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A novel method for quantitative monitoring of transplanted islets of Langerhans by positive contrast magnetic resonance imaging

Lindsey A Crowe, PhD¹, Frederic Ris, MD², Sonia Nielles-Vallespin, PhD³, Peter Speier, PhD³, Solange Masson², Mathieu Armanet, PhD², P Morel, MD², Christian Toso, MD, PhD², Domenico Bosco, PhD², Thierry Berney, MD^{2,†}, and Jean-Paul Vallee, MD, PhD^{1,†}

¹ Department of Radiology, University of Geneva School of Medicine and Geneva University Hospitals, Geneva, Switzerland ² Cell Isolation and Transplant Center, Department of Surgery, University of Geneva School of Medicine and Geneva University Hospitals, Geneva, Switzerland ³ Siemens AG Medical Solutions, Erlangen, Germany

Abstract

The Automatic Quantitative Ultrashort Echo Time imaging (AQUTE) protocol for serial MRI allows quantitative in-vivo monitoring of iron labeled pancreatic islets of Langerhans transplanted into the liver, quantifying graft implantation and persistence in a rodent model.

Rats (n=14), transplanted with iron-oxide loaded cells (0–4000 islet equivalents, IEQ), were imaged using a 3D radial ultrashort echo time difference technique (dUTE) on a Siemens MAGNETOM 3T clinical scanner up to 5 months post-surgery. In-vivo 3D dUTE images gave positive contrast from labeled cells, suppressing liver signal and small vessels, allowing automatic quantification.

Position of labeled islet clusters was consistent over time and quantification of hyperintense pixels correlated with the number of injected IEQs ($R^2 = 0.898$, $p < 0.0001$), and showed persistence over time (5 months post-transplantation). Automatic quantification was superior to standard imaging and manual counting methods, due to the uniform suppressed background and high contrast, resulting in significant timesavings, reproducibility and ease of quantification. 3D coverage of the whole liver in the absence of cardiac/respiratory artifact provided further improvement over conventional imaging.

This imaging protocol reliably quantifies transplanted islet mass and has high translational potential to clinical studies of transplanted pancreatic islets.

Keywords

MRI; ultrashort TE; rat; islet; iron oxide; quantification

Correspondence Address and affiliation 1: Lindsey A Crowe, ¹Department of Radiology, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva, Switzerland. Tel +41 22 37 25212/Fax +41 22 37 27072, Lindsey.crowe@hcuge.ch.

Full Correspondence Addresses for authors

²Department of Surgery, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva, Switzerland.

³Cardiovascular MR, Siemens AG Healthcare Sector, Erlangen, Germany.

[†]T Berney and J-P Vallée contributed equally to this work as co-senior authors.

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Introduction

Treatment of type 1 diabetes by islet of Langerhans transplantation has given excellent short-term results, but long-term attrition occurs (approximately 20% of patients insulin free 5 years after transplantation) (1–5). For efficient treatment regimes against islet rejection, to allow intervention before any clinical symptoms manifest, in-vivo non-invasive quantification and monitoring of transplanted cells is needed. Traditional quantitative modalities for cellular imaging are not suitable for serial or clinical examinations. Positron Emission Tomography suffers from very short contrast agent half-lives, precluding study of progression over time. Histology, whilst accurate, is not a method for clinical follow-up; and because of the sparse distribution of these cells, the yield of biopsy is too low for efficient graft assessment (6). Bioluminescence (7), used in rodents can characterize the model, but does not have the penetration depth for human studies.

