-175.7) cm$^2$ on contrast scans. After stratifying for tube voltage, the mean difference in SMA between non-contrast and contrast was 7.0 ± 7.5 cm$^2$ (95% confidence interval (CI) 3.5 to 10.5 cm$^2$; corresponding to 4.9 ± 5.2%) for scans made with a different tube voltage and 2.3 ± 1.7 cm$^2$ (95% CI 1.6 to 3.1 cm$^2$; corresponding to 2.0 ± 1.4%) for scans made with the same tube voltage (table 2). Within the latter group, scan pairs within made with a different slice thickness did not bias the results (after exclusion of these 5 scan pairs, the SMA of the scan pairs with the same tube voltage was 2.8 ± 1.7 cm$^2$).

The Bland Altman plots (figure 2) show the limits of agreement between non-contrast and contrast scans and indicate higher agreement, i.e. smaller limits of agreement, for scans made with the same tube voltage than for scans made with a different tube voltage. Because the Bland Altman plot of scans made with a different tube voltage showed two outliers, post hoc analyses were done to assess whether the outliers could have been identified beforehand. The outliers were not identifiable based on scan characteristics, so there was no reason to exclude the outliers from the analyses. The ICC was 0.952 for scans made with a different tube voltage and 0.997 for scans made with the same tube voltage (table 2).
Figure 2 Bland Altman plots of skeletal muscle area (SMA) measured on contrast minus non-contrast scan against mean of both measurements. The horizontal lines correspond to the mean difference (middle) and 95% limits of agreement (upper and lower line). Left: scans made with a different tube voltage (n=20, non-contrast scan at 100 kVp and contrast scan at 120 kVp). Right: scans made with the same tube voltage (n=21, both at 120 kVp).

Table 2 Results of skeletal muscle area (SMA) and muscle radiation attenuation (MRA) measured on paired non-contrast and contrast single CT slices

<table>
<thead>
<tr>
<th></th>
<th>Non-contrast</th>
<th>Contrast</th>
<th>Difference</th>
<th>ICC</th>
<th>SEM</th>
<th>SDD&lt;sub&gt;95&lt;/sub&gt;</th>
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<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Mean ± SD&lt;sup&gt;#&lt;/sup&gt;</td>
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<td></td>
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<td><strong>SMA (cm&lt;sup&gt;2&lt;/sup&gt;)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Total (n=41)</td>
<td>127.5 (114.3-162.7)</td>
<td>131.1 (116.0-175.7)</td>
<td>4.6 ± 5.8</td>
<td>0.978</td>
<td>5.2</td>
<td>14.4</td>
</tr>
<tr>
<td>Different tube voltage (n=20)*</td>
<td>142.9 (116.1-173.9)</td>
<td>147.3 (122.2-183.2)</td>
<td>7.0 ± 7.5</td>
<td>0.952</td>
<td>7.2</td>
<td>19.9</td>
</tr>
<tr>
<td>Same tube voltage (n=21)*</td>
<td>118.0 (111.0-155.4)</td>
<td>119.7 (112.7-160.2)</td>
<td>2.3 ± 1.7</td>
<td>0.997</td>
<td>2.0</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>MRA (HU)</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Total (n=41)</td>
<td>37.7 (31.1-45.1)</td>
<td>41.8 (35.5-47.1)</td>
<td>4.1 ± 8.6</td>
<td>0.495</td>
<td>6.7</td>
<td>18.5</td>
</tr>
<tr>
<td>Different tube voltage (n=20)</td>
<td>43.4 (36.7-46.9)</td>
<td>42.8 (36.4-48.1)</td>
<td>1.3 ± 11.3</td>
<td>0.207</td>
<td>7.9</td>
<td>21.8</td>
</tr>
<tr>
<td>Same tube voltage (n=21)</td>
<td>33.3 (28.6-39.0)</td>
<td>40.6 (34.2-45.9)</td>
<td>6.7 ± 3.2</td>
<td>0.682</td>
<td>5.3</td>
<td>14.6</td>
</tr>
</tbody>
</table>

ICCs: intraclass correlation coefficient. SEM: standard error of measurement. SDD<sub>95</sub>: smallest detectable difference at a 95% confidence level. IQR: interquartile range. SD: standard deviation.

*Different tube voltage: non-contrast 100 kVp, contrast 120 kVp. Same tube voltage (both 120 kVp).

<sup>#</sup>Difference: mean difference of the paired measurements of contrast and non-contrast scan.
The median value of MRA was 37.7 (IQR 31.1-45.1) and 41.8 HU (IQR 35.5-47.1) on respectively non-contrast and contrast scans. The mean difference in MRA between non-contrast and contrast scans was 1.3 ± 11.3 HU (95% CI -4.0 to 6.6 HU) for scans made with a different tube voltage and 6.7 ± 3.2 HU (95% CI 5.3 to 8.2 HU; 5.8 ± 2.8 HU) for the scan pairs with the same slice thickness) for scans made with the same tube voltage. Although for scans made with the same tube voltage the mean difference in MRA was higher, the limits of agreement between non-contrast and contrast scans were smaller, corresponding to higher agreement (figure 3). For scans made with a different tube voltage, the ICC was 0.207 and for scans made with the same tube voltage the ICC was 0.682 (table 2).

