Reproducibility of normalized apparent diffusion coefficient measurements on 3.0-T diffusion-weighted imaging of normal pancreas in a healthy population

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Abstract
To prospectively compare the reproducibility of normal pancreas-normalized apparent diffusion coefficient (ADC) measurements for the normal pancreas and mean normalized ADCs at different pancreatic anatomic locations.

In total, 22 healthy volunteers underwent pancreatic 3.0-T magnetic resonance (MR) imaging, including axial diffusion-weighted (DW) imaging with 3 b values used (0, 400, and 800 s/mm²) and with the respiratory-triggered (RT) technique. The mean ADCs from 3 regions of interest (ROIs) in 5 anatomic locations (head [H], body [B], and tail [T] of pancreas and spleen [S]) and erector spinae muscles [M]) were calculated. The pancreas-normalized ADC was defined as the ratio of the ADC for the pancreas to the ADC for the spleen or erector spinae muscle. Reproducibility of ADCs and normalized ADCs was assessed by the Bland–Altman method. The ADC and normalized ADC data were analyzed by repeated-measures ANOVA.

Mean ADC and normalized ADC values did not differ (P > .05) with repeated measurements at the different pancreatic anatomic locations. Reproducibility of pancreas-normalized ADC measurements in each of the 3 pancreatic anatomic locations was better with the erector spinae muscle rather than the spleen used as a reference. Mean ADC and normalized ADC values significantly differed between the 3 pancreatic segments (H: 1.36 × 10⁻³ mm²/s, B: 1.38 × 10⁻³ mm²/s, T: 1.25 × 10⁻³ mm²/s, P = .022; H/S: 1.75, B/S: 1.78, T/S: 1.59, P = .009; H/M: 0.91, B/M: 0.95, T/M: 0.85, P = .008). Mean ADC values and normalized ADC values showed a trend to decrease from the pancreatic head to tail.

Our preliminary results suggest that normalized ADC measurements for the pancreas show good intra- and interobserver reproducibility, the erector spinae muscle is a better choice than the spleen for calculating normalized ADC values for the pancreas, and the normalized ADC values are lower for the pancreatic tail than other pancreatic segments.

Abbreviations: ADC = apparent diffusion coefficient, B = body of pancreas, CI = confidence intervals, DW = diffusion-weighted, H = head of pancreas, LOA = limits of agreement, M = erector spinae muscle, MR = magnetic resonance, ROI = region of interest, RT = respiratory-triggered, S = spleen, T = tail of pancreas.

Keywords: normalized apparent diffusion coefficient, pancreas, reproducibility

1. Introduction
Advances in MRI have led to the application of diffusion-weighted (DW) imaging for the detection and characterization of pancreatic and other lesions.[1–4] DW imaging is used to measure the random motion of water molecules in biologic tissues. Increased cellularity and intact cell membranes inhibit this free movement of water in the intracellular and extracellular spaces. Oncologic applications have been of particular interest because lipophilic cell membranes in hypercellular tumor tissue serve as barriers to free diffusion in the intracellular and extracellular spaces.[5,6] Many studies have shown that apparent diffusion coefficient (ADC) measurements are helpful in differentiating benign pancreatic lesions from malignant ones and can be used as imaging biomarkers to assess treatment response.[7–11] However, before ADC measurements can be considered a reliable and discriminating tool, the range of ADC values within normal tissue, such as the pancreas, as well as their reproducibility should be investigated. Despite a high degree of reported intra- and interobserver reproducibility in ADC measurements, ADC values for the same tissue may substantially vary between 2 patients and between 2 examinations in the same patient.[12] To limit the possible influence of MR scanners of different field strength and vendors at different sites on the resulting ADC, researchers have used a normalized ADC to improve characterization of pathologic conditions with DW-MRI.[13,14] In the pancreas, the normalized ADC has been defined by using the adjacent pancreatic parenchyma or other organs as references.[13,14] The adjacent parenchyma or other organs are assumed to be subject to the same magnetic field heterogeneity and susceptibility effects as the pancreatic lesion. Normalization might decrease the
potential influence of some factors on ADC calculation, such as different MR scanners, field strengths, b values and patient variability, and so on. However, the reproducibility in normalized ADC measurements of the normal pancreas has not been reported. This prospective study aimed to compare the reproducibility of normal pancreas-normalized ADC measurements and to compare the normalized ADC values at different pancreas anatomic locations in healthy individuals by using the spleen and erector spinae muscles as references.