Islets cells do not naturally show contrast from liver, however they can be loaded ex-vivo with iron oxide, before the transplantation procedure. The presence of paramagnetic iron oxide particles inside the islet cells produces strong localized regions of signal loss on conventional MR images by destroying the homogeneity of the surrounding magnetic field. Preliminary reports suggest the potential of magnetic resonance (MR) imaging for non-invasive serial monitoring for islet cells, including immune rejection (16), and similar applications (8–15). Clinical MRI of iron-labeled islet grafts have been successfully achieved in our recent pilot study (17). Despite the clear visibility of signal loss on the images, and efficiency of the iron oxide labeling and cell transplantation studies, this method cannot be easily applied to quantitative or serial studies (17, 18). Several issues hamper quantification of such signal loss: similar areas of hypointensity can be created by other structures in the same region such as vessels; quantification of signal loss, or saturation, is much less feasible than measuring hyperintense signal that is significantly different from the background noise level; and finally background liver signal is heterogeneous. As signal variation makes automatic thresholding to identify islet cells impossible, previous reports of MR quantification of iron labeled islet cells are restricted to manual counting or semi-quantitative methods for number of clusters (19, 20). These conventional methods count variable size clusters as one spot, neglecting the possible greater cell numbers in larger clusters, as well as suffering from operator variability. MR sequences for positive contrast rather than hypointensity for iron-loaded cells have been the subject of intense research, as positive contrast methods improve the detection of iron oxide labeled cells and may help to solve quantification difficulties. A standard positive contrast method (21–24) has not yet emerged.

Ultrashort echo time (UTE) imaging (25, 26) has been proposed as a technique for enhancement of localized regions of iron uptake (27) and a 3D radial UTE sequence has been reported for brain and skeletal muscle imaging (28, 29). To the best of our knowledge, this is the first report of 3D-UTE for positive contrast of iron-oxide labeled islet cells in vivo. We introduce a new automated quantification method for in-vivo islet cell monitoring based on UTE. The potential advantages of this novel AQUTE method (Automatic Quantitative Ultrashort Echo Time imaging) are reported and the sequence is validated on 3 levels: correlation of quantification with number of IEQs injected; agreement between manual counting and thresholding in difference UTE (dUTE) to ensure accuracy of the new positive contrast quantification method; and imaging of iron oxide labeled islet cell graft persistence in a non-rejecting rat model.

Methods

Animals

Sprague-Dawley (SD) rats (300–500g) were purchased from Janvier (Le Genest, France) and used as islet donors and recipients. SD rats are outbred animals, and therefore mild rejection may occur in the SD-to-SD combination. Animals were kept in our institutional animal facilities with constant access to food and water. All procedures were performed under protocols approved by the institutional animal care committee and state veterinary authorities.

Islet isolation and labeling

Islets were isolated by collagenase digestion of the pancreas, Ficoll purification (30), and culture in Dulbecco's Modified Eagle Medium (1mM sodium pyruvate, 10% fetal calf serum, 11mM glucose, 100U/mL penicillin, 100mg/mL streptomycin; Gibco, Switzerland). A clinically-approved SPIO contrast agent, (carbodextran-coated ferucarbotran nanoparticles, Resovist® Schering, Switzerland) was chosen according to our previous studies (9) for incubation (37°C, 24 hours, 5% CO₂ atmosphere, 250 µg iron/ml), followed by 3 culture medium washes and re-suspension in 3ml aliquots. Islets had a purity >80%, limiting non-endocrine labeling (12). Labeled islets were used immediately, as in the clinical protocol.

Islet transplantation

Recipients (n=14, 3 per group) were transplanted with 500, 1000, 2000 or 4000 islet equivalents (IEQs) plus 2 sham saline injection controls. 2000 IEQs is equivalent to 5000 IEQ/kg, representing a clinically relevant ideal. The transplant protocol was optimized for labeling and graft efficiency (9). Intraportal transplantation was via 22-G catheter (Optiva2, Johnson & Johnson, Switzerland) under anesthesia (Isoflurane®, Abott AG, Switzerland) after median laparotomy. A fibrinogen/thrombin sponge (TachoSil®, Nycomed, Switzerland) secured haemostasis of the portal vein.

Imaging

During imaging, animals were anesthetized (Isoflurane®, respiration rate 40/minute). Images were acquired on a clinical 3T scanner (MAGNETOM Trio, a Tim system, Siemens AG, Germany) using a wrist coil, the rat placed feet-first prone. Scanning was at days 1, 8 and 15 after surgery with long-term follow-up of a sub-group at 62, 104 and 146 days (n=6, 4 and 3 respectively – see table 2). Other animals from each group were removed for histological study.

