Interindivudual variation in abdominal subcutaneous and visceral adipose tissue: influence of measurement site

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The single-image protocol employed to quantify abdominal AT makes the assumption that the distribution of SAT and VAT in the abdomen is similar between individuals. In other words, it is assumed that the ranking or quantification of subjects for either tissue determined at one level of the abdomen (e.g., L4–L5) is the same as that obtained at another level (e.g., L3–L4). This assumption was recently examined by Thomas and Bell (14), who reported marked interindividual variation in the distribution of VAT across the abdominal region in premenopausal women. On the basis of these observations, the authors concluded that the use of a single image to determine the quantity of VAT in premenopausal women should be interpreted with caution. These observations are consistent with those of Greenfield et al. (1), who performed a similar analysis in 19 premenopausal women.

These previous studies (1, 14) suffered from several limitations. Among them are the use of small sample sizes and failure in consideration of the inherent measurement error when interpreting changes in VAT ranking at different levels of the abdomen. For example, the subjects in the Greenfield et al. (1) study had extremal low values for VAT area (mean 33 cm² at L4–L5), and thus a small variation (e.g., ~3 cm²) in image analysis due to technical error may have had a significant influence on their resultant rankings. In addition to these limitations, the influence of measurement site on the ranking of abdominal SAT deposition between subjects was not considered and thus is unknown. Furthermore, whether measurement site influences the ranking of SAT or VAT deposition in men is also unknown. Finally, whether a change in ranking position at different levels of the abdomen alters the prediction of total SAT and VAT is unknown. If one assumes that quantifying the total amount of AT in the abdomen is a primary consideration, then the authors concluded that the use of a single image to measure AT was appropriate. However, it is assumed that the ranking or quantification of subjects for either tissue determined at one level of the abdomen (e.g., L4–L5) is the same as that obtained at another level (e.g., L3–L4). This assumption was recently examined by Thomas and Bell (14), who reported marked interindividual variation in the distribution of VAT across the abdominal region in premenopausal women. On the basis of these observations, the authors concluded that the use of a single image to determine the quantity of VAT in premenopausal women should be interpreted with caution. These observations are consistent with those of Greenfield et al. (1), who performed a similar analysis in 19 premenopausal women.

The objective of this study was to evaluate the influence of measurement site on ranking and prediction of abdominal SAT and VAT. To accomplish this, we examined large samples of men with wide variation in adiposity by using both CT and MRI. The change in ranking position was evaluated after consideration of the technical limits (e.g., measurement error) of the respective imaging method.

MATERIALS AND METHODS

Subjects. For analysis using CT, subjects included 100 Caucasian men selected from a large sample (n = 433) of men who had received a medical examination at the Cooper Clinic in Dallas, Texas. For

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analysis using MRI, subjects included 100 Caucasian men selected from a large sample (n = 347) of men who participated in body composition studies at Queen’s University in Kingston, Canada. To be included in the present study, subjects had to have VAT areas at the L4-L5 level within ±2 SDs of the mean of the entire subject pool of male subjects at the Cooper Clinical (CT) or Queen’s University (MRI) to ensure that no outliers were included in the study populations. All subjects gave their fully informed and written consent to participate in accordance with the ethical guidelines of the respective institutional review boards.

**Acquisition of CT images.** Axial images of the abdominal region were obtained in an electron-beam CT scanner (Imatron, General Electric, Milwaukee, WI) using a standard protocol (6). Subjects were examined in a supine position with their arms extended above their heads. Approximately 40 contiguous transverse images (6-mm thickness) were acquired from the midregion of the iliac crest to the caudal region of the heart. Images were obtained using 130 kV and 630 mA with a 480-mm field of view and a 512 × 512 matrix, resulting in a pixel size of 0.78 mm². For each subject the CT images corresponding to the L1-L3 and L3-L4 vertebral disk spaces were selected using anatomic landmarks (5).

