Protocol for measurement of liver fat by computed tomography

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EMERGING EVIDENCE SUGGESTS that excess deposition of lipid in the liver may act as an “ectopic” site of fat distribution that independently predicts insulin resistance (18) and dyslipidemia (1, 11). The prevalence of excess liver fat or “fatty liver” approximates 34% in the general population (17) and 48% in obese cohorts (2). These observations underscore the importance of liver fat measurement in studies that seek to understand the health implications of complex obesity phenotypes. Although liver biopsy is often considered the gold standard for measurement of liver fat, it can be painful (5) and has notable mortality risk (6). Proton magnetic resonance spectroscopy is noninvasive and offers researchers a reliable tool for measuring liver fat in vivo. However, application of magnetic resonance spectroscopy requires exceptional technical expertise and is restricted to liver fat measures in specified regions of interest. Radiological imaging techniques such as computed tomography (CT) are obtained routinely in clinical settings, are easily interpreted without need for sophisticated image-analysis software, and provide estimates of liver fat that correlate well with needle biopsy, especially in subjects with increased liver fat (16). On the other hand, CT employs ionizing radiation, and thus protocols need to be developed that measure the tissues of interest and limit exposure.

The identification of liver fat by CT as a predictor of health risk was first described by Banerji et al. (1) and Goto et al. (7) in 1995. The CT method employed realizes that the lower the mean liver attenuation or CT number in Hounsfield units (HU), the lower the tissue density and hence the greater the fat content. Therefore, liver density (e.g., attenuation in HUs) is inversely related to liver fat and thus is a surrogate for it (15). However, although extremely low HU values have been measured in livers infiltrated with fat, an overlap exists between normal and abnormal liver HU values (13). Therefore, the absolute liver density determined by CT may not be sensitive for predicting abnormal liver fat content. Because a constant relationship exists between liver and spleen attenuation in individuals with normal livers, the ratio of mean liver to spleen attenuation values is used as an index of liver fat, as originally described by Piekarshi et al. (14) in 1980. Obtaining a CT image that contains both liver and spleen presents a challenge; variations exist not only in the vertical positioning of the spleen relative to the liver but also in positioning of both organs within the abdominal cavity. A multi-image approach is not feasible because of excess exposure (8). This implicates a single CT image approach; however, a single-image protocol at the level of the abdomen that routinely identifies the liver and spleen has yet to be firmly established. Furthermore, once obtained, it is important to determine whether the distribution of fat throughout the liver is uniform: an observation with direct implications for those that determine liver fat by measuring only small portions or regions of interest of the liver on the CT image (e.g., biopsy, magnetic resonance spectroscopy, and CT).

The aim of this study was twofold: first, to determine an optimal location for simultaneous imaging of both liver and spleen, and second, to document liver and spleen attenuation characteristics and determine whether variation exists, and if so, whether it is of a magnitude that should alter the protocol employed to measure liver fat.

METHODS

Subject testing. Subjects were men and women who underwent a medical examination at the Cooper Institute in Dallas, TX, between 1995 and 2001. Although race data for all subjects was unavailable, ~98% of attendees at the Cooper Institute during this time were Caucasian. None of the subjects were smokers or had a history of diabetes mellitus, cardiovascular disease, stroke, or cancer. All gave informed consent before participation in the examination according to the ethical guidelines of The Cooper Institute Institutional Review Board. Medical examinations included an abdominal CT scan, comprising 40 contiguous images with 6-mm thickness, from at least T11 to K7L 3N6 (e-mail: rossr@post.queensu.ca).

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Address for reprint requests and other correspondence: R. Ross, School of Physical and Health Education, Queen’s Univ., Kingston, Ontario, Canada K7L 3N6 (e-mail: rossr@post.queensu.ca).
Landmark revealed that the image at T12–L1 provided optimal results for our initial observation using the T12–L1 intervertebral space as a landmark identifying the T12–L1 intervertebral space, we analyzed the CT image 12 mm superior, and 12 mm inferior were cross-validation sample of 130 men and 113 women, the three images on the frequency of successfully imaging both liver and spleen. “missing” slightly above or below the target landmark had an effect mm superior and 12 mm inferior to T12–L1 to observe whether subjects (Table 1).

