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Samuel Harrison Schlager March 31, 2016

# The Validation of DECT as a Quantitative Measure of Gaucher Infiltration

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2016

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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Gaucher's disease is one of the most common orphan diseases affecting approximately one in 50,000 people born by conservative estimations. The cells of individuals afflicted with Gaucher's disease have dysfunctional β-glucocerebrosidase, which leads to a buildup of the substrate glucosylceramide in the cell. This primarily affects the liver, spleen, and bone marrow in individuals with the less severe type 1 classifications, while neurons are also affect in the more severe type 2 and 3 classifications. Treatment options are available for type one individuals, but there currently is no quantitative diagnostic assessment of infiltration of Gaucher cells in tissues. Most radiological assessments use MRI in a semi-quantitative fashion to assess Gaucher's disease. However, semi-quantitative have a limited diagnostic capacity. Here we propose and validate that DECT is a more precise diagnostic alternative with quantitative abilities through the use of phantoms as well as genetically modified mouse livers and spleens. Ability to distinguish between red and yellow bone marrow using DECT was also attempted though those results proved inconclusive.

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## Introduction

### *Gaucher's Disease*

Gaucher's disease is one of the most prevalent orphan diseases effecting one person in 50,000 being born with the disease (Gruber et al., 2011). It is one of the best-understood monogenic disorders that is the result of the negative affects of over 300 mutations of the GBA gene. This leads to a variety of problems related to glucocerebrosidase's catalytic function, intracellular stability, and subcellular trafficking (Grabowski, 2008). The phenotypic result of the GBA mutation is a deficiency of the enzyme β-glucocerebrosidase. Gaucher's always presents as an autosomal recessive disorder where the deficiency in β-glucocerebrosidase results in the buildup of enzyme substrate glucocerebroside builds up primarily in the cells of the liver, spleen, and bone marrow inhibiting the function of these tissues (Guggenbuhl et al., 2008). Individuals with Gaucher's disease often display anemia, thrombocytosis, bone structure issues, hepatosplenomegaly, and sometimes neuro-involvement (Grabowski, 2008).

While there are over three hundred variants of the GBA mutation, clinically there are three relevant types of Gaucher's disease. Type one is characterized by an absence of neurological involvement and by far the most common type (Muller et al., 2010). Types two individuals often present with severe neurological involvement and as a result die in early infancy, usually before two years of age due to complications associated with neural death. Type three individuals display neurological symptoms as well but tend to be less severe, and usually survive into mid adulthood (Grabowski, 2008). The most common genetic mutations affecting

type one individuals, as reported by the International Collaborative Gaucher Group Registry, are the N370s, L444P, and 84GG substitutions (Nagral 2014). Interestingly the N370s and L444P variants have been linked to Parkinson's disease (Yu et al., 2015). The relationship between Gaucher's disease and Parkinson's disease is not fully understood but more research into the mechanism of Gaucher's disease could provide valuable information into the pathology of Parkinson's disease.

*Enzyme Replacement Therapy and Substrate Reduction Therapy*

At the moment the most common treatment options for individuals with enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). Enzyme replacement therapy consists of the purification of functional β-glucocerebrosidase and an intravenous injection of the enzyme to be absorbed by affected cells (Barton et al., 1991). The enzyme is then able to travel to the lysosome of the affected cells and restore proper function. Substrate reduction therapy utilizes a drug, administered orally, which prevents the initial steps of the β-glucocerebrosidase pathway so no substrates ever form (Platt and Jeyakumar, 2008). With these two treatments the disease has become manageable for type one individuals (Muller et al., 2010). However, there is still not a very good mechanism for radiologists to assess the progression of the disease, or the efficacy of the treatment. Very little is understood about how efficiently the current administrations of the therapy are. One study using Ceredase® found that the enzyme only had a half-life of about fourteen hours in the bone marrow (Jmordiak and Futerman, 2005). Furthermore, there is evidence that bone marrow infiltration may still be present in patients receiving ERT (de Fost et al., 2008) A better way for radiologists to assess the progression of the

disease and treatment is need to understand if this time frame is adequate for treatment of the pathology within the bone marrow.

The inability to specifically assess the progression of Gaucher cell infiltration has inhibited clinician's ability to assess their patients in any quantitative capacity. Similarly it has inhibited researchers from further analyzing the disease in vivo in humans through any radiological capacity. It is also apparent what benefits the further examination of the progression of the disease would have on further understanding the mechanism of the disorder.

*Currently Used Diagnostic Techniques*

While clinical treatments of Gaucher's disease have demonstrated a very real ability to manage the disorder in type one individuals, radiological imaging methods have not advanced concurrently in their ability to assess the progression of the disease or the efficacy of the treatment. The most common quantitative imaging methods for evaluating the involvement of bone in Gaucher's disease are dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) (Komninaka et al., 2015; Poll et all., 2002). DXA quantitative measures the bone mineral density but does not assess cellular changes in the bone marrow, where the Gaucher cells are. It also does not measure a real density nor assess trabecular bone architecture reducing its ability to predict true material strength (Bolotin, 2007). Due to the dynamic nature of Gaucher's disease, a generalized measurement of bone density may not be the best way to assess infiltration of Gaucher cells since Gaucher cell infiltration often leads to a reconversion of yellow bone marrow to red bone marrow as a compensation technique for anemic symptoms. While this leads to atypical marrow configurations in an afflicted individual it does not seem to show clear

density changes in any specific capacity. DXA is useful in measuring generalized osteopenia and changes in bone density as a result of extended ERT, but lacks the ability to measure local changes in Gaucher infiltration (Maas, Poll, and Terk, 2002). This limits the use of DXA to a very limited frame of reference.

MRI is more commonly used to assess Gaucher's disease. Quantitative chemical shift imaging is very sensitive but is not widely available and thus its use has been limited. Conventional MRI techniques using a T-1 and T-2 weighted spin echo sequence have only been able to assess Gaucher cell infiltrate based on an abnormally low signal intensity (Maas, Poll, an Terk, 2002). The issue in style of assessment is that it is only a relativistic way of assessment. It is semi-quantitative, not standardized, and poorly reliable. While conventional MRI does provide some information for clinical use, it is ultimately too vague of a technique to be implemented in any meaningful way in the assessment of Gaucher's disease. Thus, a truly quantitative assessment for measuring skeletal complications and treatment response in Gaucher patients is still needed.

*Proposed Radiological Assessment Techniques*

Infiltration of Gaucher cells changes the composition of bone marrow creating an opportunity for quantitative imaging methods. The amount of infiltration directly reflects skeletal involvement and reductions in Gaucher cells directly reflect treatment response. $^{1}$H MR spectroscopy ($^{1}$H MRS) and dual-energy CT (DECT) calculate bone marrow fat fraction from fat to water ratios. Changes in yellow marrow due to Gaucher cell infiltration can be measured.

Low amounts of yellow marrow reflect high infiltration and increasing amounts of yellow marrow over time suggests a successful treatment response.