**Acquisition of MRI images.** Whole body MRI data were obtained with a 1.5-T General Electric scanner using an established protocol (11). Images were obtained using a T1-weighted spin-echo sequence (repetition time = 210 ms, echo time = 17 ms) with a 480-mm field of view and a 256 × 256 matrix, resulting in a pixel size of 1.88 mm². Briefly, the subjects lay in the magnet in a prone position with their arms placed straight overhead. With L4-L5 used as the point of origin, 10-mm-thick transverse images were acquired every 40 mm from hand to foot, resulting in a total of ~46 images for each subject. Only the five images extending from 5 cm below to 15 cm above L4-L5 were used for the present analyses. The total time required to obtain all images was ~30 min, and the series of images obtained in the abdominal region were acquired in 26 s, during which time the subjects were required to hold their breath.

**Segmentation of SAT and VAT.** The CT and MRI data were transferred electronically to a stand-alone computer workstation for analysis using specially designed image-analysis software (Slice-O-Matic, Tomovision, Montreal, Canada). The model used to segment the various tissues is fully described and illustrated elsewhere (4, 12). Briefly, a multiple-step procedure was used to identify tissue area (cm²) for a given CT or MRI image. In the first step for the CT images, a threshold was selected for AT (~190 to ~30 Hounsfield units) and lean tissue (>0 Hounsfield units) on the basis of the gray-level histograms of the images (3). In the first step for the MRI images, a filter distinguished between different gray-level regions on the images and lines were drawn around the different regions by using a watershed algorithm (4). In the second step for both CT and MRI, the observer labeled the different tissues by assigning them different codes. In the final step, each of the CT and MRI images were reviewed by an interactive slice-editor program, which allowed for verification and, where necessary, correction of the segmented results. For both CT and MRI, the original gray-level image was superimposed on the segmented (e.g., color coded) image using a transparency mode to facilitate the corrections. The area (cm²) of SAT and VAT in each image was computed automatically by summing the AT pixels and multiplying by the individual pixel surface area.

**Derivation of SAT and VAT volume.** For CT, total SAT and VAT volume was determined by using ~40 contiguous transverse images extending from approximately the L5–S1 to the T10–T11 intervertebral spaces. The volume (cm³) of SAT and VAT for each image was calculated by multiplying the respective tissue area (cm²) by the slice thickness (6 mm). Total SAT and VAT volume was calculated by summing the errors of the individual pixels. For MRI, total SAT and VAT volume was determined using the five transverse images extending from 5 cm below to 15 cm above L4-L5. The volume (cm³) of SAT and VAT for each image was calculated by multiplying the respective tissue area (cm²) by the slice thickness (10 mm). The volume of SAT and VAT for the space between two consecutive slices (4 cm) was calculated with the use of a mathematical algorithm provided elsewhere (4). Volume (liters) was converted to mass units (kg) by multiplying the volumes by the assumed constant density of 0.92 for AT (13).

**CT and MRI measurement error.** Interobserver reliability values were used to determine whether regional variations in SAT and VAT ranking were accounted for by the technical limits (e.g., measurement error) of the respective imaging method. The interobserver reliability was determined by comparing the results of two observers’ analyses of the same L4-L5 images for 40 subjects for both CT and MRI area (cm²) measures. As expected, the interobserver error for SAT was low [coefficient of variation (CV) ~1%] for both CT and MRI measures. The interobserver error for VAT was higher for MRI (CV ~10%) by comparison to CT (CV ~3%).

**Anthropometric variables.** At both laboratories, body mass was measured on a balance scale to the nearest 0.1 kg with the subjects dressed in light clothing and barefoot, and standing height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer.