Through L5, including all or most of the liver, CT images were obtained using an electron-beam CT scanner (Imatron, General Electric, Milwaukee, WI). The image-acquisition protocol used 130 kV and 630 mA with a 48-cm field of view and a 512 × 512 matrix. The full abdominal scan results in a patient radiation exposure of ~640 mrem. Body weight and height were measured using a standard physician’s scale and stadiometer, and body mass index (BMI) was calculated using the weight in kilograms divided by the height in meters squared. Waist circumference was measured at the level of the umbilicus using a plastic tape measure.

**Determination of optimal image location.** A preliminary investigation was undertaken to determine the location of an easily identifiable, single axial image that would most frequently provide simultaneous visualization of liver and spleen. Axial images at the intervertebral spaces and the midpoints of the vertebral bodies within the region of T11–T12 and L1–L2 were visually inspected for the presence or absence of liver and spleen in a sample of 118 male and 76 female subjects (Table 1).

**Cross-validation of optimal image location.** Our initial analysis revealed that the image at T12–L1 provided optimal results for our sample of men and women (see RESULTS). We cross-validated our initial observation using the T12–L1 intervertebral space as a landmark for imaging liver and spleen in a second sample of 130 men and 113 women. Acknowledging the possibility for slight deviations in identifying the T12–L1 intervertebral space, we analyzed the CT image 12 mm superior and 12 mm inferior to T12–L1 to observe whether “missing” slightly above or below the target landmark had an effect on the frequency of successfully imaging both liver and spleen.

**Effect of image level on liver and spleen attenuation.** On the cross-validation sample of 130 men and 113 women, the three images identified at T12–L1, 12 mm superior, and 12 mm inferior were analyzed for liver and spleen attenuation characteristics using specialized image-analysis software (Tomovision, Montreal, Canada). Lines were manually drawn around the perimeter of the liver and spleen on each image to calculate the mean HU value for each organ. Mean HU values were obtained at each level to compare the differences in attenuation that might occur with subtle differences in locating T12–L1.

**Regional variation in tissue attenuation.** We sought to further investigate liver and spleen tissue attenuation characteristics by dividing the liver and spleen at the T12–L1 level into quartiles from anterior to posterior, and then subdividing each of the quartiles into medial and lateral regions, as depicted in Fig. 1. Our primary purpose in subdividing the images into these eight regions was to map regional variation in liver and spleen attenuation so that a recommendation could be made as to whether one region or another better represented the whole, and thus it be a better-suited location for placement of a region of interest when assessing liver fat by CT. The images 12 mm superior and 12 mm inferior to T12–L1 were analyzed in a similar fashion to determine whether attenuation patterns differed in regions slightly above or below the targeted image.

**Reliability in image analysis.** Two analyzers performed blinded assessments of the mean liver and spleen attenuation in 48 subjects (24 men and 24 women), chosen randomly from the 243 subjects used in the cross-validation analysis. The subjects varied widely in age [46.5 yr (SD 7.3)], body mass index [26.8 kg/m² (SD 5.2)], and waist circumference [89.1 cm (SD 17.0)] in a manner similar to the original sample. Interobserver coefficients of variation for liver and spleen attenuation (HU) were 2.9% (1.6 HU) and 4.8% (2.3 HU), respectively.

**Statistical analyses.** Data are presented as means (SD). Independent t-tests were used to assess gender differences. Univariate general linear modeling with repeated measures was used to determine the effect of landmarking on mean liver and spleen attenuation and to determine regional variation in organ attenuation compared with the whole. Bonferroni adjustment was used post hoc to correct for the multiple comparisons. Logistic regression was used to determine whether variance in anthropometric measures could explain potential differences in optimal image location. All statistical procedures were performed using SPSS version 12.0 (SPSS, Chicago, IL).