2. Materials and methods

2.1. Study population

This prospective study was approved by the research ethics committee of Zhongnan Hospital of Wuhan University, and written informed consent was obtained from each participant. In total, 24 healthy volunteers were referred for MRI of the pancreas between February 2018 and April 2018. The inclusion criteria for this study were: no history of drug abuse, pancreatitis, diabetes, alcohol abuse, chronic hepatic disease, hepatic steatosis or abdominal surgery, and no medication use during the study; and normal appearance of the pancreas on ultrasonography (no focal or diffuse pancreas disease, including mild steatosis). The exclusion criteria were: a history of or diffuse pancreas disease, including mild steatosis). Two participants did not complete the study because of poor image quality due to an obvious breathing-motion artifact (n = 2). In total, 22 participants successfully completed the imaging examinations (Table 1).

2.2. MRI protocol

All participants underwent MRI with a 3.0-T MR imager (Ingenia, Philips Medical Systems Nederland BV, The Netherlands) with a dedicated 16-channel body matrix coil and a 32-channel spine coil. DW images were acquired by using the fat-suppressed single-shot echo-planar imaging pulse sequence with a respiratory-triggered (RT) technique (with an air-filled pressure sensor capable of measuring respiratory-induced pressure changes fixed to the hypochondrial region via a respiration belt around the participant). Each volunteer was imaged using the standardized protocol for each participant was b values 0, 400, and 800s/mm2; repetition time/echo time, 1500/69 ms; matrix size, 128 × 153 mm; section thickness, 5 mm; intersection gap, 1 mm; voxel size, 2.2 × 2.3 × 5.0 mm; field of view, 280 × 356 mm; number of axial sections, 20; acquisition time, 120s.

2.3. Image analysis

MR images were transferred to a workstation (Philips IntelliSpace Portal) for postprocessing. The ADCs for each DW imaging series were automatically calculated by the MR system and displayed as corresponding ADC maps. According to the monoeXponential Stejskal and Tanner model,[15] the attenuation, A, could then be simply formulated as $A = S_0 e^{-b \times \text{ADC}}$, where $S$ is the signal intensity measured at a specific b value and $S_0$ is the signal intensity at $b = 0$mm2/s. Hence, ADC maps can be constructed by using a minimum of 2 b values according to the following equation: $ADC = \ln \left( \frac{S_2}{S_1} \right) / \left( b_2 - b_1 \right)$, where $b$ is the degree of diffusion sensitization (at least 2 b values; $b_1$ and $b_2$), and ADC (expressed in $10^{-3} \text{mm}^2/\text{s}$), and $S_1$ and $S_2$ are the tissue signal intensity on DW MR images at corresponding $b_1$ and $b_2$. ADC corresponds to the slope of the signal decay by using a logarithmic scale according to the b values used. Thus, voxels that show a steeper slope of signal attenuation with increasing b values will have higher ADCs (indicating higher water diffusivity) compared with voxels that show a gradual slope of signal attenuation.

ADCs were measured by 2 radiologists working independently (XD, JZ; readers 1 and 2 with 10 and 8 years of clinical experience in pancreas MR imaging, respectively). Regions of interest (ROIs) were drawn on each segment of the DW images obtained with $b = 0$mm2/s. The ROIs were transferred automatically from the $b_0$ image to the pixel-based ADC map for ADC calculation.

Table 1

| Volunteer demographics. | No. of volunteers | Mean Age (y) |
|-------------------------|------------------|--------------|
| volunteers              |                  |              |
| Men                     | 10               | 40.8 ± 9.4 (25–53) |
| Women                   | 12               | 39.4 ± 6.4 (33–54) |

* Data are mean ± standard deviation, with ranges in parentheses.