A challenge is that Gaucher patients frequently develop anemia and thus conversion of yellow marrow to red. This is particularly applicable to imaging evaluations of children whose bone marrow is principally red at birth but with maturation converts to the adult yellow marrow distribution (Maas, Poll, and Terk, 2002). Routine MRI cannot differentiate red marrow from Gaucher cell infiltrates (Pastores and Hughes, 2000). Gaucher cells are composed of 10% protein, 10% cholesterol, 10% phospholipids, and 70% glycolipids. More than 90% of these glycolipids are glucosylceramide (Ebato et al., 1980). Red marrow is 40% fat, 40% water, and 20% protein; yellow bone marrow is 80% fat, 15% water, and 5% protein (Malkeiwicz and Dziedzic, 2012). Analysis of fat and water content presents an opportunity to quantify marrow infiltrated by Gaucher cells. MR spectroscopy and material-selective DECT may not only be used to quantify fat percentage but may be successful in differentiating Gaucher infiltrates from red marrow.

*Alternative Radiological Techniques*

With the understanding that semi-quantitative assessment MRI methods are inadequate for assessment of disease extent in bone and treatment response, we propose to develop and validate a quantitative assessment for measuring skeletal complications and treatment response in Gaucher patients. CT and MR imaging provide a promising ability to measure fat percentages in the bone marrow.

$^{1}$H MRS also provides an alternative radiological assay. $^{1}$H MRS utilizes a spectrum of magnetic pulses and analyzes the resulting signals from hydrogen protons. Previous studies have used $^{1}$H MRS has been implemented as a way to diagnose the infiltration of malignant cell types in red ventral bone marrow. This technique diagnosed the tissue type based on fat and water ratios (Kugel et al. 2001). Based on the principle of this method in assessing infiltration of malignant cells in bone marrow, there is a strong potential that Gaucher cells would be differentiable based on the same principle of fat and water ratios affecting the red bone marrow signal. Kugel et al. did find that there were age and sex related confounding variables associated with this study. This is problematic since Gaucher's affects a wide variety of individuals of different backgrounds. $^{1}$H MRS scans are limited to a single voxel, preventing extensive multi-dimensional analysis. It also is unable to detect bone marrow density (Bredella et al., 2015). For this reason CT seems to be a more promising diagnostic tool.

Quantitative Computer Tomography (QCT) is an incredibly informative radiological assay. The three-dimensional outline provided by QCT is able to further analyze bones in a way that DXA cannot. While both are able to measure the density of bones based on x-rays, QCT is also able to quantify bone marrow density within the skeletal envelope in association with bone strength (Lang, 2010). Given the rapidly adapting bone marrow conditions as well as the increased propensity for fracture in patients with Gaucher's disease, this style of analysis could provide great insight into the mechanistic working of Gaucher's disease. Additionally QCT offers a promising ability to differentiate between red bone marrow, yellow bone marrow, and Gaucher cells based on density as well as by analyzing the fat and water content. QCT is able to determine density and specific gravity of samples collected (Brooks et al., 1980). Since it takes a more involved three-dimensional x-ray scan than DXA it can be more localized, which could in

turn provide selective analysis that DXA simply cannot provide (Lang, 2010). Selective energy quantitative CT (SEQCT) could be implemented to differentiate between Gaucher cells, red bone marrow, and yellow bone marrow based on fat and water content. SEQCT is able to analyze tissues based on specific energy signatures such as water and fat (Glüer and Genant, 1989). Red bone marrow and yellow bone marrow have significantly differing fat and water contents so SEQCT should be able to differentiate between the two tissue types. Furthermore, previous studies have already demonstrated DECT can be used to measure marrow adipose tissue content as well as density (Brendella et al., 2015). Since Gaucher cells are characterized by the buildup of glycocylceramide, a glycolipid, DECT theoretically could differentiable red marrow, yellow marrow, and Gaucher infiltrate based on fat content.

*Gaucher's Disease Animal Model*

An important consideration for establishing a new method of analysis is developing a way to validate that method. Since Gaucher's disease is a rare condition, a proper animal model for Gaucher's disease is needed for further examination of the disease without extracting tissue samples from living and already hindered humans with the condition. Mice are the most well established animal model for Gaucher's disease. There are several well-established lines of mice, including D409H, V394L, and N370S point mutations, which present similarly to the human pathology of Gaucher's disease.

Mice present two primary obstacles when considering their use for radiological assays. The first is that mice have a very minimal amount of bone marrow that is easily extractable. Marrow must be collected from hollow tubular bones so as to limit the amount of extraneous

bone fragments found in the tissue, which would influence the density results. Mice simple lack a large enough amount of bone marrow to be used in any practical way. For this reason a mouse's liver or spleen work better as a source of Gaucher cells. Human liver and spleens have a specific gravity of 1.05 g/cm$^3$ and 1.06 g/cm$^3$ respectively (McCullough, 1975). Bone marrow has a specific gravity of slightly less than 1.06 g/cm$^3$ (Van Beusechem et al., 1992). Based on this both should be passable alternatives to bone marrow. The second consideration is how representative the mouse pathology is to the human pathology.

Mice with N370S, D409V, and V394L point mutations do have some pathological differences compared to humans. For example the N370S mutation in mice, which is analogous to a common human type one mutation, results in a skin permeability problem that results in death of the offspring within the first day of birth (Sun et al., 2011). While this is the most similar mutation to that of human Gaucher's disease, the skin permeability issue makes the pathology present very differently. The prolonged affects of Gaucher's Disease, such as the build up of glucosylceramide in the liver, spleen, and bone marrow, is never observed. This makes these mice poor representatives of the pathology of Gaucher's Disease. The D409V mutation seems to present similarly to Gaucher's disease but with too much enzymatic activity. For this reason it is often paired with a null allele to allow for the buildup of Gaucher cells. Similarly the V394L variant has been associated with a milder form of the disease that does not build up significant amounts of Gaucher cells (Sun et al., 2011).

Due to the need for the accumulation of glucosylceramide seen in the later stages of Gaucher's disease, the N370S mutant mice were not very good representatives for radiological assessment. Instead the D409V/null mice were selected based on their extended lifespan, accumulation of glycosylceramide in cells. The D409V/null mice live approximately two years.

This allows them the time necessary for Gaucher cells to develop and infiltrate the liver and spleen. D409V/null mice were also shown to have the highest accumulation of Gaucher's cells within their spleen and liver compared to any other variant (Sun et al., 2013). For these reasons, Sun et al. suggested the D409V/null mouse model was the most representative of the pathology of Gaucher's disease.

**Hypothesis**

A use of a quantifiable radiological assessment for the infiltration of Gaucher cells is evident based on the reasons discussed above. To improve the lives of Gaucher patients earlier detection of disease complications and more sensitive detection of treatment response is needed to more effectively determine who needs intervention, what kind and for how long. Advanced imaging techniques provide these capabilities. Currently, one of the most promising sites for assessment of Gaucher cell infiltration is in the bone marrow since it has limited exposure to enzymes provided by enzyme replacement therapy (Jmordiak and Futerman, 2005).

DXA and MRI are the currently used diagnostic tools, though they are limited in their ability to measure localized levels of Gaucher cell infiltration based on limitations with specificity (Mass, Poll, and Terk, 2002). For this reason CT is proposed as an alternative radiological assessment of Gaucher's disease based on infiltration of the bone marrow. It is hypothesized that localized DECT density measurements of tissues can accurately quantify disease burden in bone and treatment response in bone of adult Gaucher patients. Based on this hypothesis we predict that if localized DECT density measurements of tissues can accurately quantify disease burden in bone and treatment response in bone of adult Gaucher patients then it

should be able to distinguish accurately between red marrow and yellow marrow as well as other disease infiltrated tissues. Furthermore we hypothesized that fat to water ratio measurements of tissues will accurately quantify fat compositional differences between Gaucher infiltrates and other tissues. Based on this hypothesis we predict that if fat to water ratio measurements of tissues will accurately quantify fat compositional differences between Gaucher infiltrates and other tissues then DECT should be able to distinguish between different infiltrated tissues based on fat to water ratios.