### RESULTS

**Optimal image location.** Our preliminary investigation sought to determine a level most likely to contain a cross section of both liver and spleen in 118 men and 76 women [age: men 52.9 yr (SD 9.0); women 59.9 yr (SD 10.6), BMI: men 27.8 kg/m² (3.3); women 24.5 kg/m² (SD 4.9)]. Frequency of appearance of liver and spleen at each of the selected vertebral landmarks is summarized in Table 1. The liver, being a much larger organ than the spleen, is seen at each of the

<p>| Table 1. Presentation of liver and spleen on five CT images obtained at selected vertebral landmarks |
|-------------------------------------------------|-------------------------------------------------|
| Vertebral Landmark | Men (n = 118) | Women (n = 76) |</p>
<table>
<thead>
<tr>
<th>Liver</th>
<th>Spleen</th>
<th>%</th>
<th>Liver</th>
<th>Spleen</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T11–T12</td>
<td>118</td>
<td>102</td>
<td>86</td>
<td>76</td>
<td>43</td>
</tr>
<tr>
<td>T12</td>
<td>118</td>
<td>114</td>
<td>97</td>
<td>76</td>
<td>64</td>
</tr>
<tr>
<td>T12–L1</td>
<td>118</td>
<td>106</td>
<td>90</td>
<td>76</td>
<td>67</td>
</tr>
<tr>
<td>L1</td>
<td>118</td>
<td>95</td>
<td>81</td>
<td>76</td>
<td>56</td>
</tr>
<tr>
<td>L1–L2</td>
<td>118</td>
<td>73</td>
<td>62</td>
<td>76</td>
<td>35</td>
</tr>
</tbody>
</table>

*No. of subjects; CT, computed tomography. *Percentage of subjects in n,

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Fig. 1. Identification of 8 regions in liver and spleen at T12–L1.
vertebral landmarks in all 194 subjects. The lower appearance rate of the spleen at a given landmark reflects its smaller size and variable location between subjects. A higher percent score indicates an increase in likelihood that both liver and spleen are visible at a given landmark.

The two landmarks with the highest percentage of liver and spleen appearance were the midpoint of T_{12} and the T_{12}–L_{1} intervertebral space. Because appearance rates were comparable in men and women at both sites, and because an intervertebral space is more readily identified than the midpoint of a vertebral body, the T_{12}–L_{1} intervertebral space was selected as the landmark for subsequent analyses.

**Cross-validation of optimal image location.** A separate sample of 130 men and 113 women, for which scans of the entire liver were available, was selected from a larger database. There was a relatively wide range of age and obesity in the sample selected, and the men differed from the women for most variables (Table 2).

Similar to the preliminary sample, liver was present in all three images for all cross-validation subjects. Both liver and spleen were visible at the T_{12}–L_{1} intervertebral space in 94% (122 of 130) of men and 96% (108 of 113) of women. For the image 12 mm superior to T_{12}–L_{1}, liver and spleen were observed in 94% of subjects independent of sex; the image 12 mm inferior contained both liver and spleen in 88 and 92% of men and women, respectively. Consideration of the influence of age, BMI, and visceral adiposity on the identification of the optimal image location revealed that BMI was the only variable with an independent influence (P < 0.05) on the subtle differences observed in men and women. A BMI > 25.0 kg/m² in our cross-validation sample resulted in a reduction of simultaneous liver and spleen imaging from 98 to 91% in men and from 97 to 91% in women at the T_{12}–L_{1} landmark. For all subjects combined (n = 437), liver and spleen were visible for 403 (or 92%) at the T_{12}–L_{1} level.

**Effect of image level on liver and spleen attenuation.** Mean attenuation values (e.g., HU) from liver and spleen derived from the three images analyzed in the cross-validation sample were compared to observe any differences that may be a function of obtaining an image slightly above or below the T_{12}–L_{1} level. A consistent, stepwise increase in tissue density of both liver and spleen was evident from the image 12 mm inferior to T_{12}–L_{1}, and then from T_{12}–L_{1} to the image 12 mm superior (Fig. 2). The increases from level to level were statistically significant (P < 0.001) independent of sex, and they were an average magnitude of 1.5 HU (2.5%) in liver and 2.0 HU (3.9%) in spleen.

