Quantification of Blood Gd-DTPA Levels: Implications on Dosing in dGEMRIC

by

Marisa Hori

Submitted to the Department of Electrical Engineering and Computer Science
in Partial Fulfillment of the Requirements for the Degrees of
Bachelor of Science in Electrical Engineering and Computer Science
and Master of Engineering in Electrical Engineering and Computer Science

at the Massachusetts Institute of Technology

August 16, 2005

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August 16, 2005

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ABSTRACT

Delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC) is a novel technique that allows early diagnosis of osteoarthritis (OA). Under the current protocol, subjects are injected 0.2mmol of an MRI contrast agent (Gd-DTPA\(^2^-\), Berlex Imaging, Wayne, NJ) per kilogram of body weight. Because the distribution volume of Gd-DTPA\(^2^-\) is affected by body composition, subjects with high Body Mass Index (BMI) may effectively be dosed higher compared to low BMI subjects. In this study, 0.2mmol of Gd-DTPA\(^2^-\) per kilogram of body weight was injected into 17 subjects with varying BMI. Their blood Gd-DTPA\(^2^-\) levels were measured at 15, 30, 45, 60, 90, and 120 minutes post-injection. Although there was a wide scatter in Gd-DTPA\(^2^-\) levels both across the subjects and within subjects of similar BMI, results indicated a positive relationship between blood Gd-DTPA\(^2^-\) levels and BMI. It was determined that this effect could lead to over-pronounced OA severity for high BMI subjects. However, further experiments are needed to understand the scatter to better quantify the effect BMI could have on dGEMRIC.

Thesis Supervisor: Deborah Burstein
Title: Associate Professor of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School
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I. Introduction

Osteoarthritis (OA) is a degenerative disease of the articular cartilage that affects millions of people in the United States. The diagnostic tool used today to detect OA utilizes x-ray imaging technology. However, signs of OA are visible only after substantial physical breakdown of the cartilage has already occurred. Molecular degradation of the cartilage tissue in the joints cannot be detected using x-ray technology. Thus, researchers are exploring alternative imaging techniques that can detect OA at its early stages, before significant damage is already done.

One such alternative technique that is currently under development is called “delayed gadolinium enhanced magnetic resonance imaging of cartilage” (dGEMRIC). It is a non-invasive method that uses magnetic resonance imaging (MRI) technology to indirectly quantify the molecular content of cartilage and thus detect abnormalities in articular cartilage. The basis for dGEMRIC relies on the theory that the amount of negatively charged contrast agent gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA$^2$) that distributes in cartilage is inversely related to the amount of negatively charged glycosaminoglycan (GAG) molecules [1]. Clinical applications of dGEMRIC have been investigated through several pilot studies. These studies have indicated the validity of dGEMRIC as a potential diagnostic tool for OA [2]. However, the dGEMRIC method is still under development; a number of issues need to be addressed before further advances can be made on the path to the clinical use of dGEMRIC.

One issue that needs to be investigated is the way in which the contrast agent dose is determined. In order to be able to compare relative amounts of GAG molecules across patients using dGEMRIC, the amount of contrast agent in the blood must be the same across all subjects. To
achieve this, the current dGEMRIC protocol doses subjects according to their total body weight, with the expectation that the blood levels of Gd-DTPA\textsuperscript{2-} will be constant across a range of subjects of varying weight. However, studies have indicated that obesity affects body composition and therefore alter the distribution volumes of drugs. Because obesity is one risk factor of OA, it is of particular importance to be able to accurately dose dGEMRIC subjects of varying body compositions. The aim for this project was to examine the effects of body composition on dGEMRIC contrast agent dosing.
II. Background

A. Osteoarthritis

Articular cartilage is a connective tissue that covers the bone at the joints and is lubricated by the synovial fluid. Its main functions are to distribute the load within the joint and to allow smooth movement of the joints. It is composed of the extra cellular matrix (ECM) with chondrocyte cells embedded within. Major components of the ECM are glycosaminoglycan (GAG) macromolecules that are kept in place in a tight network of collagen fibrils. Under physiological conditions, GAG has negatively charged side chains that give cartilage some of its compressive strength and mechanical stiffness.

The ECM is constantly degraded and synthesized, a process regulated by chondrocyte cell activity. In normal adults, this process is kept in balance to maintain physiological function of cartilage. In OA subject, there is an imbalance between the degradation and synthesis process that eventually causes a depletion of GAG macromolecules. Decrease in GAG macromolecular content leads to deterioration of normal physiological function of cartilage tissue. With progression of OA, physical erosion and damage to cartilage tissues become evident. Genetic, environmental, metabolic, and biochemical factors have been considered as causes for OA.

Quantification of GAG molecules within cartilage tissue has been studied as a potential diagnostic tool for identifying OA in patients; by assessing GAG content, one can implicitly describe the functional state of articular cartilage tissue [3, 4]. GAG depletion occurs in early stages of OA, before physical breakdown of the cartilage tissue have occurred. Therefore,
assessing GAG content can lead to early diagnosis of OA, allowing possible therapeutic treatment before the disease becomes more difficult to treat.

**B. Basic MRI and Contrast Agent Theory**

In the presence of a strong external magnetic field, protons spin at the Larmor frequency and possess a net longitudinal magnetization parallel to the external magnetic field. When a radio frequency (RF) pulse is introduced, more protons start to spin anti-parallel to the external magnetic field, and therefore, the net longitudinal magnetization decreases. As soon as the RF pulse is turned off, the longitudinal magnetization begins to increase back to its original value by an exponential process with a characteristic time constant, $T_1$ relaxation time. The longitudinal magnetization intensity is measured indirectly using receiver coils, and the corresponding $T_1$ relaxation time constant is calculated from this signal. The $T_1$'s at different locations of an object can be measured using gradient coils to give a spatially mapped image of the $T_1$'s. Since $T_1$ can depend on tissue composition, it is possible to obtain an “image” of an object with heterogeneous tissue composition.