The novel AQUETE method (Automatic Quantitative Ultrashort Echo Time imaging) comprised a dUTE acquisition and automatic threshold quantification. The dual-echo UTE sequence includes a 60µs non-selective RF pulse, 40µs switch delay and 100% asymmetric acquisition from the centre of k-space with read-out during the gradient ramp-up (28, 29) with 3D isotropic resolution 0.375mm (in-plane and slice thickness), 12cm FOV, 35000 radial projections, echo times TE(1)/TE(2) 0.07ms/5.7ms, repetition time TR 9.6ms and 10° flip angle (FA). Subtraction (dUTE) gave quantifiable positive contrast for AQUETE. Respiratory triggering used a pressure pad system (SA Instruments Inc., USA; 8 minutes including respiratory pauses, efficiency 75%). A conventional 2D-GRE (gradient-echo) signal loss sequence (TE/TR/FA = 7ms/1490ms/40°, 1 signal average, 3 minutes) was acquired with the same pixel resolution, but a minimum 2mm slice thickness.

Image Analysis

Signal-to-noise ratio (SNR) was calculated as (mean signal)/(noise standard deviation), and mean cell-to-liver contrast (CNR), in relation to the liver signal, as:

$$\frac{SNR_{liver} - SNR_{islet}}{SNR_{liver}}.$$

Average values, taken for three islet clusters and liver regions (day 8 and 15) for each IEQ (n=24) are given in Table 1. The same regions were averaged over several animals and time points to give a mean representation of signal intensity and contrast proved by this sequence in relation to the validated 2D protocol. This was not intended as a serial comparison.

Quantitative assessment for comparison of IEQs used threshold measurement of the number of pixels containing hyperintense dUTE signal. An intensity threshold was applied to the liver avoiding the vena cava (Osirix Open source <http://www.osirix-viewer.com/>, Matlab, MathWorks, USA). The threshold value was defined as the mean liver signal plus 50 units, corresponding to approximately double the intensity. This threshold lies between the maximum intensity of background liver and the labeled islet cells.

Histology

Histology was carried out on all animals at time of sacrifice. Under general anesthesia, the liver was perfused with NaCl 0.9% via the portal vein and fixed with intra-portal perfusion of 10% formalin. Dehydration was performed in successive ethanol baths, followed by 100% Xylol bath prior to paraffin embedding. Liver sections were cut in the same plane as the MR image orientation and stained for insulin (Guinea-pig antibody anti-insulin A564, Dako, Switzerland) and iron (Prussian Blue, Sigma, Switzerland).

Statistics

Statistical analysis used SPSS software (SPSS Inc. 17.0, Chicago, IL). Results were expressed as mean $\pm$ SEM (standard error of the mean). Variables were compared with the one-way ANOVA test where appropriate. Correlation was tested by determining the Pearson correlation coefficient R and Fisher slope comparison (all animals and time points) with statistically significant difference defined as $p < 0.05$.

Seven studies' regions were redrawn on original images to test repeatability. Statistical analysis for this included calculation of the correlation between the two sets of data and a Bland-Altman plot.

Results

Islet imaging using 3D-dUTE

3D radial UTE imaging provided isotropic resolution with high image quality without any artifacts in all rats for both TE(1) 0.07 and TE(2) 5.7ms as shown in Figures 1 and 2. The ultrashort TE sequence provided high-resolution images suitable for anatomical delineation of the liver. The small vessels did not change signal intensity between the two echoes, unlike the iron-loaded islets that appeared dark and clearly different from the high intensity liver parenchyma only at TE(2). The dUTE series demonstrated bright spots corresponding only to islets as small liver vessels were suppressed. Figure 1 shows coronal 3D ultrashort TE(1), 3D TE(2) and dUTE images with maintained anatomical information, background suppression and positive islet contrast. A complete 3D series is provided in supplementary material. Due to the isotropic resolution, spherical clusters could be identified with

confidence in all 3 orthogonal planes, over multiple slices. Cluster size varied throughout an individual, but clusters were generally observed in 2–3 slices indicating a diameter of 0.7–1mm.

Comparison of image quality: 2D-GRE vs 3D-dUTE MR sequences

The validated in-house protocol is used to compare with the proposed protocol. Signal measurements for 2D-GRE and 3D-UTE images are presented in Table 1. The level of the background liver signal (SNR) was less uniform on the 2D-GRE MR sequence than dUTE, with dUTE also providing higher contrast (CNR). Out of 15 slices for the 2D-GRE sequence, at least 3 slices in all animals showed severe motion artifacts near the heart and signal loss close to the intestines. These artifacts, not present with the 3D-dUTE sequence, precluded islet identification (Figure 2). In addition, with 3D acquisition, the partial volume effect was significantly reduced as up to 70 thin slices are obtained over the liver region.