**Figure 3** Bland Altman plots of muscle radiation attenuation (MRA) measured on contrast minus non-contrast scan against mean of both measurements. The horizontal lines correspond to the mean difference (middle) and 95% limits of agreement (upper and lower line). Left: scans made with a different tube voltage (n=20, non-contrast scan at 100 kVp and contrast scan at 120 kVp). Right: scans made with the same tube voltage (n=21, both at 120 kVp).

**Discussion**

This study is the first describing agreement between skeletal muscle measurements (SMA and MRA) of non-contrast and contrast scans. Agreement between non-contrast and contrast measurements was excellent for SMA and poor (different tube voltage) to good (same tube voltage) for MRA. Use of a contrast agent was associated with higher SMA and MRA, possibly due to the presence of the contrast agent in skeletal muscle.

For SMA, ICCs between the results of non-contrast and contrast CT scans were excellent, with the highest agreement when the paired scans were made with the same tube voltage. The difference in SMA was 7.0 ± 7.5
cm² for scans made with a different tube voltage and 2.3 ± 1.7 cm² for scans with the same tube voltage. In observational studies, both cross-sectional SMA and longitudinal change in SMA were associated with clinical outcomes. According to the present study results, the difference between non-contrast and contrast scans is substantial for scans made with a different tube voltage and relatively small for scans made with the same tube voltage. When comparing SMA between patient populations or analysing longitudinal change of SMA, it should be noted that the measurement can be influenced by use of a contrast agent and by tube voltage. Therefore, scans should preferably be performed with similar scan conditions to minimize measurement error.

Agreement of MRA between non-contrast and contrast scans was poor for scans pairs made with a different tube voltage and good for scan pairs with the same tube voltage. The mean difference was smaller for scans made with a different tube voltage (different tube voltage 1.3 ± 11.3 HU and same tube voltage 6.7 ± 3.2 HU), which may be explained more photoelectric effect and consequently greater attenuation in the non-contrast 100 kVp scans than in the 120 kVp contrast scans. This may have partly eliminated the increase in radiation attenuation caused by the contrast agent.

Differences in results of SMA and MRA between the non-contrast and contrast CT scan may be caused by other factors than contrast. These factors may be real or process-related. Real, physical factors that may reduce MRA are muscle fat infiltration, glycogen content and water content. Since the non-contrast and contrast scans were made within a short time interval, it is highly unlikely that a change in muscle fat- or glycogen content has occurred. Muscle water content might have been influenced by process-related confounders. An increase in muscle water content could be present as a consequence of intravascular fluid administration, although the degree of uptake of this small amount of fluid in skeletal muscle is expected to be negligible. Moreover, muscle water content might have changed due to laying supine, leading to fluid redistribution. In a previous study 120 min of bed rest resulted in a decrease in muscle area of the lower limb (1.9% and 5.5% for respectively the thigh and calf muscle) and increase of radiodensity (only for the calf muscle; 4.8%). Because in the abdominal region there is less fluid redistribution and participants in our study laid supine less than 10 min, we expect the effect of fluid redistribution is negligible in our study. Other process-related factors that could have biased the results, like different slice location and observer error, were minimized in different ways. A consistent location of the slice pairs was achieved by including only the CT slices of individuals in whom the slices of non-contrast and contrast scans were exactly at the same level. This was independently assessed by two researchers. Intra-observer error was limited by analysing both scans of each individual by the same observer at the same time.
using a split screen, to ensure equivalent analyses of skeletal muscle on non-contrast and contrast scans. Since bias by these factors was confined, the remaining measurement difference is most likely associated with image acquisition and more specifically, the result of the use of the contrast agent.

To assess the influence of the use of a contrast agent more precisely, more research is needed. A more optimal study design would be to compare a group which was scanned without and with use of a contrast agent to a group which was scanned twice without the use of a contrast agent, leaving all other variables constant. The same design would be useful to assess the effect of tube voltage. In the current retrospective study routinely conducted CT scans were used and this design was not possible, however this study indicates that both use of a contrast agent and tube voltage do influence SMA and MRA. Therefore, when comparing between individuals or within individuals between time points, it is preferred to use CT-scans made with similar scan conditions concerning use of a contrast agent and tube voltage. However, this is not always feasible when using routine CT scans.

**Conclusion**

Skeletal muscle measurements can be slightly influenced by the use of contrast agent. To minimize measurement error, image acquisition (use of contrast agent, tube voltage) of the scans should be similar and be reported. When image acquisition differs, the smallest detectable difference should be taken into account.

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**Conflict of interest**

The authors have no conflicts of interest.
References