## Materials and Methods

### *Validation of DECT with standards*

In order to validate the DECT technique, a standard was needed as a point of comparison. 8 ml polyethylene test tubes were loaded with mixtures of soybean oil and water. One tube was filled with 5 ml soybean oil and another with 5 ml of water. A beaker was filled 15 ml soybean oil and 5 ml water to produce a 3:1 ratio. Soy based Lecithin was added to the mixture to allow for the two substances to combine and the beaker was mixed using a magnetic stir vane for ten minutes. Once the mixture was homogenized, 5 ml of this mixture were then loaded into a polyethylene test tube. This process was repeated to produce a 1:1 oil to water mixture and a 1:3 oil to water ratio.

The tubes were loaded into a hard plastic aparatus, which has the same specific gravity as bone. This allowed the scanned images to resemble soft tissue confined inside tubular bone. The

samples were then scanned using GE 3T signa HD scanner and a Discovery CT750 HD scanner. This provided a point of comparison for the diagnostic capabilities of CT. Images were taken with a GE 3T signa HD scanner using standard knee protocols. The tubes were loaded laterally providing a small axis image. CT images were taken using a Discovery CT750 HD scanner using standard knee protocol. The tubes were loaded laterally providing a small axis image.

*Analysis of phantoms*

Once scanned the images of the phantoms were analyzed using the program Centricity software. Cross sections of the samples collected using MRI were analyzed based on density. The average density of the section was measured as well as the standard deviation based on centricity. The CT samples were also measured for density based on centricity. In addition to density, the CT scans also analyzed the samples for their water and oil content.

*Validation of DECT with marrow samples*

Red and Yellow bone marrow extracted from tubular bones of goats were analyzed to validate technique's ability to differentiate between the two types of soft tissue within the bone. Cross sections of goat legs were obtained from Georgia Halal Meat. The bones were frozen for preservation purposes. Before loading into the test tubes the bones were allowed to thaw for easier extraction. Then a sterile spatula was used to scoop out the marrow from the bone. Approximately 5 ml of red bone marrow and 5 ml of yellow bone marrow were extracted and placed in polyethylene test tubes. In addition to the goat samples, bovine yellow bone marrow

was also collected. Frozen cross sections of bovine femur were provided by farm burger. One large cross section was allowed to thaw and its contents were directly loaded into an 8 ml test tube. A sterile wooden skewer was used to allow the air to escape from the bottom of the tube while the sample was loaded. The samples were then placed into the plastic apparatus. Images were taken with a GE 3T signa HD scanner using standard knee protocols. The tubes were loaded laterally providing a small axis image. CT images were taken using a Discovery CT750 HD scanner using standard knee protocol. The tubes were loaded laterally providing a small axis image.

*Analysis of marrow samples*

The marrow scans were measured using the MRI and CT software. The MRI software looked specifically at the average density of the marrow samples and average density as well as the standard deviations of the data was collected based on the program. The CT data was analyzed according to density. Here the average density and standard deviation was also collected. In addition to density readings the oil and water content of the samples were also analyzed based on the program.

*Mouse model*

D490V/null mice were produced by back-crossing D490V/null mice with *GBA1* null/WT mice. Mice were stored in micro-isolators following the institutional guidelines under Cincinnati Children's Hospital Research Foundation Institutional Animal Care and Use Committee

(IACUC). Five individuals, one 380 days old, one 383 days old, and three 384 days old, were selected and sacrificed for organ extraction. Livers and spleens were collected from the mice after perfusion with saline, loaded into 8 ml polyethylene test tubes, and stored at -80 °C (as specified by Dr. Sun). Three wild type mice were also sacrifice and had their livers and spleens extracted. Two D490V/null mouse liver test tubes were loaded, each containing two liver masses from D490V/null mice. Additionally a single test tube containing one D490V/null mouse spleen was loaded. Similarly two wild type mouse liver test tubes were loaded, one containing liver masses from two separate individuals and one containing a single mouse's liver. Both tubes were similar in total liver mass contained. A single tube containing a wild type mouse spleen was also sent. Organs were then shipped to Emory University Orthopedic and Spine Center for imaging purposes.

*Imaging of mouse model specimens*

Spleen samples were loaded into the plastic apparatus and imaged side by side for comparative purposes. Similarly, all four test tubes containing liver samples were loaded next to each other for comparison. Images were taken with a GE 3T signa HD scanner using standard knee protocols. The tubes were loaded laterally providing a small axis image. CT images were taken using a Discovery CT750 HD scanner using standard knee protocol. The tubes were loaded laterally providing a small axis image.

*Analysis of mouse model images*

Images of the organs of the mouse model organs were examined using centricity. The program assessed the samples based on their densities. The density readings of the D490V/null spleen were compared to that of the wild type spleen. Similarly, the density readings of the D490V/null liver samples were compared to those of the wild type liver samples. CT images were separately examined for density readings. Again, the density readings of the D490V/null spleen were compared to that of the wild type spleen and the density readings of the D490V/null liver samples were compared to those of the wild type liver samples. Additionally, the CT software examined water and fat in the samples. Water and fat readings of the D490V/null spleen were compared to that of the wild type spleen. Water and fat reading were also used to compare D490V/null liver samples with wild type liver samples.

## Results

### *Phantom Trial*

The MRI results of the phantom trial show that a stepwise decrease in fat signal (T1) and increase in water signal (T2) as samples contained larger proportions of water compared to oil. The roughly linear progression of the phantoms, a negative slope in T1 (Fig 1C) and a positive slope in T2 (Fig 1D) provided evidence that the machine's diagnostic software was working properly. The large error bars show the limitations in MRI's quantitative ability to diagnose density and fat content. This is why most MRI is only done in a semi-quantitative fashion (Maas, Poll, and Terk, 2002). In both trials the three part water one part oil tube was removed from the data set based on an inability for the water and oil to remain miscible.