A contrast agent is a chemical substance that is used to alter the $T_1$ and hence provide contrast in tissues that otherwise might have had uniform $T_1$'s. One such contrast agent commonly used in clinical and *in vitro* MRI studies is Magnevist (Berlex Imaging, Wayne, NJ), which is the chemical Gd-DTPA$^2$-. Gd-DTPA$^2$- decreases $T_1$ relaxation time of the medium it accumulates in areas where there is a higher concentration of Gd-DTPA$^2$- will have shorter $T_1$. **Equation 1** describes the relationship between the enhanced $T_1$ values and Gd-DTPA$^2$- concentration:
Equation 1

$$[\text{Gd-DTPA}^{2-}] = \frac{1}{r} \left( \frac{1}{T_1} - \frac{1}{T_1^0} \right)$$

where $[\text{Gd-DTPA}^{2-}]$ is the gadolinium concentration, $T_1$ is the enhanced $T_1$, $T_1^0$ is the $T_1$ of the substance without contrast agent, and $r$ is the constant of Gd-DTPA$^{2-}$ relaxivity. The measured $(1/T_1)$ value (minus the original, non-enhanced $(1/T_1^0)$) is proportional to Gd-DTPA$^{2-}$ concentration. In another words, areas of tissue with shortened $T_1$ are areas that have higher concentrations of Gd-DTPA$^{2-}$. By measuring $T_1$ after contrast agent penetration, it is straightforward to determine the corresponding concentration of Gd-DTPA$^{2-}$ by using Equation 1 if $r$ and $T_1^0$ are known.

C. Theoretical Basis of dGEMRIC

dGEMRIC is a molecular imaging technique that is capable of indirectly indicating functional abnormalities in cartilage tissue. It is based upon known properties of articular cartilage tissue in conjunction with basic MRI theory. As discussed earlier, by assessing GAG content in cartilage, one can implicitly describe the functional state of articular cartilage tissue. How then might one measure GAG content in cartilage using MRI technology?

For in vitro applications of dGEMRIC, cartilage tissue is bathed in a solution of Gd-DTPA$^{2-}$ and is allowed some time for the contrast agent to diffuse through the tissue. In clinical applications of dGEMRIC, Gd-DTPA$^{2-}$ is injected intravenously and allowed some time for it to penetrate into the cartilage tissue from the vasculature via the bone surface and the synovial surface. GAG molecules, fixed within the ECM, have negatively charged side chains. Therefore, when
negatively charged contrast agent Gd-DTPA\(^{2-}\) is introduced in cartilage tissue, it distributes inversely to the GAG concentration; areas of lower GAG concentration will have lesser net negative charge, and therefore, more Gd-DTPA\(^{2-}\) will distribute into that area. When the cartilage tissue is imaged with MRI, areas with higher concentration of Gd-DTPA\(^{2-}\) accumulation will indicate shorter T\(_1\) times, whereas areas with lower concentration of Gd-DTPA\(^{2-}\) accumulation will indicate longer T\(_1\) times. With Gd-DTPA\(^{2-}\)-enhanced MR images, one can infer spatial distribution of GAG in human cartilage tissue [1].

More specifically, how do we relate an MR image of cartilage to its GAG distribution? For \textit{in vitro} applications of dGEMRIC where cartilage samples are submerged in a solution of Gd-DTPA\(^{2-}\) in saline, GAG content can be quantified using a series of equations. The following flow chart (Figure 1) summarizes the steps involved in dGEMRIC analysis:

**Figure 1**

\[ \text{FCD} = -2[\text{Na}^+]_\text{bath} \left( \sqrt{\frac{[\text{Gd-DTPA}^{2-}]_\text{tissue}}{[\text{Gd-DTPA}^{2-}]_\text{bath}}} - \sqrt{\frac{[\text{Gd-DTPA}^{2-}]_\text{bath}}{[\text{Gd-DTPA}^{2-}]_\text{tissue}}} \right) \]

\[ [\text{Gd-DTPA}^{2-}] = \frac{1}{r} \left( \frac{1}{T_1} - \frac{1}{T_0} \right) \]

\[ [\text{GAG}] = \text{FCD} \left( \frac{502.5 \text{g/mol}}{2} \right) \times 10^{-3} \]
First, \([\text{Gd-DTPA}^2]\) within the cartilage tissue can be derived from measured \(T_1\) data using **Equation 1** given the relaxivity constant and the non-altered \(T_1\), \(T_1^0\).

Then, from \([\text{Gd-DTPA}^2]\), the tissue fixed charge density (FCD) distribution in the ECM can be calculated using principles of electroneutrality and the Donnan theory [5]. The electroneutrality principle states that the total amount of negative charge and positive charge within a compartment must be equal. The cartilage tissue is submerged in saline solution, with sodium (\(\text{Na}^+\)) and chloride (\(\text{Cl}^-\)) as the dominant charged ions. Electroneutrality principle applied to the tissue compartment and bath compartment gives the following relationship:

\[
\left[\text{Na}^+\right]_{\text{bath}} - \left[\text{Cl}^-\right]_{\text{bath}} = 0
\]

\[
\left[\text{Na}^+\right]_{\text{tissue}} - \left[\text{Cl}^-\right]_{\text{tissue}} + \text{FCD} = 0
\]

where \([\text{Na}^+]_{\text{bath}}\) and \([\text{Cl}^-]_{\text{bath}}\) are the ion concentrations in the bathing solution, \([\text{Na}^+]_{\text{tissue}}\) and \([\text{Cl}^-]_{\text{tissue}}\) are ion concentrations within the cartilage tissue, and FCD is the fixed charge density associated with the Gd-DTPA\(^2\)-bound GAG molecules within the ECM.