**Statistics.** All statistical procedures were performed by using SPSS version 11.0 (SPSS, Chicago, IL). The relationship between CT and MRI measures of SAT and VAT area at the two levels of the abdomen with SAT and VAT mass derived using multiple images were analyzed by using linear regression techniques. Subject rankings were based on increasing SAT and VAT areas at the two levels (e.g., L4-L5 and L5-L6 for CT, L4-L5 and L4-L5 + 5 cm for MRI). For each AT area value, a range of values was calculated by taking into account the appropriate technical error (%) of the respective imaging method. Each AT area range (lowest to highest) was then compared with the other individuals’ AT area range (lowest to highest) to determine possible ranking ranges. Finally, for each subject, the possible ranking range at one measurement level (e.g., L4-L5) was compared with the ranking range at the other measurement level (e.g., L5-L6). For example, a subject with a CT-VAT area of 109.5 cm² at L4-L5 was ranked 35th out of 100. Assuming a ±3% error of measurement, the true value of 109.5 cm² may have ranged anywhere from 106.3 to 112.8 cm². Because we assumed that the same measurement error (3%) was present in all individuals, which would have also influenced their VAT area and individual ranking, the subject ranked 35th may have been ranked anywhere from 31st to 41st on the basis of VAT area at L4-L5. Furthermore, in this example, the same subject had a CT-VAT area of 125.6 cm² at L4-L5, which ranked him 37th with a potential ranking range of 35th to 39th once measurement error (3%) was accounted for. Because the potential ranking ranges at L4-L5 (31st to 41st) and L5-L6 (35th to 39th) overlap, this indicates that his ranking was the same at both levels. However, if this subject had CT-VAT area of 93 cm² at L4-L5, he would have been ranked 22nd with a potential ranking range of 19th to 24th. Because the ranking ranges at L4-L5 (31st to 41st) and L5-L6 (35th to 39th) do not overlap in this scenario, this indicates that his ranking difference exceeded the expected ranking range (ERR) between the two levels.

On the basis of differences in ranking between the two levels of measurement, each subject was categorized into one of three groups for both SAT and VAT measures for both imaging modalities: 1) those who had no change in ranking (e.g., ranked 35th at both L4-L5 and L5-L6), 2) those who had a change in ranking that was within the ERR (e.g., potential ranking ranges at L4-L5 and L5-L6 overlapped), and 3) those who had a change in ranking that exceeded the ERR (e.g., potential rankings at L4-L5 and L5-L6 did not overlap).

**RESULTS**

**Subject characteristics.** Subject characteristics for the CT and MRI groups are given in Tables 1. The subjects varied widely in age, body mass index, and abdominal AT for both the
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>CT (n = 100)</th>
<th>MRI (n = 100)</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age, yr</td>
<td>50 ± 8</td>
<td>30–67</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27 ± 4</td>
<td>21–36</td>
</tr>
<tr>
<td>SAT mass, kg</td>
<td>3.4 ± 1.2</td>
<td>0.6–7.3</td>
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<tr>
<td>SAT area, cm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L₄–L₅</td>
<td>228 ± 81</td>
<td>35–487</td>
</tr>
<tr>
<td>L₄–L₅</td>
<td>220 ± 80</td>
<td>42–503</td>
</tr>
<tr>
<td>VAT mass, kg</td>
<td>2.9 ± 1.5</td>
<td>0.3–6.0</td>
</tr>
<tr>
<td>VAT area, cm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L₄–L₅</td>
<td>140 ± 60</td>
<td>20–273</td>
</tr>
<tr>
<td>L₄–L₅</td>
<td>169 ± 83</td>
<td>12–348</td>
</tr>
</tbody>
</table>

n. No. of subjects; CT, computed tomography; MRI, magnetic resonance imaging; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

For MRI, the image was obtained at a level 5 cm above L₄.

Influence of measurement site on ranking of SAT. Intra-subject variation in SAT at two levels measured by CT (L₄–L₅ and L₃–L₄) and MRI (L₄–L₅ and L₄–L₅ + 5 cm) is shown in Fig. 1. For CT, the average difference in ranking for SAT at the two levels was 7.2 ± 6.6 positions (range 0–30), and the average difference in SAT area at the two levels was 5.7 cm². Thus the average ranking change of 7.2 positions translated to an ~41-cm² (15%) difference in MRI-SAT area. Ninety-two of the 100 subjects changed rank position when CT-SAT values at L₄–L₅ were compared with those at L₃–L₄. For 76 of these 92 subjects, the change in rank for CT-SAT exceeded the ERR. For these 76 subjects, the average ranking change of 9.1 positions translated to an ~52-cm² (25%) difference in MRI-SAT area.