2.4. Statistical analysis

ADCs were expressed as median (Q1–Q3) and range, and were tested first with the Kolmogorov-Smirnov test for normality. Data were compared with the Wilcoxon signed rank test. The reproducibility of ADCs and normalized ADCs were evaluated by the Bland-Altman method.[16] ADCs and normalized ADCs were compared between segments with the Kruskal–Wallis test; P value less than the Bonferroni-corrected significance value of .017 (.05/3) indicated a statistically significant difference. Statistical analyses involved use of SPSS v23.0 (SPSS, Inc, Chicago, IL) and MedCalc (MedCalc, Mariakerke, Belgium). Differences were considered significant at $P < .05$. 

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3. Results

3.1. The Kolmogorov–Smirnov test

According to the Kolmogorov–Smirnov test, ADCs and normalized ADCs did not conform to normal distribution, but ADC differences and normalized ADC differences between repeated measurements were normally distributed.

3.2. Intraobserver variability

The mean absolute difference (bias) and 95% confidence intervals (CIs) for the mean difference (limits of agreement, LOA) between ADCs for the head (H), body (B), and tail (T) of the pancreas and spleen (S) and erector spinae muscles (M) were $-0.00 \times 10^{-3}$ [$-0.10-0.09] \text{mm}^2/\text{s}$, $-0.01 \times 10^{-3}$ [$-0.07-0.05] \text{mm}^2/\text{s}$, $-0.00 \times 10^{-3}$ [$-0.11-0.10] \text{mm}^2/\text{s}$, $0.01 \times 10^{-3}$ [$-0.09-0.10] \text{mm}^2/\text{s}$, and $-0.01 \times 10^{-3}$ [$-0.13-0.10] \text{mm}^2/\text{s}$, respectively. Intraobserver reproducibility of ADC measurements for each of the 5 anatomic locations was similar. The LOA were 0.060–0.115 $\times 10^{-3}\text{mm}^2/\text{s}$.

The mean absolute difference (bias) and 95% CIs for the mean difference (LOA) between normalized ADCs for the H/S, H/M, B/S, B/M, T/S and T/M were $-0.04 \times 10^{-3}$ [$-0.32-0.25]$, 0.01 [$-0.10-0.12]$, $-0.04 \times 10^{-3}$ [$-0.32-0.24]$, 0.00 [$-0.08-0.09]$, $-0.03 \times 10^{-3}$ [$-0.27-0.22]$, and 0.01 [$-0.08-0.10]$, respectively. Within the 95% LOA, the maximum absolute value of the difference between the ADC values measured by reader 1 for the H/S, H/M, B/S, B/M, T/S and T/M were 0.13, 0.05, 0.14, 0.05, 0.22 and 0.07, respectively. The difference between the 2 measurements by reader 1 was at most 0.22, which is clinically acceptable, so the 2 repeated measurements by the same reader had good intraobserver reproducibility. The intraobserver reproducibility of normalized ADCs for each of the 3 pancreatic anatomic locations were better when using the erector spinae muscle than the spleen as a reference. The LOA were (0.085–0.110) and (0.245–0.285), respectively (Table 2).

The ADC values calculated for the 5 anatomic locations and normalized ADC values calculated for the 3 pancreatic segments during the 2 repeated measurements for reader 1 are in Table 3. We found no significant differences in ADC and normalized ADC measurements for each of the 3 pancreatic segments.

3.3. Interobserver variability

The mean absolute difference (bias) and 95% CIs for the mean difference (LOA) between ADCs for the H, B, and T of the pancreas and S and M were $-0.02 \times 10^{-3}$ [$-0.12-0.08] \text{mm}^2/\text{s}$, $0.00 \times 10^{-3}$ [$-0.09-0.10] \text{mm}^2/\text{s}$, $-0.00 \times 10^{-3}$ [$-0.08-0.07] \text{mm}^2/\text{s}$, $0.01 \times 10^{-3}$ [$-0.09-0.11] \text{mm}^2/\text{s}$, and $-0.00 \times 10^{-3}$ [$-0.18-0.18] \text{mm}^2/\text{s}$, respectively. Interobserver reproducibility of ADC measurements for each of the 5 anatomic locations were similar. The LOA were 0.075–0.180 $\times 10^{-3}\text{mm}^2/\text{s}$.