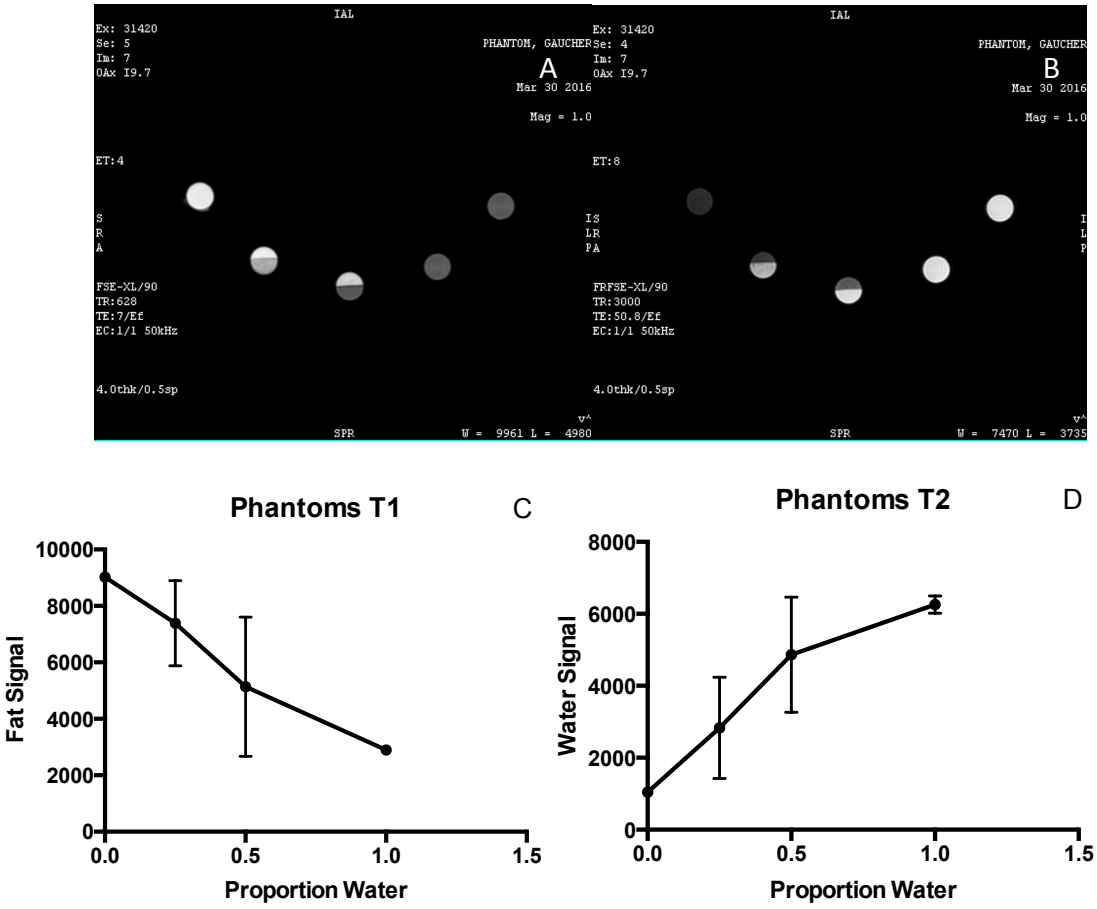


**Figure 1. MRI phantom scan:** Test tubes were loaded from left to right with all oil, ¼ water, ½ water, ¾ water, and all water. MRI images of A) a T1 weighted scan examining fat signal and B) a T2 weighted scan examining water signal. C) Fat signal values based on signal intensity of the T1 test and D) water signal values based on signal intensity of T2 test

The DECT results provided a much cleaner difference in water content based on density readings. A clear linear progression was established based on density readings (Fig 2B). Compared to the MRI results for both T1 (Fig 1C) and T2 (Fig 1D), which had very large areas of uncertainty, the CT provided a much more definitive point based on imaging results. CT data

concerning fat to water ratios were not consistent with the phantoms used. Due to this, fat analysis with CT was not done on any other samples.

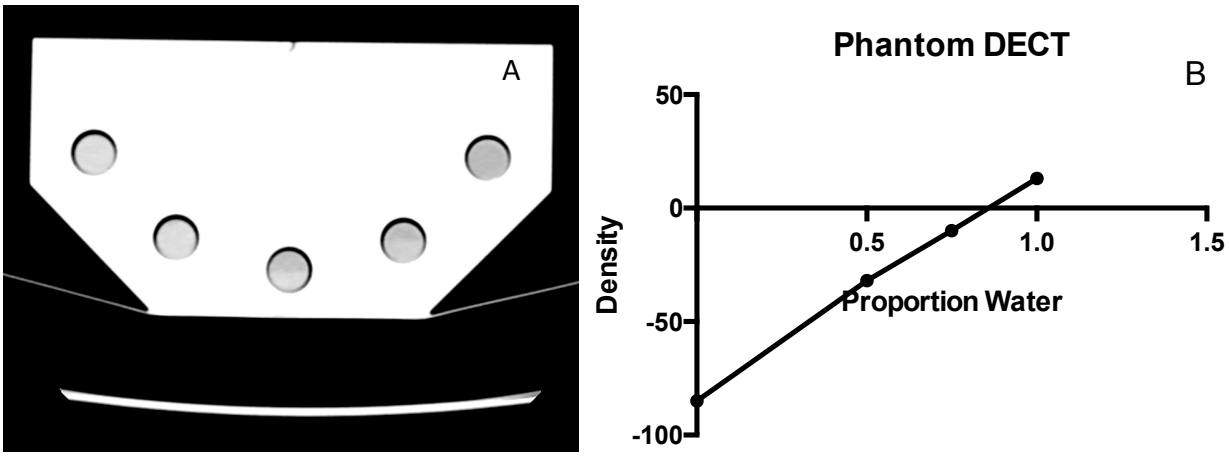


**Figure 2. DECT phantom scan:** Test tubes were loaded from left to right with all oil, ¼ water, ½ water, ¾ water, and all water. A) Image of DECT with signals and B) density values based on DECT scan

*Marrow results*

The marrow analysis was inconclusive for both the T1 (Fig 3A) and T2 (Fig 3B) weighted scans. Neither scan was able to provide a significant difference based on fat or water content. The cow marrow appeared much lower than expected on both assays indicating that the freezing process may have impacted the tissue. For this reason cow marrow results were not included