Partial volume effects increased with slice thickness causing blurring even when in-plane resolution was kept constant. In terms of spatial resolution, the 3D-dUTE MR sequence had a clear advantage over the 2D-GRE MR sequence as in the latter the slices were thicker than the cluster diameter and did not allow reformatting for registration of serial images. Comparison was by visual counting (the only possibility for the 2D-GRE sequence) therefore with possible operator variability. The visual counting of the clusters on both the 3D-UTE and 2D-GRE sequences was linearly related ($y = 0.999x + 2.43$, $R^2=0.98$, $p<0.0001$) with a slope value indicating that the same number of islets was counted by both sequences. Over 60 slices, there were 42–76 clusters, for 1000 IEQs transplantation. A more accurate count of smaller clusters was expected with the higher resolution of the 3D dUTE sequence, however, 2D-GRE could have a higher count due to other hypointense species giving false clusters.

Islet quantification with AQUTE

AQUTE provided quantification, by automatic thresholding, of high contrast dUTE images with uniform background suppression. This pixel threshold method gave a physically relevant value related to the labeled islet cell volume when summed over isotropic resolution 3D images. Figure 2 illustrates the AQUTE quantification method for total islet volume (the total number of pixels enhanced over the whole liver, representative of the total number of iron-labeled islet cells), and the relative advantages of 3D-dUTE over the traditionally used 2D-GRE signal loss images. Comparing a visually similar slice (Figure 2), even on relatively good quality conventional signal loss 2D images, approximately a quarter of the thresholded pixels originated from non-uniform background and hypointense vessels with the 2D-GRE sequence by comparison to the 3D-dUTE. Due to the varying high intensity background with the 2D-GRE sequence, it was also not possible to choose a standard threshold value, with slices containing motion artifact.

There was a significant correlation between the total islet volume measured by **AQUTE** and the manual count for the 3D-dUTE sequence for days 8 and 15 combined ($R^2=0.82$, $p<0.0001$) despite manual counting not quantifying the size of clusters and becoming very difficult and time consuming at high IEQ. A divergence at high values of IEQ was due to increased difficulty of manual counting. Table 2 gives individual values, correlations and statistical significance of agreement for these analyses and the following. Comparing the mean quantification by AQUTE, there was also significant difference between groups of 0, 1000, 2000 and 4000 IEQs by ANOVA ($p<0.0001$). The number of hyperintense pixels measured by AQUTE correlated linearly with the number of injected IEQs over all rats, on days 8 and 15 combined ($R^2 = 0.898$, $p<0.0001$) as shown on Figure 3.

Islet follow-up

Clusters could be monitored over time (Figure 4i–iv), including for long-term follow-up with a recognizable pattern of islet distribution. The signal within islet cells persisted and was relatively constant over the first 3 time-points from day 8 to day 62 (Figure 4v). Regressions between AQUTE measurements and injected IEQ were used to confirm consistency in quantification at different time points. As reported in Table 2, there was no difference between the slopes measured at day 8, 15 or 62 (Fisher test $p > 0.16$). These results were confirmed within both AQUTE measurements over time and for the manual counting comparison of the 2D-GRE and 3D-UTE sequences as reported in Table 2.

Reproducibility

Liver manual contouring is the only dependant parameter of the AQUTE method. It was repeated to assess the variability of the method. The reproducibility of the region drawing on the AQUTE threshold method is illustrated by a strong correlation between two independent sets of measurements ($y = 0.96x + 27.3$, $R^2 = 0.994$, $p < 0.0001$), with a slope close to 1 and with no significant difference between paired data ($p = 0.27$, $n = 7$). A Bland Altman plot is shown in Figure 5. The variability between measures was 7.5% (± 5.5).