The mean absolute difference (bias) and 95% CIs for the mean difference (LOA) between normalized ADCs for the H/S, H/M, B/S, B/M, T/S, and T/M were $-0.04 \times 10^{-3}$ [$-0.30-0.22]$, $-0.02 \times 10^{-3}$ [$-0.22-0.18]$, $-0.02 \times 10^{-3}$ [$-0.30-0.27]$, $-0.00 \times 10^{-3}$ [$-0.11-0.11]$, $-0.02 \times 10^{-3}$ [$-0.26-0.21]$, and $-0.01 \times 10^{-3}$ [$-0.19-0.17]$, respectively (Fig. 2). Figure 2

### Table 2

| Reproducibility | H/S | H/M | B/S | B/M | T/S | T/M |
|----------------|-----|-----|-----|-----|-----|-----|
| **Intraobserver** | $-0.04 (0.285)$ | 0.01 (0.110) | $-0.04 (0.280)$ | 0.00 (0.085) | $-0.03 (0.245)$ | 0.01 (0.090) |
| **Interobserver** | $-0.04 (0.260)$ | $-0.02 (0.200)$ | $-0.02 (0.285)$ | $-0.00 (0.110)$ | $-0.02 (0.235)$ | $-0.01 (0.180)$ |

Data in parentheses are 95% LOA.

ADC = apparent diffusion coefficient, B = body of pancreas, H = head of pancreas, LOA = limits of agreement, M = erector spinae muscle, S = spleen, T = tail of pancreas.

* The ratio of different anatomical sites.

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Figure 1. Diffusion-weighted (DW) MRI of the pancreas. (A) Three circular regions of interest (ROIs) (arrow) were placed on the pancreatic head by using a DW trace image obtained with $b = 3 \text{mm}^2/\text{s}$ for optimal placement. (B) The ROIs (arrow) were transferred automatically from the $b_0$ image to the pixel-based apparent diffusion coefficient (ADC) map for ADC calculation.
shows that 1/22 (4.5%) of the points were outside the 95% LOA. Within the 95% LOA, the maximum absolute value of the difference between the ADC values measured by 2 independent readers for the H/S, H/M, B/S, B/M, T/S, and T/M were 0.20, 0.11, 0.23, 0.06, 0.14, and 0.08, respectively. The difference between the 2 measurement results for 2 independent readers was at most 0.23, which is clinically acceptable, so the 2 repeated measurements by 2 independent readers were better when using the erector spinae muscle than the spleen as a reference. The LOA were (0.110–0.23), which is clinically acceptable, so the 2 repeated measurements by 2 independent readers were better when using the erector spinae muscle than the spleen as a reference.

ADC values (\(\times 10^{-3}\) mm\(^2\)/s) and normalized ADC values for the 3 anatomic locations during two repeated measurements by the same reader (intraobserver comparison) and 2 independent readers (interobserver comparison).

| Location | Reader 1 | Reader 2 | \(P\) value | Reader 1 | Reader 2 | \(P\) value |
|----------|----------|----------|-------------|----------|----------|-------------|
| ADC      | First measurement | Second measurement | | First measurement | Second measurement | |
| Head     | 1.36 (1.23–1.56) | 1.37 (1.25–1.57) | .856 | 1.35 (1.26–1.59) | 1.37 (1.26–1.59) | .205 |
| Body     | 1.37 (1.27–1.66) | 1.39 (1.29–1.57) | .228 | 1.37 (1.27–1.66) | 1.37 (1.27–1.66) | .826 |
| Tail     | 1.25 (1.16–1.38) | 1.28 (1.16–1.38) | .958 | 1.25 (1.14–1.41) | 1.25 (1.14–1.41) | .889 |
| Spleen   | 0.80 (0.75–0.87) | 0.78 (0.72–0.88) | .679 | 0.78 (0.72–0.88) | 0.78 (0.72–0.88) | .369 |
| Muscle   | 1.53 (1.42–1.57) | 1.52 (1.43–1.58) | .091 | 1.52 (1.43–1.58) | 1.52 (1.43–1.58) | .267 |