The Donnan theory is based on the electrochemical equilibrium principal between two interfaces; it governs the relationship between the internal cartilage tissue ion concentrations to the external saline bath ion concentration. With the addition of Gd-DTPA\(^2\) ions to the Na\(^+\) and Cl\(^-\) ion-dominated bath and tissue compartments, the following relationship holds:
\[
\frac{[\text{Na}^+]_{\text{tissue}}}{[\text{Na}^+]_{\text{bath}}} = \frac{[\text{Cl}^-]_{\text{bath}}}{[\text{Cl}^-]_{\text{tissue}}} = \frac{[\text{Gd}\cdot\text{DTPA}^{2-}]_{\text{tissue}}^{1/2}}{[\text{Gd}\cdot\text{DTPA}^{2-}]_{\text{bath}}^{1/2}}
\]

The following equation combines the theory of electroneutrality and Donnan with empirical data to give the FCD (Equation 2):

**Equation 2**

\[
\text{FCD} = -2[\text{Na}^+]_{\text{bath}} \left( \frac{\sqrt{[\text{Gd}\cdot\text{DTPA}^{2-}]_{\text{tissue}}}}{\sqrt{[\text{Gd}\cdot\text{DTPA}^{2-}]_{\text{bath}}}} - \frac{\sqrt{[\text{Gd}\cdot\text{DTPA}^{2-}]_{\text{tissue}}}}{\sqrt{[\text{Gd}\cdot\text{DTPA}^{2-}]_{\text{bath}}}} \right)
\]

where FCD is the fixed charge density, \([\text{Na}^+]_{\text{bath}}\) is the sodium concentration in the bath, \(\sqrt{[\text{Gd}\cdot\text{DTPA}^{2-}]_{\text{tissue}}}\) is the Gd-DTPA\(^{2-}\) concentration in tissue, and \(\sqrt{[\text{Gd}\cdot\text{DTPA}^{2-}]_{\text{bath}}}\) is the Gd-DTPA\(^{2-}\) concentration in the bath [5]. Finally, to calculate the GAG concentration in the tissue, the following conversion equation can be used (Equation 3):

**Equation 3**

\[
[\text{GAG}] = \text{FCD} \left( \frac{502.5\text{g/mol}}{2} \right) \times 10^{-3}
\]

**Equation 3** assumes 2 moles of charge per one mole of GAG, and that GAG has a molecular weight of 502.5g/mol.
By repeating these steps for each pixel on the $T_1$ map, an image of [GAG] distribution can be obtained from a Gd-DTPA$^{2-}$-enhanced MRI image.

Ideally, *in vivo* clinical applications of dGEMRIC would follow the same set of equations (Equation 1 ~ Equation 3) as described above in the *in vitro* applications. However, absolute quantification of GAG in cartilage is not possible because the relaxivity constant $r$, the $T_1^0$ constant, and the Gd-DTPA$^{2-}$ concentration in the *bathing* solution (physiological fluid surrounding cartilage tissue) for *in vivo* environments cannot be easily measured nor fixed. Bashir et al have validated that, although the absolute GAG content cannot be computed accurately from the $T_1$ measurements, there is a good correlation between measured $T_1$ with GAG content using *in vivo* dGEMRIC techniques [1]. They verified that low $T_1$ was an indication for low GAG content, and vise versa. Therefore, instead of computing for GAG, *in vivo* clinical applications of dGEMRIC often use $T_1$ (termed as the “$T_1$ dGEMRIC Index”), as a measurement of relative GAG content in cartilage.

**D. Pharmacokinetics of Gd-DTPA$^{2-}$**

Gd-DTPA$^{2-}$ is a hydrophilic ionic contrast agent that shortens $T_1$, and is commonly used in clinical MRI. Prior studies of Gd-DTPA$^{2-}$ pharmacokinetics have shown that plasma Gd-DTPA$^{2-}$ concentrations for intravenously administered Gd-DTPA$^{2-}$ declines bi-exponentially in two phases [6]. The following is the result from Weinmann’s study, where plasma Gd-DTPA$^{2-}$ concentration is plotted over time after injecting healthy male subjects with 0.25mmol of Gd-DTPA$^{2-}$ per kg body weight (*Figure 2*).
During the initial distribution phase, Gd-DTPA$^{2-}$ rapidly distributes in the vascular system and diffuses into the extracellular water compartment of the body. In Weinmann’s study, they found that the mean-half life during this distribution phase was 0.20 +/- 0.13 hours. During the elimination phase, Gd-DTPA$^{2-}$ is filtered and excreted unmetabolized through the kidneys, with a plasma elimination mean-half life of 1.58 +/- 0.13 hours.

**E. Body Composition and Obesity**

For a normal individual, total body water (TBW) is roughly 60% of body weight [7]. TBW can be subdivided into two separate compartments: extracellular water (ECW) and intracellular water...
(ICW). Gd-DTPA₂⁻ cannot cross cell membranes, and therefore is contained within the ECW compartment of the body. For that reason, the following discussion will focus on the ECW compartments of the body.