Influence of measurement site on ranking of VAT. Intra-subject variation in VAT at the two levels measured by CT (L₄–L₅ and L₃–L₄) and MRI (L₄–L₅ and L₄–L₅ + 5 cm) is shown in Fig. 2. For CT, the average difference in ranking for VAT at the two levels was 6.6 ± 5.6 positions (range 0–23) and the average difference in VAT for a one-position change in ranking was 2.6 cm². Thus the average ranking change of 6.6 positions translated to a ~17-cm² (12%) difference in CT-VAT area. Ninety-one of the 100 subjects changed rank position when CT-VAT values at L₄–L₅ were compared with those at L₃–L₄. For 36 of these 91 subjects, the change in rank for CT-VAT exceeded the ERR. For these 36 subjects the average difference in ranking for CT-VAT at the two levels was 11.6 ± 5.7 positions (range 3–23). Thus the average ranking change of 11.6 positions translated to an ~30-cm² (22%) difference in CT-VAT area.

For MRI, the average difference in ranking for VAT at the two levels was 7.6 ± 7.6 positions (range 0–41), and the average difference in VAT area for a one-position change in ranking was 2.8 cm². Thus the average ranking change of 7.6 positions translated to a ~19-cm² (23%) difference in MRI-VAT area.
positions translated to a ~21-cm² (16%) difference in MRI-VAT area. Ninety-five of the 100 subjects changed rank position when MRI-VAT values at L₄–L₅ were compared with those measured at 5 cm above L₄–L₅. For 8 of these 95 subjects, the change in rank for MRI-VAT exceeded the ERR. For these eight subjects, the average difference in ranking for MRI-VAT at the two levels was 20.3 ± 13.5 positions (range 4–41). Thus the average ranking change of 20.3 positions translated to an ~57-cm² (42%) difference in MRI-VAT area.

Relationship between SAT and VAT area (cm²) and corresponding rank position. The average difference in ranking for SAT between two levels was 1.8 positions. For these eight subjects, the average difference in ranking for VAT exceeded the ERR. For these eight subjects, the change in rank for VAT (Fig. 5) translated to an ~22-cm² (16%) difference in MRI-VAT area.

DISCUSSION

A principal finding of this study was that the ranking of men for abdominal SAT and VAT deposition was dependent on the level of the abdomen at which these depots were measured. However, despite the interindividual variation in ranking, the ability to predict SAT and VAT mass using single images obtained at the L₄–L₅, L₃–L₅ + 5 cm, and L₃–L₄ levels was comparable.

Two previous studies report that the ranking of subjects for VAT is influenced by measurement site. Thomas and Bell (14) report marked variability for the ranking of MRI-VAT in a sample of 59 premenopausal women. This observation confirmed an earlier study by Greenfield et al. (1), who report similar findings for CT-VAT in a sample of 19 relatively lean premenopausal women. Our findings reinforce and extend these earlier observations and suggest that the ranking of men for both VAT and SAT varies according to the site of measurement and, therefore, that the distribution of both tissues varies on an individual basis.

To put the observed change in ranking position in perspective, we considered whether the change in position was within or outside the ERR given the technical limitations of the radiological methods. For SAT (Fig. 2), although the frequency of change in rank position was reduced after allowance for measurement error, significant ranking differences (e.g., outside the ERR) at the two levels persisted independent of imaging modality. On the other hand, for MRI- and CT-VAT, the vast majority of those who changed rank position did so within the measurement error (e.g., inside the ERR). These disparate observations are likely explained by the fact that, for both MRI and CT, the error attributed to the measurement for SAT (1%), and hence the ERR, was smaller than for VAT (3–10%). In other words, the frequency of change in rank position that is outside the expected range is a consequence of the size of that range, which itself is a function of the technical limitations inherent to the measurement of that tissue. This is also true when the differences observed between imaging methods are compared. For example, because the standard error of measurement for VAT by MRI (~10%) is greater than CT (~3%), 87 of the 95 subjects who changed rank for MRI-VAT at the two levels were within the expected range, whereas 55 of the 91 subjects who changed rank for CT-VAT were within the expected range. However, although CT measures of VAT yielded greater changes in ranking between methods, the average difference in VAT area for a one-position change in ranking was similar for CT and MRI (2.6 cm² for CT-VAT vs. 2.8 cm² for MRI-VAT). Nevertheless, the implication of these observations is that the limitations of the imaging method should be considered when determining the extent to which a change in rank position for SAT or VAT between two levels of the abdomen has occurred.