normalized ADC

| Location | First measurement | Second measurement | \(P\) value | Reader 1 | Reader 2 | \(P\) value |
|----------|------------------|------------------|-------------|----------|----------|-------------|
| H/S†     | 1.72 (1.62–1.95) | 1.75 (1.64–1.98) | .592 | 1.78 (1.68–1.93) | 1.78 (1.68–1.93) | .280 |
| H/M‡     | 0.91 (0.85–1.01) | 0.91 (0.84–0.98) | .321 | 0.92 (0.85–1.01) | 0.92 (0.85–1.01) | .760 |
| B/S†     | 1.77 (1.65–1.98) | 1.79 (1.65–2.01) | .433 | 1.80 (1.65–2.02) | 1.80 (1.65–2.02) | .672 |
| B/M‡     | 0.96 (0.87–1.04) | 0.93 (0.88–1.03) | .309 | 0.95 (0.88–1.03) | 0.95 (0.88–1.03) | .474 |
| T/S†     | 1.62 (1.42–1.75) | 1.63 (1.47–1.73) | 1.00 | 1.60 (1.45–1.82) | 1.60 (1.45–1.82) | .537 |
| T/M‡     | 0.84 (0.77–0.91) | 0.85 (0.76–0.93) | .330 | 0.83 (0.73–0.92) | 0.83 (0.73–0.92) | .470 |

The ADC values calculated for the 5 anatomic locations and normalized ADC values calculated for the 3 pancreatic segments during the 2 repeated measurements by 2 independent readers are in Table 3. ADC and normalized ADC measurements did not differ for each of the 3 pancreatic segments.

3.4. ADC and normalized ADC values for different anatomic pancreas locations

We found a significant difference in ADC values among the 3 pancreatic segments (H: 1.36 \(\times 10^{-3}\) mm\(^2\)/s; B: 1.38 \(\times 10^{-3}\) mm\(^2\)/s; T: 1.25 \(\times 10^{-3}\) mm\(^2\)/s; \(P = .022\)), because of a lower median ADC value at the pancreatic tail than the head and body (Table 4, Fig. 3).

We also found a significant difference in normalized ADC values among the 3 pancreatic segments (H: 1.75; B: 1.78; T: 1.59; \(P = .009\); H/M: 0.91; B/M: 0.95; T/M: 0.85; \(P = .008\)), because of lower median normalized ADC values at the pancreatic tail than the head and body (Table 4, Fig. 3).

4. Discussion

Intra- and interobserver reproducibility of ADC measurements have been reported with 1.5- or 3.0-T examination for different pancreatic segments in patients free of pancreatic disease.[17] The
results of our study show that ADC measurements within the 5 anatomic locations were reproducible on both an intra- and interobserver basis in a healthy population. Our data can be used as a reference in future studies for the range of pancreatic DW imaging errors, which could be more frequently measured to help detect and characterize pancreatic abnormalities.

The ADCs in the 4 pancreatic segments at 1.5 and 3.0 T are relatively homogeneous\textsuperscript{[17–19]} with a mean ADC of 1.611 \times 10^{-3} \text{mm}^2/\text{s} (range: 0.16–3.01). These variations in ADCs may result from differences in patient populations, imaging sequences, the specific b values used for ADC calculation, or other technical parameters of the data acquisition\textsuperscript{[20–22]}. Two studies found lower ADCs in the pancreatic tail than other pancreatic segments\textsuperscript{[17,23]}.

Our study also showed that the ADC value was lower for the pancreatic tail than the pancreatic head and body. The reasons for this result may be greater blood supply to the pancreatic head and body and more capillary network distribution than in the tail. The pancreatic tail is mainly...
**Table 4**  
*P* values of paired comparisons of ADC values and normalized ADC values obtained from the 3 pancreatic segments.

| Segments          | *P* value | *P* value |
|-------------------|-----------|-----------|
| ADC               |           |           |
| Head vs Body      | .181      | .542      |
| Head vs Tail      | .014      | .041      |
| Body vs Tail      | .003      | .009      |
| Normalized ADC    |           |           |
| H/S vs B/S        | .244      | .733      |
| H/S vs T/S        | .005      | .015      |
| B/S vs T/S        | .002      | .005      |
| Normalized ADC    |           |           |
| H/M vs B/M        | .139      | .418      |
| H/M vs T/M        | .008      | .026      |
| B/M vs T/M        | .001      | .003      |

Comparisons were made using the Kruskal-Wallis test, and a *P* value less than the Bonferroni-corrected significance value of .017 (.05/3) or a *P* value < .05 was considered to indicate a significant difference (*P* values equal to *.05*).