The approximate normal value of ECW is 20% of body weight [7]. However, with obesity, this typical value can be altered. Obesity is often objectively defined by body mass index (BMI) greater than 30, where BMI is defined as \((\text{body weight}) / (\text{height})^2\) in units of kg/m².

Waki et al.’s study looked at the ECW content of obese and non-obese subjects. In their study, they measured the weight and ECW of 26 non-obese (BMI=21.2 +/- 2.4) and 39 obese (BMI=46.9 +/- 6.5) subjects [8]. The reported measurements are indicated in Table 1.

<table>
<thead>
<tr>
<th>Population</th>
<th>Non-Obese</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BMI</td>
<td>21.2 +/- 2.4</td>
<td>46.9 +/- 6.5</td>
</tr>
<tr>
<td>Mean Body Weight (kg)</td>
<td>56.6 +/- 4.6</td>
<td>124.2 +/- 18.8</td>
</tr>
<tr>
<td>Mean ECW (Liters)</td>
<td>12.1 +/- 1.1</td>
<td>19.7 +/- 4.2</td>
</tr>
</tbody>
</table>

From these values, the estimated fraction of ECW per body weight for the obese and non-obese population were determined by dividing the mean ECW by the mean body weight. To a large approximation, non-obese subjects with an average BMI of 21.2 have a fraction of ECW per body weight of 0.214 L/kg, and obese subjects with an average BMI of 46.9 have a fraction of ECW per body weight of 0.159 L/kg. In another words, their study quantitatively indicated a decreased fraction of ECW per body weight for obese subjects (0.159) as compared to non-obese subjects (0.214). Because Gd-DTPA₂⁻ distributes into the ECW compartments of the body and
plasma is part of the ECW compartment, a decrease in ECW would suggest increased concentration of Gd-DTPA$^{2-}$ in plasma where as an increase in ECW would suggest decreased concentration of Gd-DTPA$^{2-}$ in plasma.

To observe the general trend between the fraction of ECW per body weight and BMI, let us assume a linear relationship between fraction of ECW per body weight and BMI. Using values from Waki’s study, the following plot and equation describes the general relationship between BMI and the fraction of ECW per body weight (Figure 3).

![Figure 3](image)

**Figure 3**

BMI vs (ECW/Body Weight)

\[ y = -0.00214 \times x + 0.259 \]

Extrapolating from the above equation, the fraction of ECW per body weight is 0.216 L/kg and 0.163 L/kg for a subject with BMI of 20 and 45, respectively. Assuming that the volume of distribution is the ECW volume, and that the subjects are given a loading dose of 0.2mmol per
kg body weight, the expected initial [Gd-DTPA²⁻] in plasma in a subject would be given as follows:

\[
[Gd\cdot DTPA^{2-}] = \frac{\text{dose}}{\text{volume of distribution}}
\]

\[
= \frac{(0.2 \text{mmol per kg Body Weight}) \cdot (\text{Body Weight})}{\text{ECW}}
\]

\[
= \frac{(0.2 \text{mmol per kg Body Weight}) \cdot (\text{Body Weight})}{(\text{Liters of ECW per kg Body Weight}) \cdot (\text{Body Weight})}
\]

\[
= \frac{(0.2 \text{mmol per kg Body Weight})}{(\text{Liters of ECW per kg Body Weight})}
\]

If the fraction of ECW per body weight were 0.216 L/kg, as would be expected for a subject with BMI of 20, the expected [Gd-DTPA²⁻] would be 0.93 mM using the equation above. If the fraction of ECW per body weight were 0.159 L/kg, as would be expected for a subject with BMI of 45, the expected [Gd-DTPA²⁻] would be 1.26 mM. Using these values, it is predicted that subjects with BMI of 45 who are dosed Gd-DTPA²⁻ proportionally to their body weight would result in higher blood levels by a factor of 1.35 compared to subjects with BMI of 20.
III. Purpose of Research

A. Motivation

In an attempt to fix the bath concentration for in vivo experiments, the current clinical dGEMRIC protocol administers Gd-DTPA$^{2-}$ at a dose proportional to body weight (0.2mmol/kg). This is done with the assumption that the distributing volume is directly proportional to body weight. However, as discussed earlier, body weight may not be an accurate predictor of distributing volume; obesity affects body composition and therefore may alter the fraction of total body weight that is ECW and hence the distribution volumes of intravenous drugs. Consequently, this may result in higher effective doses of Gd-DTPA$^{2-}$ in obese subjects. Higher effective doses of Gd-DTPA$^{2-}$ may lead to misrepresentative exaggeration in the level of OA severity. The motivation behind the study was to determine by how much higher obese subjects were receiving effective doses of Gd-DTPA$^{2-}$. BMI is an index used commonly to quantify the severity of obesity. The goal for this project was to examine the effect of BMI on blood level of Gd-DTPA$^{2-}$ in order to estimate the equilibrating bath concentration so that we can gain a better understanding of how the current dosing for dGEMRIC can be improved.