VARIATION IN RANKING OF ABDOMINAL ADIPOSE TISSUE

Fig. 2. Intrasubject variation in visceral adipose tissue (VAT) area and ranking at 2 levels of the abdomen. Bars represent the VAT area at L₃–L₄ (CT, A) or L₄–L₅ + 5 cm (MRI; B) in 100 men ranked from lowest to highest according to VAT area at L₄–L₅. Insets: number of subjects who had no Δ, Δ within ERR, or Δ outside ERR at the 2 levels. Changes within or outside of the expected ranking range were determined by considering the technical error of CT (3%) and MRI (10%) measures of VAT.

Fig. 3. Intratissue variation in SAT area and ranking at 2 levels of the abdomen. Bars represent the SAT area at L₄–L₅ + 5 cm (CT, A) or 5 cm above (MRI; B) in 100 men ranked from lowest to highest according to SAT area at L₄–L₅. Insets: number of subjects who had no Δ, Δ within ERR, or Δ outside ERR at the 2 levels. Changes within or outside of the expected ranking range were determined by considering the technical error of CT (3%) and MRI (10%) measures of SAT.

Fig. 4. Intrasubject variation in SAT area and ranking at 2 levels of the abdomen. Bars represent the SAT area at L₄–L₅ (CT, A) or 5 cm above (MRI; B) in 100 men ranked from lowest to highest according to SAT area at L₃–L₄. Insets: number of subjects who had no Δ, Δ within ERR, or Δ outside ERR at the 2 levels. Changes within or outside of the expected ranking range were determined by considering the technical error of CT (3%) and MRI (10%) measures of SAT.

Fig. 5. Intrasubject variation in VAT area and ranking at 2 levels of the abdomen. Bars represent the VAT area at L₃–L₄ (CT, A) or L₄–L₅ + 5 cm (MRI; B) in 100 men ranked from lowest to highest according to VAT area at L₄–L₅. Insets: number of subjects who had no Δ, Δ within ERR, or Δ outside ERR at the 2 levels. Changes within or outside of the expected ranking range were determined by considering the technical error of CT (3%) and MRI (10%) measures of VAT.

Fig. 6. Intratissue variation in SAT area and ranking at 2 levels of the abdomen. Bars represent the SAT area at L₄–L₅ + 5 cm (CT, A) or 5 cm above (MRI; B) in 100 men ranked from lowest to highest according to SAT area at L₃–L₄. Insets: number of subjects who had no Δ, Δ within ERR, or Δ outside ERR at the 2 levels. Changes within or outside of the expected ranking range were determined by considering the technical error of CT (3%) and MRI (10%) measures of SAT.

Fig. 7. Intrasubject variation in VAT area and ranking at 2 levels of the abdomen. Bars represent the VAT area at L₃–L₄ (CT, A) or L₄–L₅ + 5 cm (MRI; B) in 100 men ranked from lowest to highest according to VAT area at L₄–L₅. Insets: number of subjects who had no Δ, Δ within ERR, or Δ outside ERR at the 2 levels. Changes within or outside of the expected ranking range were determined by considering the technical error of CT (3%) and MRI (10%) measures of VAT.
abdomen reflects a biological truth or is the consequence of technical error. This is particularly true for CT- and MRI-measured VAT.

Apart from the differences in subject ranking of SAT and VAT attributable to measurement error, the clinical significance of the magnitude of the differences observed is unclear. For example, the average SAT ranking change of about eight positions for CT and seven positions for MRI translated to only ~38 cm² (16%) and ~41 cm² (15%), respectively. Similarly, the average VAT ranking change of seven positions for CT and eight positions for MRI translated to only ~17 cm² (12%) and ~21 cm² (15%), respectively. The relatively small variation likely explains why the prediction of SAT and VAT mass (kg) by the corresponding SAT and VAT area (cm²) values at the levels measured were comparable (Figs. 4 and 5). If it is assumed that a primary objective when measuring SAT and VAT deposition using a single image is to quantify the true mass of the tissue, our findings suggest that either level of the abdomen (L₄–L₅ or L₃–L₄) provides excellent surrogate measures of the total mass. This observation is reinforced by our finding that SAT and VAT values at the L₄–L₅ level relate well to the corresponding values at L₃–L₄ or L₄–L₅ + 5 cm (Fig. 3).