Histology

Histological analysis was performed on liver sections after in vivo imaging in order to ascertain the concordance of MRI spots and iron-labeled transplanted islets (Figure 6). Iron (Prussian blue stain) was only present within or around the islet cells, and that insulin production (immunohistochemistry) was still functioning within marked cells. In particular, no iron was seen without an islet cell. Location of islets corresponded to MRI. Statistical correlation was not attempted, however, histological results confirmed the previously reported validation (9).

Discussion

To the best of our knowledge, this is the first report of 3D-UTE for positive contrast of iron-oxide labeled islet cells in vivo. The advantages of 3D radial UTE imaging are high contrast, uniform background and the absence of motion artifacts enabling automated quantification of iron loaded cells as demonstrated on a longitudinal study of islet cell transplantation in rodent. The AQUTE method is efficient on 3 levels: correlation with manual counting and with IEQ, as well as demonstration of persistent quantification over time even at high IEQ numbers.

UTE retains anatomical information in fully registered multiple echoes. It is less sensitive to large susceptibility or fat/water shift artifacts (25). The acquisition of two echoes, varying only in the signal from the iron labeled cells allows positive contrast from the labeled cells on a homogeneous background. UTE provides signal from the shortest $T2^*$ species, and dUTE intensity is linear with iron concentration (27) in contrast to signal loss images where there is no clear correlation between the level of signal loss and the iron concentration. The proposed AQUTE method is based on the difference image, dUTE, suppressing the entire background signal, automatic thresholding of the islet cell clusters and quantification with time of positive contrast while maintaining the anatomical information in the original images. Second echo times can be adjusted to avoid signal saturation as there is no initial signal loss in the UTE image and the difference image is therefore not merely an inversion of a negative contrast image. Therefore, UTE appears to be the sequence of choice for imaging of iron oxide labeled cells. As an added advantage, the radial imaging technique necessary for the ultrashort echo has a diffuse distribution of artifacts, without wraparound,

making it robust to flow and motion, and ideal for small field-of-view (FOV) demonstrated by comparison with a standard 2D-GRE sequence.

AQUTE provides a simple automatic thresholding of uniform background suppressed, high dUTE images with high contrast between islet cells and the rest of the liver and vessels, for quantification of the total size of all clusters. Previously published work mainly includes manual counting of clusters (17, 31), but attempts have been made at developing quantification of hypointensity. Percentage signal loss is described in mice, though this would not be applicable to the sparse distribution in patients or models with fewer cells (32, 33). Jiao et al. (34) describe calculation of 'iron volume' from differences in relaxation time and diameter of hypointense spots, serially assessing single clusters in normal and diabetic rats. Unlike our study, however, this suffers from difficulty identifying islet clusters among other hypointense signals. Thresholding enables quantification of grouped clusters and avoids difficulties at high IEQ. Automation reduces operator input, and sources of variability. Variable cluster size and distribution is accounted for. Complex classification methods should also be efficient with the 3D-UTE images, but require more analysis than a simple intensity threshold and would enable further study.

The advantages and limitations of other positive contrast methods for iron oxide have been described (21–24). Limitations include loss of anatomical information, complex reconstruction and post processing techniques, and significant artifacts and sensitivities to perturbations in the magnetic field. UTE has been reported in relatively large regions of iron oxide uptake in tumors (35), though in this case the method is reported as modification of relaxation time calculation rather than a positive contrast method. Positive contrast has been reported only in-vitro, in endothelial progenitor or cancer cells, with 2D imaging, or with anatomical distortion (36–38).

As demonstrated by the absence of difference in the correlation slope of quantification with different sequences and timepoints, there was little change over long-term follow-up. However, one animal (500 IEQ) had sudden islet loss (2 months) and one animal (4000 IEQ) that showed an important decrease at 3.5 months. As the rats are not inbred (not true syngeneic model) some slight rejection is expected in the first weeks. It is possible that clusters may move at early times within the liver and 2 clusters may separate giving a higher count at later times. Clusters could be multiple in a small area, but always unique in a portal space. The distribution is random over the liver. The percentage of the total transplanted islets detected is $9.3\% \pm 3.42$ according to our previous study (9). As reported on Table 2, quantification shows reasonable agreement with Jiao et al. who described 62.5 clusters for 1000 injected IEQs (34). The 3D-dUTE images show islet distribution over the whole liver, with no preference for a single lobe. Apart from 2 animals, islet persistency and the longevity of iron within living cells in this model are well demonstrated by the long-term follow-up. Even though there is good correlation of pixels to IEQs, intragroup variation may be explained by labeling and transplantation efficiency. Factors known to affect islet survival after transplantation are known (39–44). Damage during the isolation or transplant procedure, ischemia-reperfusion-like injury, acute inflammation, non-regeneration and exhaustion are possible explanations for loss. Iron toxicity and immunity play no part in this model. Serial imaging may provide an early indication of partial graft loss, as suggested by a recent report (45). Once detectable changes in insulin occur, it is already too late to save the graft by immunosuppressant therapy.