B. Hypothesis

From previous studies of obesity, body composition, and pharmacokinetics of Gd-DTPA$^{2-}$, the following hypothesis was proposed for this project: when subjects are administered Gd-DTPA$^{2-}$ dosed by their body weight, obese subjects will effectively be dosed higher compared to non-obese subjects, and this will be observable in the blood levels of Gd-DTPA$^{2-}$. Besides testing this hypothesis, the goal of the research was to determine how this hypothesized effective higher dose in obese subjects could affect the outcome of the dGEMRIC technique.
IV. Materials/Methodology

Seventeen subjects of varying BMI were recruited and consented according to standard protocol. Blood was drawn a few minutes prior to Gd-DTPA\textsuperscript{2−} injection ("PreDose" sample). The subjects were given a single intravenous (IV) dose of Gd-DTPA\textsuperscript{2−} at 0.2mmol per kg body weight, the current standard dosage for the dGEMRIC protocol. The IV line was flushed with normal saline. Most subjects had blood samples drawn at 15, 30, 45, 60, 90, and 120 minutes post injections. However, several blood draws were missed or skipped for some of the subjects. 13 subjects had blood drawn from the same arm of injection while 4 subjects had blood drawn from the opposite arm of injection. For each blood sample, plasma was separated by centrifugation, and about 1mL of plasma was stored below freezing in 5mL tubes.

For each sample, $T_1$ was measured with inversion recovery magnetic resonance (MR) spectroscopy using an 8.5 Tesla MR magnet (Bruker Instruments, Billerica, MA). A varying set of 10 to 15 inversion times were chosen, depending on expected $T_1$. For each measurement, samples were brought to room temperature prior to measurements. $T_1$ for each plasma sample was calculated using ParaVision curve fitting software (Bruker Biospin, Billerica, MA).

60 minutes post-injection plasma samples from 12 subjects were sent for inductively coupled plasma (ICP) analysis (Elemental Analysis, Lexington, KY) to determine the gadolinium (Gd) concentrations using a method used previously in other Gd-DTPA\textsuperscript{2−} pharmacokinetic studies. This ICP analysis was done for two reasons: first, to calculate the appropriate relaxivity constant, r, that is required to calculate [Gd-DTPA\textsuperscript{2−}] from $T_1$ using Equation 1, and second, to confirm that the MR spectroscopy is a reasonable method for indirectly measuring Gd-DTPA\textsuperscript{2−}.
concentration in plasma. In addition to the plasma samples, saline samples hand-pipetted with known concentrations of Gd (1.0mM and 0.5mM) were also sent in for ICP analysis as a validation procedure for the ICP method itself.

T$_1^0$, the T$_1$ without contrast agent, was taken to be the mean of the T$_1$ of pre-dose plasma samples. A plot of (1/T$_1$) versus Gd concentration was generated using the data from the subset of samples that were analyzed with both ICP and MR spectroscopy (60 minutes post-injection plasma samples from 12 subjects). For this plot, the measured values from MR spectroscopy were used for T$_1$, and the ICP data was used for the Gd concentration. The relaxivity constant, r, was calculated from the slope of the regression line through the 12 data points and (1/T$_1^0$), the y-intercept.

Using T$_1^0$ and r values obtained through the aforementioned measurements and calculations, T$_1$ of each plasma sample was converted to [Gd-DTPA$^{2-}$] using Equation 1. For each subject, [Gd-DTPA$^{2-}$] was plotted as a function of post-injection time. For the 60 minutes post-injection plasma samples, the % difference between the Gd concentrations from ICP analysis and the Gd concentration from MR spectroscopy measurements were calculated.

For each of the post-injection time points (15, 30, 45, 60, 90, and 120 minutes), plasma [Gd-DTPA$^{2-}$] from the MR spectroscopy experiments was plotted as a function of each subject’s BMI. A linear regression line was obtained for each plot to observe the general trend between BMI and blood Gd-DTPA$^{2-}$ concentration. Using the regression lines, expected Gd-DTPA$^{2-}$ levels for BMI of 45 and 20 were extrapolated. To estimate the overall difference in blood Gd-
DTPA$^{2-}$ levels for obese and non-obese subjects, the extrapolated Gd-DTPA$^{2-}$ levels for BMI of 45 was divided by the extrapolated Gd-DTPA$^{2-}$ levels for BMI of 20. This was done to get an estimate of how much higher obese subjects were being effectively dosed compared to non-obese subjects.
V. Results

A. Sample Collection and Preparation

The range of BMI of the 17 subjects recruited for the study was from 21.5 to 40.2.

B. $T_1$ Measurement of Blood Samples

$T_1$ of each sample are indicated in the chart below (Table 2). Missed or skipped blood draws are indicated as blank spaces.