Fig. 3. Relationship between CT and MRI measures of SAT and VAT measured at 2 different levels of the abdomen. SEE, standard error of estimate.

Fig. 4. Relationship between CT and MRI measures of SAT area measured at 2 different levels of the abdomen with the corresponding mass values determined by using multiple-image protocols. See MATERIALS AND METHODS for details.
Accordingly, the change in ranking position at the levels of the abdomen measured here has little clinical importance, if it is assumed that the objective is to predict SAT and/or VAT mass.

The notion that the change in rank position for SAT and VAT deposition at the levels measured in this study would have little clinical impact is underscored by previous observations suggesting that the relationship between metabolic risk and VAT area at one level (e.g., L4–L5) is similar to those observed at a different level (e.g., L4–L5/5 cm) in obese individuals (8). It is also reported that the association between VAT volume, measured using a series of five abdominal images (5 cm below to 15 cm above L4–L5), and metabolic risk factors is similar to that observed between metabolic risk factors and VAT area (cm²) at the L4–L5 level (8, 9). Similar relationships have also been shown between metabolic risk factors with SAT area (one image) and volume (multiple images) (2, 7, 8). When combined with the findings reported here, it would appear that intersubject variations in abdominal SAT and VAT deposition have little influence on the prediction of SAT and VAT mass or the relationship between these AT variables and metabolic risk. Nevertheless, our findings here suggest that, if forced to choose, SAT and VAT area (cm²) measured at the level of L3–L4 or L4–L5 +5 cm predicts the total mass of either tissue slightly better than L4–L5.

In this study, we compared the ranking of subjects for SAT and VAT deposition by using data obtained at the L4–L5 and L3–L4 levels for CT and at L4–L5 and 5 cm above for MRI. These sites were selected because they are the sites most often reported in the literature for the measurement of abdominal adiposity (15). Although our findings suggest that interindividual variation in the ranking of SAT and VAT deposition exists between these levels, whether the variation would increase or decrease were different levels selected is unknown. For example, it is possible that the ranking of subjects for SAT and/or VAT deposition at L4–L5 relates better to corresponding rankings at L1–L2 than rankings at L3–L4. Although this may be true, assuming once again that the primary objective is to select the level of the abdomen that best predicts total SAT and VAT, rather than determine whether intersubject variation in AT distribution is altered depending on a comparison of arbitrarily selected CT or MRI images, our premise is that it would be more appropriate to perform a rigorous analysis that determines which level(s) of the abdomen is optimal for prediction of total SAT and VAT mass. To accomplish this requires identification of the anatomic regions that define abdominal SAT and VAT volume or mass. As yet, there is no consensus in this regard. Furthermore, once the anatomic regions that define the depots are established, a multiple, contiguous imaging protocol would be required to accurately measure their respective volumes. This would be a challenging study, but it is one that is required to properly identify the levels of the abdomen that best predict abdominal SAT and VAT.

In summary, the findings of this study demonstrate that the frequency of changes in rank at different levels of the abdomen due to intersubject variation in VAT and SAT deposition remains despite allowance for measurement error. However, the magnitude of the change in rank with respect to SAT and VAT area (cm²) is small and thus the ability to predict SAT and VAT mass using single images obtained at the L4–L5, L4–L5 +5 cm, or L3–L4 level is comparable. Whether our findings based on a Caucasian sample remain true for other racial or ethnic groups is unknown.

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Fig. 5. Relationship between CT and MRI measures of VAT area measured at 2 different levels of the abdomen with the corresponding mass values determined by using multiple-image protocols. See MATERIALS AND METHODS for details.
GRANTS
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