Histology verifies that the clusters seen in both 2D-GRE, and 3D-dUTE images are due to the labeled islet cells, normal liver or surgical artifact. Iron was only found in and around viable transplanted islets in agreement with a previous study (9, 45). Only a portion of intra-hepatic islets presented iron inside the cells or in the islet stroma. Free contrast agent

injected into the liver has been shown, in the case of Resovist, to clear over a few days, therefore any iron released from non-viable cells would quickly be eliminated (9). We can therefore conclude that positive signal in the images corresponds to iron in functioning cells.

Quantification using AQUTE is non-zero for control animals and is also indicated by the bias in the intercept values. This results from noise/artifact pixels that are high intensity and included in the threshold, but are not spherical islet cell cluster structures. However, spurious hyperintense pixels are <0.5% of the total and less than intragroup variation. Work-in-progress includes combination with automatic spherical segmentation combining cluster count and size to correct for false positives. Despite this systematic error, consistency of both IEQ number and temporal reproducibility, makes follow-up studies highly feasible. The volume of the signal disturbance from iron, though closer to the actual volume than in conventional signal loss imaging, will be larger than the physical volume of the islet cluster. The threshold volumes will be proportion to the volume of islet, thereby allowing quantification and correlation, but calculating the number of islets of 150 μ m diameter in relation to the cluster image diameter of around 1mm, will over-estimate the absolute number.

One limitation of this study resides in the fact that there is no direct count of individual islets, but quantification of clusters. Due to high numbers of islets and slice thickness differences, it is impossible to get a quantitative histological comparison of the complete distribution. Analysis for total iron content is difficult due to the small percentage of iron label within the liver. On cell death, iron may be metabolized to a non-paramagnetic form that is not imaged, but would still be found by elemental analysis.

This is a sequence validation study before moving on to study of more complex and realistic diabetic and rejection models to track islet loss and the efficacy of anti-rejection treatments. As previously published (17), human studies with labeled islets were possible at 1.5T. A limitation of the technique was the lack of MR sequences allowing quantification. The performance of these studies using clinical-grade contrast agents and readily available clinical 3T scanner for experimental rodent imaging shows translational potential.

Automatic quantification within suppressed background is widely applicable e.g. stem cells and molecular imaging. Larger, irregular regions of contrast uptake (e.g. atherosclerotic plaque (27)) could also benefit. Actual iron loading from signal intensity is beyond the scope of this paper and is a work-in-progress. Although pixel numbers and their intensity are available, a combined segmentation and threshold technique is needed to link intensity to size of cluster.

In conclusion, dUTE gives high-resolution 3D anatomical images with suppressed background and high iron contrast. AQUTE allows automatic quantification of iron oxide-labeled islet cells in serial images. Comparison with 2D signal loss imaging and histology show feasibility and reproducibility suited to more complex models. We observe correlation with IEQ for assessment of graft success and survival with the persistence of islets over several months. We believe that the correlation observed is representative of the signal quantified representing a total islet cluster volume in the transplants and that it can be used to assess initial success and follow long-term outcome of labeled transplanted islet cells. We would like to stress the advantages of a new sequence and the possibility of thresholding the images to allow more automated quantification and correlation. This quantification tool is designed to fit into a multidisciplinary diagnostic protocol to monitor and treat graft outcomes with minimally invasive procedures, while there is still time to salvage a failing graft.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AQUTE	Automatic Quantitative Ultrashort Echo Time imaging protocol
MRI/MR	Magnetic Resonance Imaging/Magnetic Resonance
IEQ	Islet Equivalents
UTE	Ultrashort Echo Time imaging
dUTE	Difference Ultrashort Echo Time imaging
SD	Sprague Dawley
RF	Radio Frequency
TE	Echo Time
FOV	Field Of View
TR	Repetition Time
FA	Flip Angle
GRE	Gradient Echo
SNR	Signal-to-Noise Ratio
CNR	Contrast-to-Noise Ratio