ADC = apparent diffusion coefficient, B = body of pancreas, H = head of pancreas, M = erector spinae muscle, S = spleen, T = tail of pancreas.  
The ratio of different anatomical sites.

The ratio of different anatomical sites.

composed of pancreatic islet cells, but the pancreatic head and body are mainly composed of acinar cells. The synthesis of pancreatic islet cells and secretion of the hormone are slower than for acinar cells. Our results were within the ranges of previous reported results.

ADC normalization is a promising tool to improve the accuracy of ADC measurement and diagnostic performance. At present, the pancreas-normalized ADC has been defined by using adjacent pancreatic parenchyma as a reference. However, fibrosis and fatty infiltration of the pancreas may occur with chemotherapy or in patients with underlying chronic disease. Thus, changes in pancreatic parenchyma during treatment in the absence of tumor response or tumor necrosis may result in misinterpretation of normalized ADCs when using the adjacent pancreatic parenchyma as a reference. In our study, we used the spleen and erector spinae muscle as reference sites to reduce errors, because the spleen and muscles may be less affected by treatments. As illustrated recently, one major limitation of DW-MRI is the difficulty to differentiate between pancreatic adenocarcinoma and mass-forming pancreatitis because of overlap in ADC values. The preliminary results by Barral et al suggest that normalized ADC helps characterize focal pancreatic lesions and further discriminate between pancreatic cancers and mass-forming pancreatitis.

Before the normalized ADC is used, the consistency of both an intra- and interobserver measurements must be analyzed. So far, no study has reported the reproducibility of normalized ADC measurements in pancreas. The results of our study show that normalized ADC measurements within the 3 pancreatic segments are reproducible on both an intra- and interobserver basis, and the reproducibility of normalized ADC measurements in each of the 3 pancreatic anatomic locations were better when using the erector spinae muscle than the spleen as a reference. The erector spinae muscle may be less susceptible to breathing-motion images and has less blood supply and thus less measurement error than the spleen. The pancreatic tail had a lower normalized ADC value than the pancreatic head and the pancreatic body, perhaps because the pancreatic tail has a lower ADC value, and the ADC values of the spleen and erector spinae muscles are relatively constant in a healthy population. However, for patients with portal hypertension or splenic disease, the erector spinae muscles can be selected as a reference for standardizing ADC values in the differential diagnosis of pancreatic lesions.

Our study has some limitations. First, our prospective study had a small number of participants, which may have implied selection bias. Larger studies are needed to validate our findings. Second, we used 3 b values (0, 400, and 800s/mm²) for ADC measurements. Because the perfusion fraction was not excluded and only monoeponential fitting was performed, the ADC values might have been overestimated; however, the reproducibility should remain the same. Third, to keep the imaging variables as constant and homogeneous as possible, we performed all imaging examinations with the same 3.0-T system from a single vendor. This ideal scenario may not be achievable in actual daily clinical practice. Fourth, although the quality of DW images obtained with RT technique were good, it took more time to check the imagers and our results may apply to only this specific acquisition technique. Finally, we did not evaluate the effects of different field strengths and b values on ADC measurement reproducibility. The choice and number of b values, field strength, and the analysis software used could affect the measured *ADC* values and they might also affect measurement reproducibility.

In conclusion, normalized ADC measurements of the pancreas showed good reproducibility in our preliminary study, and the erector spinae muscle is a better choice than the spleen for calculating normalized ADC values of the pancreas. When applying normalized ADC values, we should pay attention to the specific normalized ADC values in different pancreatic anatomic locations.

**Author contributions**

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