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Pre Dose</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG</td>
<td>32.9</td>
<td>2.320</td>
<td>0.129</td>
<td>0.216</td>
<td>0.268</td>
<td>0.316</td>
<td>0.422</td>
</tr>
<tr>
<td>AMM</td>
<td>40.2</td>
<td>2.304</td>
<td>0.024</td>
<td>0.191</td>
<td>0.209</td>
<td>0.241</td>
<td>0.276</td>
</tr>
<tr>
<td>SJ</td>
<td>33.4</td>
<td>2.336</td>
<td>0.011</td>
<td>0.167</td>
<td>0.210</td>
<td>0.240</td>
<td>0.304</td>
</tr>
<tr>
<td>MDR</td>
<td>33.4</td>
<td>2.331</td>
<td>0.143</td>
<td>0.196</td>
<td>0.241</td>
<td>0.297</td>
<td>0.376</td>
</tr>
<tr>
<td>LJ</td>
<td>28.1</td>
<td>2.300</td>
<td>0.181</td>
<td>0.262</td>
<td>0.307</td>
<td>0.371</td>
<td>0.552</td>
</tr>
<tr>
<td>SM</td>
<td>22.5</td>
<td>2.315</td>
<td>0.065</td>
<td>0.101</td>
<td>0.260</td>
<td>0.316</td>
<td>0.368</td>
</tr>
<tr>
<td>NDD</td>
<td>28.7</td>
<td>2.331</td>
<td>0.126</td>
<td>0.199</td>
<td>0.232</td>
<td>0.276</td>
<td>0.328</td>
</tr>
<tr>
<td>AMD</td>
<td>25.1</td>
<td>2.315</td>
<td>0.065</td>
<td>0.101</td>
<td>0.260</td>
<td>0.316</td>
<td>0.368</td>
</tr>
<tr>
<td>JM</td>
<td>24.8</td>
<td>2.230</td>
<td>0.181</td>
<td>0.222</td>
<td>0.237</td>
<td>0.308</td>
<td>0.371</td>
</tr>
<tr>
<td>PH</td>
<td>28.9</td>
<td>2.330</td>
<td>0.166</td>
<td>0.242</td>
<td>0.327</td>
<td>0.403</td>
<td>0.530</td>
</tr>
<tr>
<td>JP</td>
<td>21.5</td>
<td>2.470</td>
<td>0.100</td>
<td>0.269</td>
<td>0.306</td>
<td>0.378</td>
<td>0.459</td>
</tr>
<tr>
<td>MLD</td>
<td>22.8</td>
<td>2.374</td>
<td>0.167</td>
<td>0.226</td>
<td>0.271</td>
<td>0.310</td>
<td>0.397</td>
</tr>
<tr>
<td>GTD</td>
<td>37.2</td>
<td>2.312</td>
<td>0.061</td>
<td>0.108</td>
<td>0.185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEL</td>
<td>27.0</td>
<td>2.386</td>
<td>0.154</td>
<td>0.223</td>
<td></td>
<td>0.318</td>
<td></td>
</tr>
<tr>
<td>FJM</td>
<td>29.5</td>
<td>0.167</td>
<td>0.196</td>
<td>0.270</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFD</td>
<td>26.0</td>
<td>0.144</td>
<td>0.197</td>
<td>0.243</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>22.7</td>
<td>0.169</td>
<td>0.199</td>
<td>0.258</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean $T_1$ of plasma prior to Gd-DTPA$^{2-}$ injection ("PreDose"), $T_1^0$, was $2.333 \pm 0.055$ seconds.
C. ICP Analysis

Raw data from ICP analysis for the 60 minutes post-injection plasma from 12 subjects are shown in the table below (Table 3).

Table 3

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>NG</th>
<th>AMM</th>
<th>SJ</th>
<th>MDR</th>
<th>LJ</th>
<th>SM</th>
<th>PDD</th>
<th>AMD</th>
<th>JM</th>
<th>PH</th>
<th>JP</th>
<th>MLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICP [Gd] (mM)</td>
<td>0.518</td>
<td>0.712</td>
<td>0.632</td>
<td>0.712</td>
<td>0.588</td>
<td>0.432</td>
<td>0.617</td>
<td>0.547</td>
<td>0.719</td>
<td>0.401</td>
<td>0.540</td>
<td>0.549</td>
</tr>
</tbody>
</table>

ICP analysis of 1.0mM and 0.5mM control saline samples revealed Gd concentration of 0.9475mM and 0.4757mM, respectively. This indicated that the ICP analysis was within 6% of the hand-pipetted concentrations.

D. Relaxivity Calculation

A plot of $(1/T_1)$ versus Gd concentration using the data from the subset of samples (60 minutes post-injection plasma samples from 12 subjects) that were analyzed with both ICP and MR spectroscopy is shown in the figure below (Figure 4). The regression line and its corresponding equation are also indicated.
Figure 4

ICP [Gd] vs MRI (1/T1)

\[ y = 5.1831x + 0.4286 \]
\[ R^2 = 0.989 \]

The relaxivity constant, \( r \), was 5.183.

E. [Gd-DTPA\(^2-\)] Calculation from Relaxivity and \( T_1^0 \) Constants

The table below summarizes the Gd-DTPA\(^2-\) concentrations for each sample in units of mM calculated from Equation 1, where \( r = 5.183 \) and \( T_1^0 = 2.333 \) (Table 4).
Table 4