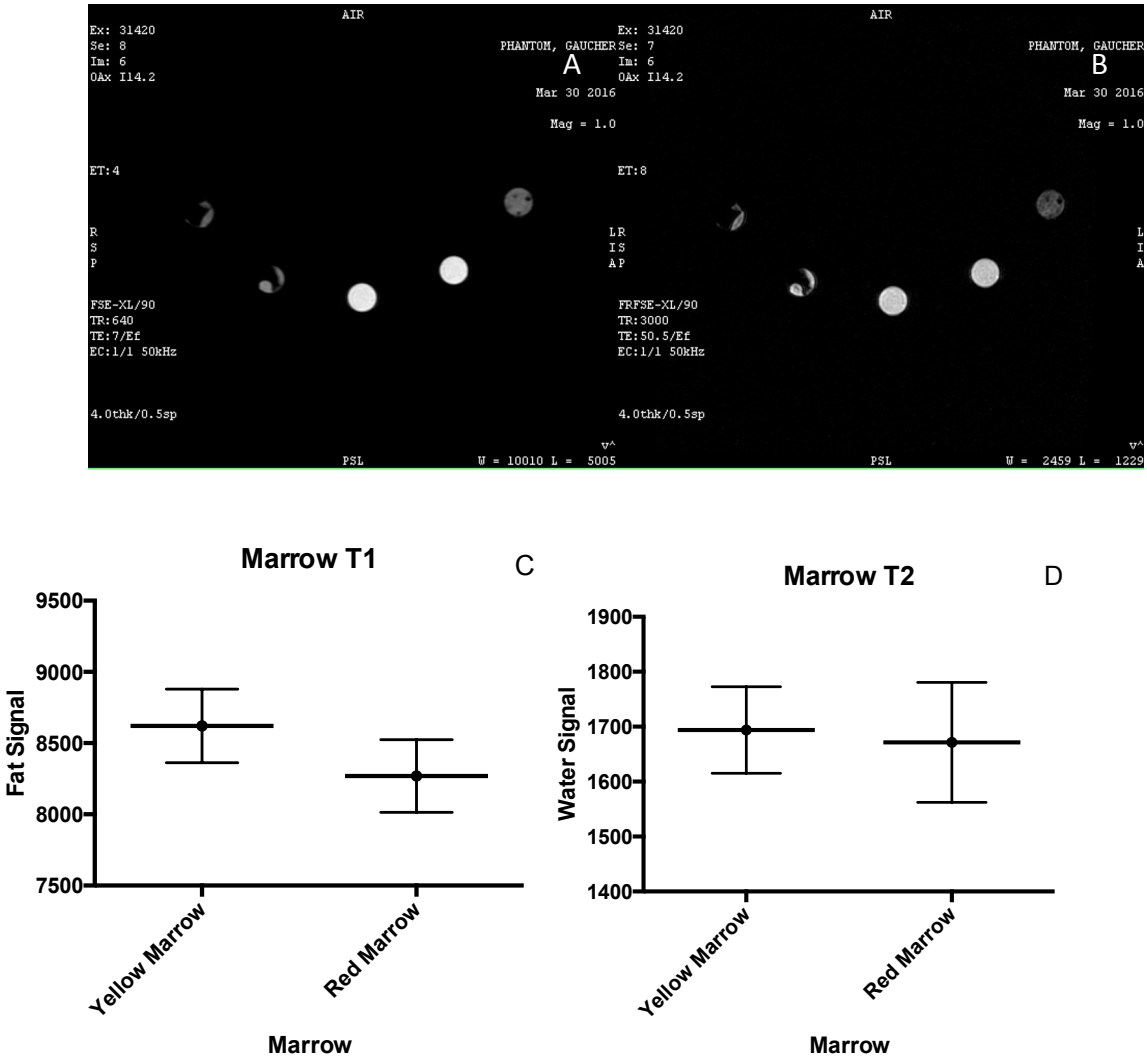


**Figure 3. MRI bone marrow scan:** Sample were loaded from left to right as wild type spleen, D490V/null spleen, goat red marrow, goat yellow marrow, and cow yellow marrow. The MRI results based A) on a T1 weighted scan examining fat signal and B) a T2 weighted scan examining water signal C) Fat signal values based on signal intensity of the T1 test and D) water signal values based on signal intensity of T2 test

The DECT results showed very similar densities for the goat red bone marrow with a density of -82 and the goat yellow bone marrow with a density of -88 (Fig 4). The cow sample contained an unusually low signal with a density of -64

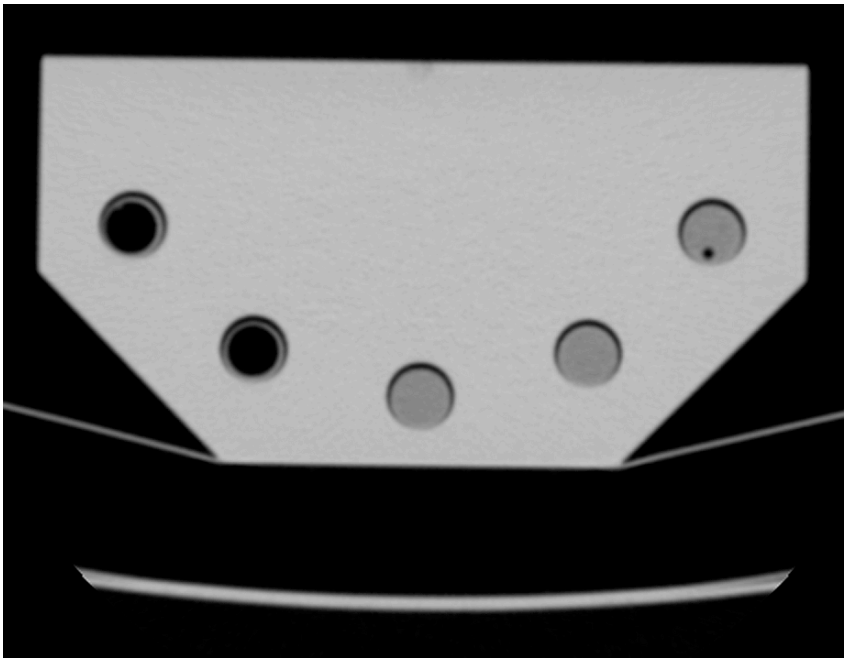

**Figure 4. DECT bone marrow:** Middle position is goat red bone marrow with a density of -82, followed by goat yellow bone marrow with a density value of -88, furthest to the right is cow bone marrow with a density value of -64. Left two positions in image contain spleen samples (discussed later)

*Mouse Model results*

Both the T1 and T2 tests showed differences when comparing the D490V/null mouse spleen with the WT spleen. Based on the T1 analysis of fat signal (Figure 5C) the infiltration of Gaucher cells leads to a measurable difference between the two spleens based on MRI analysis. According to T2 analysis of water signal (Fig 5D), the infiltration of Gaucher cells leads to a measurable difference between water content of Gacucher infiltrated spleens and wild type spleens. However, standard deviations provided by the analysis software centricity showed the difference was not statistically different (approximately one standard deviation was observed). Dark spots on the spleen in both tests reveal air pockets where the small spleen did not fill up the test tube. Readings were selectively taken from the non-dark areas.