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Figure 1. Difference ultra-short image (dUTE) principle

Cartoons and images show the principle of dUTE imaging. In vivo images show the whole FOV acquired with the liver in the centre of the image (outlined on the second echo in white and with arrow)

The simultaneously acquired dual contrast 3D radial sequence with ultrashort first echo TE(1) (i), longer echo TE(2) (ii) and dUTE with positive contrast (iii). Positive contrast on background suppressed liver is obtained in conjunction with maintained anatomical information in the individual echoes. Parameters include: Coronal images in vivo. 3D isotropic resolution 0.375mm, 12cm FOV, 35000 radial projections, TE(1)/TE(2) 0.07ms/ 5.7ms, echo spacing 9.6ms (70–110 segments depending on respiration) and optimized 10° flip angle. Subtraction of the two echoes gives dUTE images with positive contrast from labeled cells. Respiratory triggering, pausing during the short inhale/exhale, used a pressure pad and external trigger with trigger delay 150ms.

Figure 2.

Comparison between 3D difference ultra-short image (3D-dUTE) and 2D gradient echo (2D-GRE) MR sequences

In all the images, the rat liver contours are delineated by a white line drawn from the corresponding second echo of the 3D-UTE images. The column (i) demonstrates clarity and background level towards the top of the rat liver for both MR sequences, showing absence of artifacts with 3D – dUTE images (upper). The 2D-GRE images (lower) shows partial

volume for islets (arrow a), vessel flow (arrow b), motion blurring (arrow c) and slice thickness partial volume (arrow d). Threshold of enhanced pixels shows that vessel and low signal region of liver (arrows) are not highlighted in 3D-dUTE images (as seen in row ii), unlike conventional 2D-GRE images (as seen in row iii) where unwanted pixels account for an extra 25% on the resulting threshold value. The two regions are identified as artifact due to their size and shape in 3D but not in 2D. Figure 2 iv shows the box plots of the mean signal intensity of vessels, islets and liver parenchyma on 2D-GRE images (lower) and 3D-dUTE images (upper). It is rather impossible to choose an appropriate simple threshold value on the 2D-GRE image that captures only islet signal. MR images parameters are as in Figure 1.

Figure 3.

Correlation of the number of pixels detected by automated threshold results from Automatic Quantitative Ultrashort Echo Time imaging protocol (**AQUTE**) with the number of IEQs injected. AQUTE gave a physically relevant value related to the labeled islet cell volume successfully transplanted in the liver that was significantly correlated to number of injected IEQs ($R^2 = 0.898$, $p < 0.0001$). The threshold value used by AQUTE for quantification was defined as the mean liver signal plus 50 units, corresponding to approximately double the intensity.

Figure 4.

Visual consistency in position of clusters over the difference ultra-short images (dUTE) at days 1(i), 8(ii), 15(iii) and 104(iv). A single axial slice from the 3D-dUTE images for the first 3 time points and an oblique reconstruction for the follow-up at day 104 shows the same 5 clusters, with zoom of identified group. The persistency of the islet detection is further demonstrated in (v) by the quantification of all islet cells using Automatic Quantitative Ultrashort Echo Time imaging protocol (AQUTE) in initial 3 weeks. The linear trend line shows both persistence over time and differences between IEQ numbers. Mean AQUTE pixel number for each injected IEQ are reported with trend line and standard deviation error bars.

Figure 5.

Reproducibility of the Automatic Quantitative Ultrashort Echo Time imaging protocol (AQUTE). Bland Altman plot shows an excellent agreement between 2 repeated region drawing for the automatic threshold **AQUTE** in a subgroup (n=7) randomly selected from all animals and time points to illustrate the reproducibility of the liver region drawing before the automatic quantification. Plot shows mean difference between two independent measurements ± 1.96 standard deviations of the difference.