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>BMI</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG</td>
<td>32.9</td>
<td>1.413</td>
<td>0.811</td>
<td>0.637</td>
<td>0.528</td>
<td>0.375</td>
<td>0.275</td>
</tr>
<tr>
<td>AMM</td>
<td>40.2</td>
<td>7.857</td>
<td>0.927</td>
<td>0.840</td>
<td>0.718</td>
<td>0.616</td>
<td>0.518</td>
</tr>
<tr>
<td>SJ</td>
<td>33.4</td>
<td>3.425</td>
<td>0.966</td>
<td>0.711</td>
<td>0.632</td>
<td>0.490</td>
<td>0.396</td>
</tr>
<tr>
<td>MDR</td>
<td>33.4</td>
<td>18.292</td>
<td>1.073</td>
<td>0.836</td>
<td>0.721</td>
<td>0.552</td>
<td>0.450</td>
</tr>
<tr>
<td>LJ</td>
<td>28.1</td>
<td>1.267</td>
<td>0.902</td>
<td>0.718</td>
<td>0.567</td>
<td>0.430</td>
<td>0.310</td>
</tr>
<tr>
<td>SM</td>
<td>22.5</td>
<td>0.983</td>
<td>0.654</td>
<td>0.546</td>
<td>0.437</td>
<td>0.267</td>
<td></td>
</tr>
<tr>
<td>PDD</td>
<td>22.7</td>
<td>1.449</td>
<td>0.887</td>
<td>0.749</td>
<td>0.616</td>
<td>0.506</td>
<td>0.401</td>
</tr>
<tr>
<td>AMD</td>
<td>25.1</td>
<td>2.909</td>
<td>1.828</td>
<td>0.659</td>
<td>0.528</td>
<td>0.442</td>
<td>0.329</td>
</tr>
<tr>
<td>JM</td>
<td>24.8</td>
<td></td>
<td>0.983</td>
<td>0.786</td>
<td>0.731</td>
<td>0.544</td>
<td>0.437</td>
</tr>
<tr>
<td>PH</td>
<td>28.9</td>
<td>1.080</td>
<td>0.715</td>
<td>0.507</td>
<td>0.396</td>
<td>0.281</td>
<td>0.191</td>
</tr>
<tr>
<td>JP</td>
<td>21.5</td>
<td>1.847</td>
<td></td>
<td>0.635</td>
<td>0.548</td>
<td>0.428</td>
<td>0.338</td>
</tr>
<tr>
<td>MLD</td>
<td>22.8</td>
<td>1.073</td>
<td>0.771</td>
<td>0.629</td>
<td>0.540</td>
<td>0.403</td>
<td>0.288</td>
</tr>
<tr>
<td>GTD</td>
<td>37.2</td>
<td>3.106</td>
<td>1.704</td>
<td>0.960</td>
<td></td>
<td></td>
<td>0.440</td>
</tr>
<tr>
<td>BEL</td>
<td>27.0</td>
<td>1.170</td>
<td>0.762</td>
<td></td>
<td></td>
<td></td>
<td>0.524</td>
</tr>
<tr>
<td>FJM</td>
<td>29.5</td>
<td>1.073</td>
<td>0.902</td>
<td></td>
<td></td>
<td></td>
<td>0.632</td>
</tr>
<tr>
<td>PFD</td>
<td>26.0</td>
<td>1.257</td>
<td>0.897</td>
<td></td>
<td></td>
<td></td>
<td>0.711</td>
</tr>
<tr>
<td>PD</td>
<td>22.7</td>
<td>1.059</td>
<td>0.887</td>
<td></td>
<td></td>
<td></td>
<td>0.665</td>
</tr>
</tbody>
</table>

The following table summarizes the [Gd-DTPA\(^2\)] values for 60 minutes post-injection plasma samples (Table 5). Concentrations measured by both MR spectroscopy and ICP analysis are indicated. The % difference in the concentrations between the two methods is also shown.

Table 5

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>NG</th>
<th>AMM</th>
<th>SJ</th>
<th>MDR</th>
<th>LJ</th>
<th>SM</th>
<th>PDD</th>
<th>AMD</th>
<th>JM</th>
<th>PH</th>
<th>JP</th>
<th>MLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR [Gd-DTPA(^2)] (mM)</td>
<td>0.528</td>
<td>0.718</td>
<td>0.632</td>
<td>0.721</td>
<td>0.567</td>
<td>0.437</td>
<td>0.616</td>
<td>0.528</td>
<td>0.731</td>
<td>0.396</td>
<td>0.548</td>
<td>0.540</td>
</tr>
<tr>
<td>ICP [Gd] (mM)</td>
<td>0.518</td>
<td>0.712</td>
<td>0.632</td>
<td>0.712</td>
<td>0.588</td>
<td>0.432</td>
<td>0.617</td>
<td>0.547</td>
<td>0.719</td>
<td>0.401</td>
<td>0.540</td>
<td>0.549</td>
</tr>
<tr>
<td>% Difference</td>
<td>2.0</td>
<td>0.8</td>
<td>0.0</td>
<td>1.3</td>
<td>-3.6</td>
<td>1.1</td>
<td>-0.2</td>
<td>-3.5</td>
<td>1.8</td>
<td>-1.3</td>
<td>1.5</td>
<td>-1.8</td>
</tr>
</tbody>
</table>

The difference in the MR spectroscopy and ICP methods for measuring Gd concentration was less than 3.6%.
F. [Gd-DTPA\textsuperscript{2-}] vs. Time

[Gd-DTPA\textsuperscript{2-}] as a function of post-injection time for each subject is shown in the figure below (Figure 5). BMI corresponding to the plot of each subject is indicated in the legend.

**Figure 5**

![Graph showing [Gd-DTPA\textsuperscript{2-}] over Time](image)

G. BMI vs. [Gd-DTPA\textsuperscript{2-}]

For each post-injection time point, Gd-DTPA\textsuperscript{2-} concentrations plotted as a function of BMI are shown in the graphs below (Figure 6 ~ Figure 11). The linear regression line and its corresponding equation are also indicated.
Figure 6

BMI vs 15min Post Injection [Gd-DTPA²]

\[ y = 0.3803x - 7.7109 \]
\[ R^2 = 0.2406 \]

Figure 7

BMI vs 30min Post Injection [Gd-DTPA²]

\[ y = 0.0155x + 0.5361 \]
\[ R^2 = 0.069 \]
Figure 8

BMI vs 45min Post Injection [Gd-DTPA²]

\[ y = 0.0122x + 0.3582 \]
\[ R^2 = 0.3554 \]

Figure 9

BMI vs 60min Post Injection [Gd-DTPA²]

\[ y = 0.0061x + 0.4264 \]
\[ R^2 = 0.0966 \]
Figure 10

BMI vs 90min Post Injection \([\text{Gd-DTPA}^2]\)

\[
y = 0.0059x + 0.2929 \\
R^2 = 0.1361
\]

Figure 11

BMI vs 120min Post Injection \([\text{Gd-DTPA}^2]\)

\[
y = 0.0076x + 0.1388 \\
R^2 = 0.2578
\]
A summary of the slope, intercept, and R² values for the regression line for each post-injection time point are given below (Table 6):