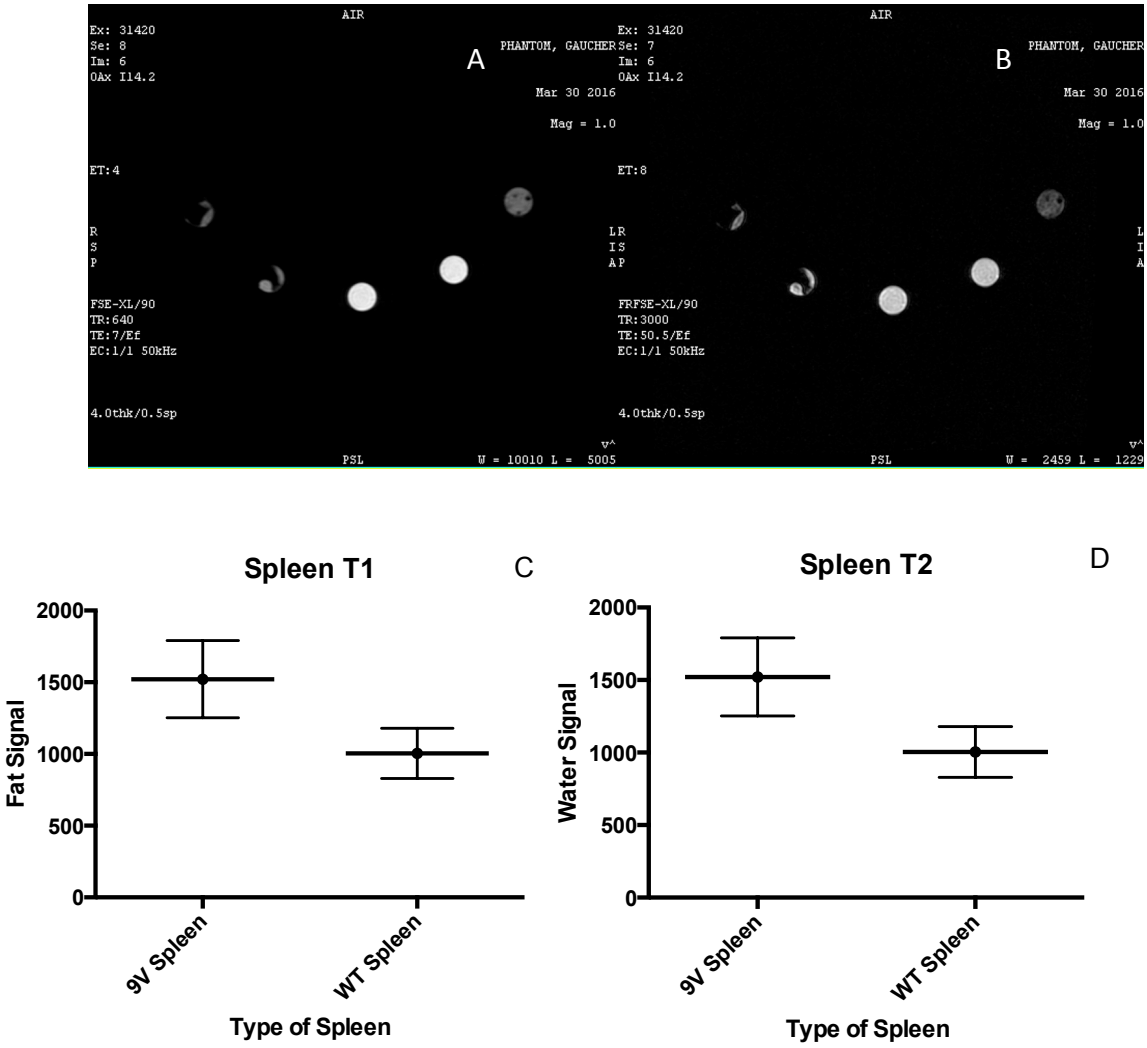


**Figure 5. MRI spleen scan:** Sample were loaded from left to right as WT spleen, 9V spleen, goat red marrow, goat yellow marrow, and cow yellow marrow. Results of MRI analysis based on A) T1 weighted scan examining fat content of D490V/null and wild type mouse spleens and B) T2 weighted scan examining water signal of D490V/null and wild type mouse spleens

DECT results of D490V/null spleen and wild type spleen showed a measurable difference in density with density values of +30 and +21 respectively (Fig 6). Compared to MRI this is a

meaningful value based on the specificity and selective nature of DECT. Similar to MRI, density measurements were selectively taken from non-dark areas of the image.

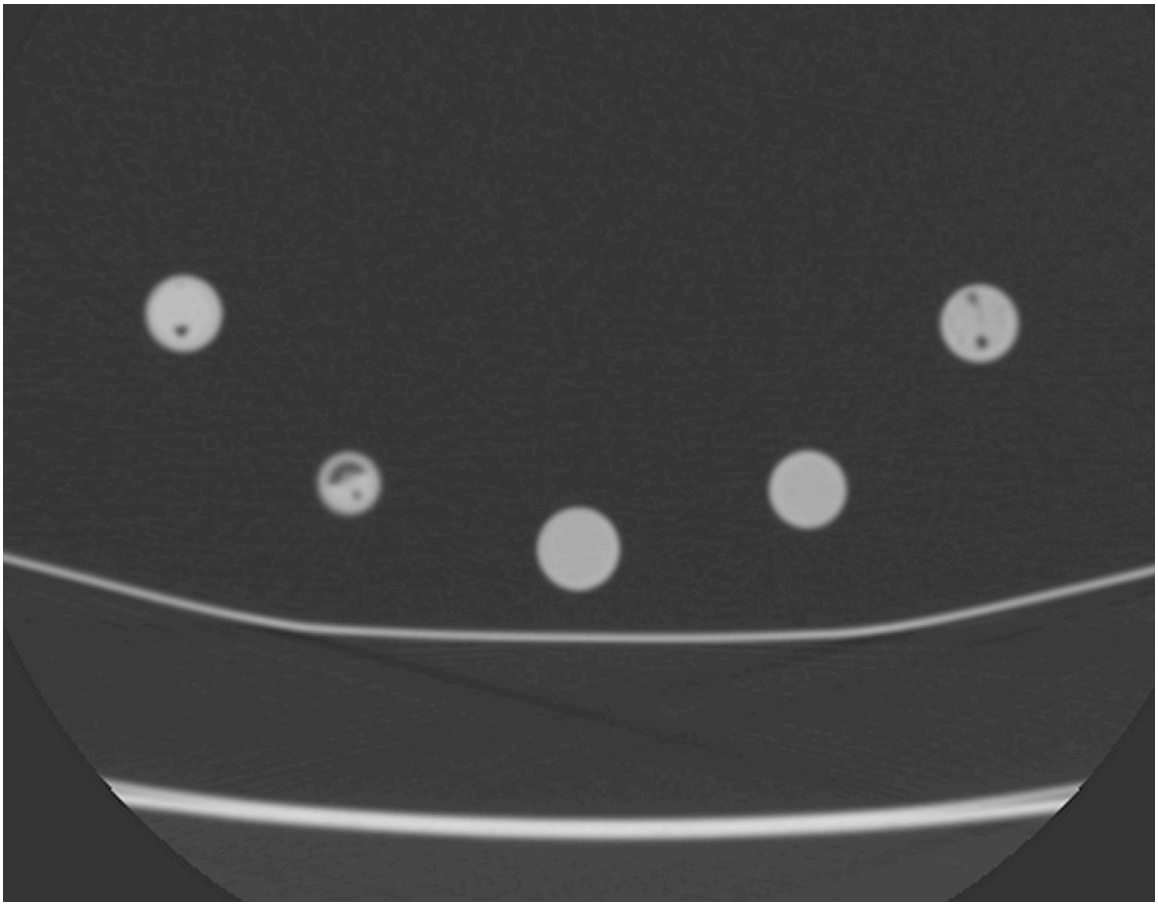

**Figure 6. DECT of spleen:** Left position is a wild type spleen with a density value of +30, 2nd to the left is a D490V/null mouse spleen with a density value of +21. The next three samples were bone marrow samples discussed earlier

The MRI results for the T1 weighted MRI showed a measurable difference in the fat signal of D490V/null mice livers when compared to wild type livers (Fig 7A). However, this also was statistically insignificant (approximately 1 standard deviation). The T2 weighted MRI was unable to distinguish between wild type livers and D490V/null livers based on water signal (Fig

7B). Interestingly the wild type had both the highest and lowest average water signal with values of 1512 and 1251 respectively based on the T2 test.