**Regional variation in tissue attenuation.** Characteristics of tissue attenuation throughout liver and spleen were assessed by subdividing the T_{12}–L_{1} image into eight regions (See Fig. 1) and then comparing each region to the HU of the entire liver or spleen image. Mean attenuation values for medial and lateral quartiles of liver and spleen at T_{12}–L_{1} are shown in Fig. 3.

The first quartiles (or anterior regions) of both liver and spleen consistently displayed attenuation values that were significantly lower than the whole on each image. In men, the medial liver regions of the second and third quartiles had increased HU values by comparison to the whole, whereas the lateral regions did not. In women, the liver attenuation was higher in both medial and lateral portions of the third and fourth quartiles, indicating a general increase in attenuation from anterior to posterior liver. In both men and women, the medial spleen was associated with decreased attenuation compared with the lateral regions. The average absolute difference of regional attenuation scores from whole liver and spleen was 1.8 HU (2.9%) and 3.4 HU (6.6%), respectively.

For the images 12 mm above and below the T_{12}–L_{1} level, with few exceptions, the pattern of attenuation for the respective regions mirrored those of T_{12}–L_{1} in relation to the whole (data not shown).

**DISCUSSION**

The primary finding was that a single CT-measured image obtained at the level of the T_{12}–L_{1} intervertebral space identified the liver and spleen in 92% (403 of 437) of the men and

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**Table 2. Characteristics of cross-validation sample subjects**

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 130)</th>
<th>Range</th>
<th>Women (n = 113)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>51.3 (8.4)</td>
<td>22.0–76.0</td>
<td>40.9 (4.2)*</td>
<td>27.0–46.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>88.0 (12.3)</td>
<td>60.7–124.3</td>
<td>65.1 (12.4)*</td>
<td>45.2–108.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.8 (3.2)</td>
<td>21.5–37.5</td>
<td>23.6 (4.3)*</td>
<td>18.6–38.1</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>94.8 (9.9)</td>
<td>74.0–123.0</td>
<td>74.5 (10.0)*</td>
<td>61.0–103.0</td>
</tr>
<tr>
<td>Visceral adipose tissue (L₄–L₅), cm²</td>
<td>147.4 (63.7)</td>
<td>20.0–388.0</td>
<td>61.2 (40.8)*</td>
<td>8.0–182.0</td>
</tr>
<tr>
<td>Mean liver attenuation, HU</td>
<td>61.2 (9.6)</td>
<td>29.0–78.2</td>
<td>60.5 (5.3)</td>
<td>46.4–73.6</td>
</tr>
<tr>
<td>Liver-to-spleen ratio</td>
<td>1.24 (0.16)</td>
<td>0.73–1.79</td>
<td>1.19 (0.13)*</td>
<td>0.88–1.79</td>
</tr>
</tbody>
</table>

n. No. of subjects; BMI, body mass index; HU, Hounsfield units. *Significantly different from men, P < 0.05.
women studied. Furthermore, we observed that the attenuation or liver fat score was relatively homogeneous, implying that little variability exists in the deposition of fat throughout the T12–L1 image. These observations suggest that the use of a CT protocol that includes a low-dose vertebral scout for location of T12–L1 intervertebral space, followed by acquisition of a single image at T12–L1, is a practical, reliable method for routine measurement of liver fat in research and clinical settings.