Figure 6.

Iron loading of functioning islet cells by histology with 2000 IEQs. 3D difference ultra-short image (3D-dUTE) image of islet is shown (i) in with the corresponding histology zoomed to same region of interest in (ii). Prussian blue stain in (iii) shows that iron uptake is within islet cells in a further zoom. The insulin antibody staining demonstrates a good correlation between insulin production from living islet cells to the iron oxide stain in (iv).

Table 1

Signal-to-noise ratio (SNR) and mean cell-to-liver contrast (CNR) showing the advantages of 3D-dUTE over 2D-GRE and the individual echoes. Mean values are given with standard deviation in parentheses, and statistical analysis by ANOVA.

	mean(s.d.)		
	SNR islets	SNR liver	CNR
3D ultrashort TE(1)	27.9 (3.4)	29.1 (2.0)	-0.05 (0.1)
3D TE(2)	12.9 (3.7)	24.2 (2.2)	-1.0 (0.6)
3D-dUTE	15.7 (3.1)	5.0 (1.4)*	2.2 (0.2)[†]
2D-GRE	27.8 (8.3)	49.2 (5.5)*	-0.4 (0.2) [†]

n=24 (3 regions per animal)

* As compared to 2D-GRE, the liver signal with 3D-dUTE is associated with low background and low variability (lower SNR mean and standard deviation, closer to noise)

p<0.0001 showing a significant difference between liver SNR for the 2 sequences. % variance/mean = 61% for 2D-GRE and 41% for 3D dUTE

[†] As compared to 2D-GRE, 3D-dUTE is associated with high cell-to-liver contrast p<0.0001 showing a significant difference between CNR for the 2 sequences.

Islet quantification using Automatic Quantitative Ultrashort Echo Time imaging protocol (AQUTE) and manual counting at different time points and for different injected IEQs.

Table 2

	dUTE					dUTE		2D GRE	
	AQUTE (pixel threshold)					manual count		manual count	
	day 8	day 15	day 62	day 104		day 8	day 15	day 8	day 15
INDIVIDUAL VALUES									
						∩	∩	∩	∩
Injected IEQs	#	#				∩	∩	∩	∩
0 a	904	1331				2	1	4	3
0 b	1799	1259				1	2	0	2
500 a	1964	2475				13	11	19	9
500 b	4295	2004	1679	2100		19	24	29	24
500 c	4247	2960				24	22	22	35
1000 a	4000	4569				49	45	42	44
1000 b	5578	4506	1923			61	66	60	76
1000 c	5327	4506	2862	2163		42	42	48	48
2000 a	3467	7565				65	73	70	61
2000 b	8773	6555	5939			77	79	83	71
2000 c	8801	7142	3813	4427		97	103	129	121
4000 a	8421	8307				160	152	164	162
4000 b	8780	11440				77	59	58	70
4000 c	12772	9237	8524	3479		131	139	130	119
REGRESSION WITH IEQ									
slope	1.99*	2.07*	2.02*	1.65		0.031	0.028†	0.028†	0.027†
intercept	2450	1951	583	968		10.9	13.2	16	16.4
R2	0.854	0.951	0.957	0.608		0.904	0.859	0.823	0.834
P	1.02E-04	1.82E-07	3.00E-03	3.92E-01		8.91E-05	8.20E-05	2.97E-04	2.09E-04

For each number of islet equivalents, the individual animals with the same IEQ are labeled a, b and c for tracking over the course of the experiment.

Comparison of individual values, by day, sequences and methods by ANOVA with no significant difference between day 8 and day 15 for dUTE threshold (#, $p = 0.764$), for dUTE manual ($\therefore$, $p = 1$), for 2D GRE manual ($\perp$, $p = 0.96$) and between all 4 manual counts ($\cap$, $p = 0.998$).

As values within a particular column vary significantly (with IEQ) the regression slope was also compared for no statistical significant difference.

- * $p > 0.16$ no significant difference between threshold vs IEQ slopes (pairwise Fisher slope comparison at all time points up to day 62)
- † $p > 0.21$, no significant difference between all manual counting vs IEQ slopes (pairwise Fisher slope comparison between both timepoints and both 2D GRE and 3D dUTE sequences)