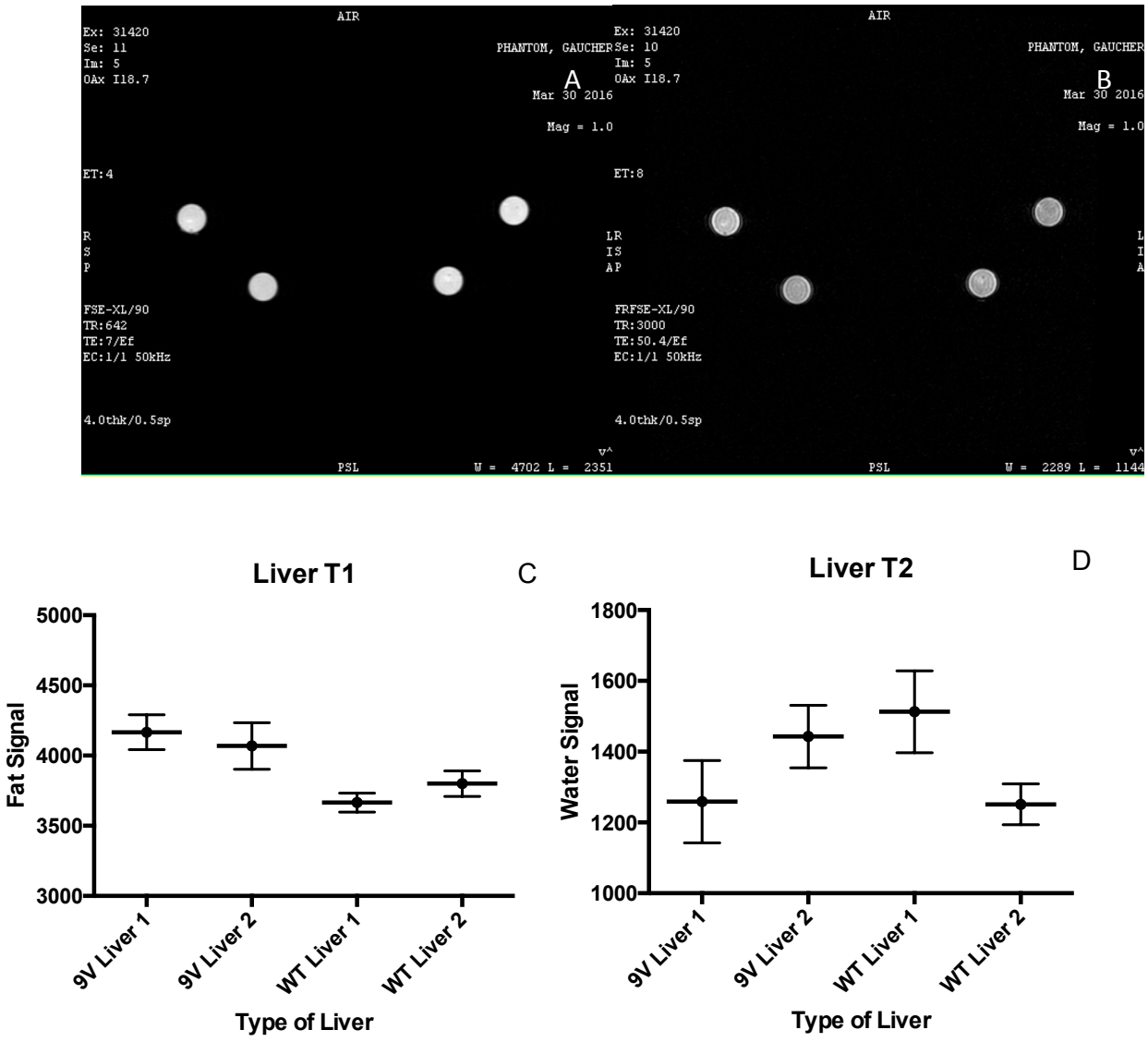


**Figure 7: MRI liver scan:** Sample were loaded from left to right as WT liver 1, WT liver 2, 9V liver 1, 9v liver 2. Results of MRI analysis based on A) T1 weighted scan examining fat content of both D490V/null and wild type mice liver and B) T2 weighted scan examining water signal of D490V/null and wild type mouse liver

DECT was able to clearly distinguish between the liver samples based on density. Both wild type livers had density readings in the mid 50s while the D490V/null livers were in the high 30s (Fig 8). Unlike the MRI results (Fig 7) DECT results provided a much clearer image and reading to distinguish Gaucher infiltration.

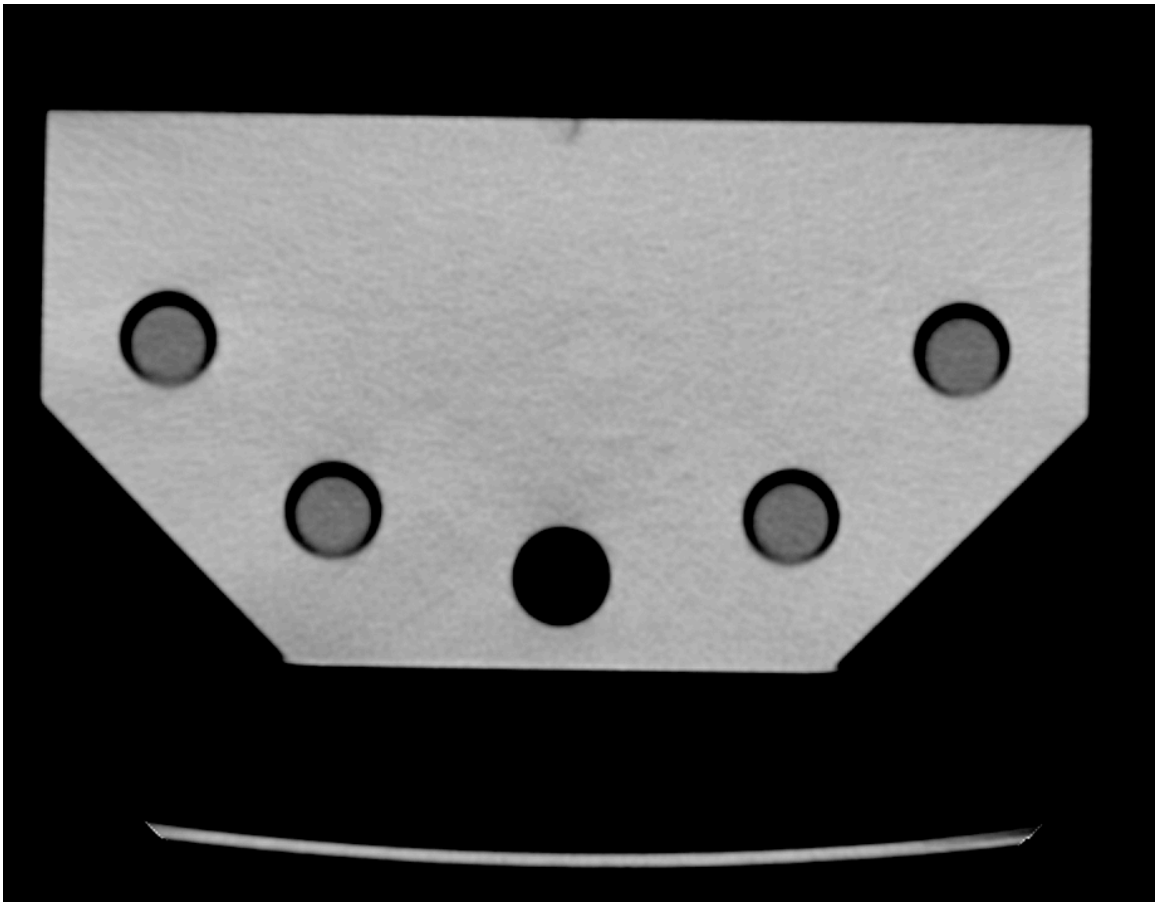

**Figure 8. DECT of liver:** Loaded from left to right WT 1 liver, WT 2 liver, open space, V9 1 liver, and V9 2 liver. Density values were +56.1, +54.5, +39.9, +37.7 respectively

**Discussion**

The results showed that DECT was able to clearly differentiate Gaucher infiltrated tissues based on density. Both the livers and the spleen of D490V/null mice had significantly different values based on densities (Fig 6 and Fig 8). The MRI results were able to differentiate between the D490V/null livers and wild type livers based on a T1 weighted scan (Fig 7C) and between the D490V/null spleen and wild type spleen based on both T1 and T2 weighted scans (Fig 5C and Fig 5D). However, these findings were not significant. This data is consistent with the semi-quantitative analysis used currently by radiologists in the analysis of Gaucher's (Maas, Poll, and Terk, 2002). Based on these findings DECT density analysis provides a much more consistent and reliable analysis of Gaucher infiltration, which in turn could lead to a standardized quantitative analysis.