The accuracy of CT to estimate liver fat in vivo by comparison to histological determination of fat from liver biopsies was first described in the early 1980s (3, 4). In those studies, the CT number (attenuation values in HUs) was a strong, inverse correlate of liver fat from biopsy samples. However, because an overlap exists between normal and abnormal liver HU values (13), the absolute liver density determined by CT may not be sensitive for predicting abnormal liver fat content. Because a constant relationship exists between liver and spleen attenuation in individuals with normal livers, it was shown that the ratio of mean liver to spleen attenuation values provides a useful index of liver fat (14). Simultaneous measurement of liver and spleen attenuation to characterize liver fat in obesity was first reported by Goto et al. (7) in a study wherein the difference in the ratio of liver to spleen attenuation values was reported to be associated with insulin clearance and insulin sensitivity. A limitation of this study is that the authors did not clearly identify the landmark employed for acquisition of the CT image. That we observed a stepwise increase in both liver and spleen attenuation from the images acquired 12 mm inferior and superior to T12–L1 underscores the importance of proper landmarking to avoid differences in attenuation attributable to positioning error. Recently, Kelley et al. (9) used T11–T12 as a landmark for imaging liver and spleen in men and women with Type 2 diabetes mellitus. In that study, the authors did not report the frequency for which the liver and spleen were observed nor whether any gender difference existed. The results of our study confirm that T11–T12 is a useful landmark in men. In women, however, the ability of the image at T11–T12 to identify the liver and spleen was substantially less by comparison to T12–L1. Hence, our recommendation is that a single axial CT image at the T12–L1 level is extremely useful for simultaneous identification of liver and spleen for both men and women with wide variation in age, visceral adiposity, and obesity. However, it is noteworthy that, while neither age nor visceral adiposity had any effect on liver and spleen appearance within the T12–L1 image, subjects with an elevated BMI had a slightly lower success rate at that level. This point may be important for future studies to consider, especially when acquiring images in a morbidly obese sample of men and women. Because our sample contained relatively few subjects with a BMI in excess of 35 kg/m², further research is required to establish whether T12–L1 remains the optimal location in these individuals.

The region-by-region analysis of liver and spleen performed in this study sheds light on patterning of tissue attenuation in both organs. To our knowledge, this is the first study to employ a systematic sampling of liver and spleen that includes a comparison from medial to lateral, quartiles from anterior to posterior, and replication of these regions on images above and below to identify attenuation patterns in liver and spleen. Our objective in characterizing these regions was to identify specific areas of the liver and spleen that may best represent the whole image so that optimal locations for regions of interest could be recommended. However, although many of the regions varied statistically from the mean attenuation of the whole liver image, the magnitude was on average <2.0 HU. From a clinical perspective, this is a minor variation in light of reports suggesting that a 14-HU increase in liver attenuation is

![Fig. 3. Mean (SD) attenuation of liver and spleen by region in men (A) and women (B).](image)
observed as a consequence of a modest 6% reduction in body weight (12). Furthermore, our findings suggest that methodologies that measure liver fat by assessment of regions of interest, including biopsy and magnetic resonance spectroscopy, need not be particular with respect to placement of the regions of interest, provided there is consistency in placement for serial measurements. Accordingly, with CT, it seems reasonable to recommend that the index of liver and spleen attenuation be derived from a mean score of the entire image. This would avoid potential bias introduced by variable placement of a region of interest and would gain the advantage of a much larger sample of tissue for estimating liver fat.

It is also important to note that with CT attenuation alone it is not possible to quantify liver fat. Because the molecular composition of lipid, water, and lean tissue within each voxel influences the resulting attenuation value, small variations in one may mask changes in the other. For example, it is entirely possible that elevations in the water component of a voxel may alter (increase) the measured attenuation, leading to the erroneous conclusion that the absolute lipid content was altered (decreased). This represents a challenge to the researcher when trying to interpret subtle changes in attenuation that may result from a given perturbation.

The findings of our study extend current knowledge with respect to CT-measured tissue characteristics of the liver and spleen and offers evidence for the improvement of methodology in CT image acquisition. Indeed, that the attenuation values within the liver are relatively homogeneous lends support to protocols that acquire liver fat measures within only a small region of interest. Furthermore, our results provide a compelling argument for the use of a single CT image at T1=−L1 as a practical, reliable method for routine measurement of liver fat in research and clinical settings.

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GRANTS

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