The phantom data further serves to demonstrate this difference in quantitative assessment of cells based on fat and water content. The large error bars show how limited MRI is at providing discrete values based on analysis (Fig 1C and Fig 1D). The DECT density based assessment provided discrete measurement consistent with the contents of the phantoms (Fig 2B). As stated earlier this ability for discrete measurements could provide radiologists with a quantitative assessment tool for the measurement of the infiltration of Gaucher cells.

Surprisingly, the red marrow and yellow marrow of goats had very similar density measurements based on DECT (Fig 4). This suggests that the marrow itself may not have been very different on a cellular level though its coloration was characteristically different. It is also possible that goat bone marrow simply is not a good substitute for human bone marrow based on potential differences in the storage of the different marrows. The cow marrow density appeared

unusual on the DECT with a density value of -64. It is likely that this is a result of the freezing procedures used by Farmburger, which seemed to remove liquid from the tissue. Cadaver marrow could be extracted and MRI and CT test could be repeated to determine the ability of both to differentiate between different marrow samples.

*Limitation and Future Directions*

The sample size of the study was very small, using only parts from five D490V/null mice and it is possible that the data from the study could be limited in applicability. However, the study did demonstrate an ability to measure differences in Gaucher infiltration based on DECT. This validates the claim that DECT could be a quantitative tool used to diagnose Gaucher infiltration. Based on this, further study could be pursued analyzing the ability of DECT to measure Gaucher infiltration in humans. DECT does expose patients to a considerable amount of radiation, which is a concern. However, improvements in the management of DECT radiation have allowed it to cause no more exposure than a normal CT (Brendella et al., 2015). Additionally, a localized focus on the marrow of one of the extremities would provide patients with a relatively benign CT while still providing important quantitative information on the progression of the disease.

Further research could also pursue $^{1}$H MRS as a quantitative diagnostic tool for Gaucher infiltration. Though it is not as widely used or comprehensive in its analytical ability, it also does not expose patients to radiation. Measurements of the infiltration of the liver and spleen could be done much more safely and often with $^{1}$H MRS, which could make it a useful alternative to DECT. Validation of $^{1}$H MRS with D490V/null mice would provide insight into its ability to

measure Gaucher cell infiltration. Further research could also be done using fat to water ratios since this techniques has been shown to have validity (Bredella et al., 2015). With adjustments to the GE program used for fat and oil analysis, this could also be a viable area for establishing a quantitative measure of Gaucher infiltration.

**Work Cited**

Bredella, Miriam A., et al. "Marrow Adipose Tissue Quantification of the Lumbar Spine by Using Dual-Energy CT and Single-Voxel 1H MR Spectroscopy: A Feasibility Study." *Radiology* 277.1 (2015): 230-235.

Barton, Norman W., et al. "Replacement therapy for inherited enzyme deficiency—macrophage-targeted glucocerebrosidase for Gaucher's disease." *New England Journal of Medicine* 324.21 (1991): 1464-1470.

Bolotin, H. H. "DXA in vivo BMD methodology: an erroneous and misleading research and clinical gauge of bone mineral status, bone fragility, and bone remodeling." *Bone* 41.1 (2007): 138-154.

Brooks, Rodney A., et al. "On the relationship between computed tomography numbers and specific gravity." *Journal of Computer Assisted Tomography* 5.4 (1981): 598.

de Fost, Maaike, et al. "Persistent bone disease in adult type 1 Gaucher disease despite increasing doses of enzyme replacement therapy."*Haematologica* 93.7 (2008): 1119-1120.

Glüer, Claus-C., and Harry K. Genant. "Impact of marrow fat on accuracy of quantitative CT." *Journal of computer assisted tomography* 13.6 (1989): 1023-1035.

Grabowski, Gregory A. "Phenotype, diagnosis, and treatment of Gaucher's disease." *The Lancet* 372.9645 (2008): 1263-1271.

Gruber, S., et al. "Magnetic resonance spectroscopy in patients with Fabry and Gaucher disease." *European journal of radiology* 79.2 (2011): 295-298.

Guggenbuhl, Pascal, Bernard Grosbois, and Gérard Chalès. "Gaucher disease." *Joint Bone Spine* 75.2 (2008): 116-124.

Jmoudiak, Marina, and Anthony H. Futerman. "Gaucher disease: pathological mechanisms and modern management." *British journal of haematology* 129.2 (2005): 178-188.

Komninaka, Veroniki, et al. "Evaluation of bone involvement in patients with Gaucher disease: a semi-quantitative magnetic resonance imaging method (using ROI estimation of bone lesion) as an alternative method to semi-quantitative methods used so far." *European journal of haematology* (2015).

Kugel, Harald, et al. "Age-and sex-specific differences in the 1H-spectrum of vertebral bone marrow." *Journal of Magnetic Resonance Imaging* 13.2 (2001): 263-268.

Lang, Thomas F. "Quantitative computed tomography." *Radiologic Clinics of North America* 48.3 (2010): 589-600.

Maas, M., L. W. Poll, and M. R. Terk. "Imaging and quantifying skeletal involvement in Gaucher disease." *The British journal of radiology* 75.suppl_1 (2002): A13-A24.

Małkiewicz, Agata, and Magdalena Dziedzic. "Bone marrow reconversion–imaging of physiological changes in bone marrow." *Polish Journal of Radiology*77.4 (2012): 45.

Muller, Maria Viviane Gomes, et al. "Quantification of glucosylceramide in plasma of Gaucher disease patients." *Brazilian Journal of Pharmaceutical Sciences* 46.4 (2010): 643-649.

Nagral, Sanjay. "Corruption in Indian Medicine, Or_ Overenthusiasm of the Marketing Department." *Economic and Political Weekly* 49.29 (2014).

Pastores, G. M., and D. A. Hughes. "Gaucher Disease. 2000 Jul 27 [Updated 2011 Jul 21]." *Gene ReviewsTM [Internet]: Seattle (WA): University of Washington, Seattle* (1993).

Sun, Ying, et al. "Substrate compositional variation with tissue/region and Gba1

mutations in mouse models–implications for Gaucher disease." *PLoS One* 8.3 (2013): e57560.

Van Beusechem, Victor W., et al. "Long-term expression of human adenosine deaminase in rhesus monkeys transplanted with retrovirus-infected bone-marrow cells." *Proceedings of the National Academy of Sciences* 89.16 (1992): 7640-7644.

Yu, Zhe, et al. "Mutations in the glucocerebrosidase gene are responsible for Chinese patients with Parkinson's disease." *Journal of human genetics* 60.2 (2015): 85-90.