<table>
<thead>
<tr>
<th>Post-Injection Time</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>0.3803</td>
<td>0.0155</td>
<td>0.0122</td>
<td>0.0061</td>
<td>0.0059</td>
<td>0.0076</td>
</tr>
<tr>
<td>Intercept</td>
<td>-7.7109</td>
<td>0.5361</td>
<td>0.3582</td>
<td>0.4264</td>
<td>0.2929</td>
<td>0.1388</td>
</tr>
<tr>
<td>R²</td>
<td>0.2406</td>
<td>0.069</td>
<td>0.3554</td>
<td>0.0966</td>
<td>0.1361</td>
<td>0.2578</td>
</tr>
<tr>
<td>P value</td>
<td>0.0538</td>
<td>0.3256</td>
<td>0.0315</td>
<td>0.2412</td>
<td>0.2641</td>
<td>0.0765</td>
</tr>
</tbody>
</table>

The following table summarizes the predicted blood Gd-DTPA²⁻ levels from the regression line for subjects with BMI of 45 and 20, and the factor of difference between the two values (Table 7). These values are obtained for 30, 45, 60, 90, and 120 minutes post-injection time points.

<table>
<thead>
<tr>
<th>Post-Injection Time</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted [Gd-DTPA²⁻] for BMI of 45 (mM)</td>
<td>1.23</td>
<td>0.907</td>
<td>0.701</td>
<td>0.558</td>
<td>0.481</td>
</tr>
<tr>
<td>Predicted [Gd-DTPA²⁻] for BMI of 20 (mM)</td>
<td>0.846</td>
<td>0.602</td>
<td>0.548</td>
<td>0.411</td>
<td>0.291</td>
</tr>
<tr>
<td>Factor</td>
<td>1.45</td>
<td>1.51</td>
<td>1.28</td>
<td>1.36</td>
<td>1.65</td>
</tr>
</tbody>
</table>
VI. Discussion of Results

A. Sample Collection and Preparation

The first 13 subjects had blood drawn from the same arm of injection. For several 15 minutes post-injection plasma samples, unusually short $T_1$ (less than 0.1 seconds) values were observed. Short $T_1$ implies high concentration of Gd-DTPA$^{2-}$. It was speculated that the high concentration of Gd-DTPA$^{2-}$ was due to improperly flushed IV lines. Remnants of the highly concentrated bolus injection of Gd-DTPA$^{2-}$ remaining in the IV line may have contaminated the 15 minutes post-injection plasma samples, resulting in unusually high concentrations. Post-injection plasma samples from 30 minutes and beyond may also have been contaminated as well; however, this is less likely, as the IV line was flushed with saline between each blood draws. For the last 4 subjects of the study, blood was drawn from the opposite arm of injection. Results for these 4 subjects indicated normal range of $T_1$ values, between 0.1 and 0.4 seconds. Therefore, it may be advisable to avoid using the same arm for injection and drawing in future pharmacokinetic studies.

B. $T_1$ Measurement of Blood Samples

The plasma samples were measured using MR spectroscopy with an 8.5T magnet. The MR spectroscopy method was chosen over MR imaging, as spatial information was not necessary. Rather, obtaining the $T_1$ of the whole sample was sufficient to obtain the appropriate [Gd-DTPA$^{2-}$]. This provided a relatively fast means to measure the $T_1$ of the plasma samples compared to imaging techniques. The mean $T_1^0$, the $T_1$ of “PreDose” plasma without any Gd-DTPA$^{2-}$, was 2.333 seconds with standard deviation of 0.055 seconds indicating little variability in $T_1^0$ values from subject to subject.
C. ICP Analysis

ICP is a tool used for detecting and analyzing trace elements and has been used in previous pharmacokinetic studies of Gd-DTPA\(^{2-}\). However, it is not a time nor cost-effective method for measuring Gd-DTPA\(^{2-}\) concentration because the equipment for ICP analysis is not readily available. MR spectroscopy is a quick and less costly process to indirectly measure Gd-DTPA\(^{2-}\) concentration in a laboratory with access to MRI magnets. The aim for ICP analysis was to validate the results from MR spectroscopy method if it matched closely to the results from the ICP method. To verify the ICP method itself, saline control samples with known amounts of Gd-DTPA\(^{2-}\) were sent for ICP analysis. The hand-pipetted saline control samples matched ICP results within 6%. This 6% difference could have been a result of an offset due to calibration of the pipette used during hand-pipetting, or it could have resulted from ICP precision. 6% is within a reasonable range; it is reasonable to conclude that ICP is a valid means to measure [Gd-DTPA\(^{2-}\)]. Therefore, the results for ICP analysis for the Gd-DTPA\(^{2-}\) concentration in plasma should be considered valid.

D. Relaxivity Calculation

Previously published relaxivity constant of Gd-DTPA\(^{2-}\) in plasma for 8.5T magnet at room temperature was found to be 3.98 [9]. Gd-DTPA\(^{2-}\) relaxivity in plasma using the MR spectroscopy T\(_1\) and ICP Gd concentration results was 5.183. The cause of this discrepancy between the previously published relaxivity and the current relaxivity is not known. Previously published relaxivity constant of Gd-DTPA\(^{2-}\) in saline for 8.5T magnet at room temperature was found to be 3.87 [9]. Gd-DTPA\(^{2-}\) relaxivity in saline using the MR spectroscopy T\(_1\) and known hand-pipetted concentrations resulted was measured to be 4.68. Similar to plasma relaxivity,
saline relaxivity using MR spectroscopy method resulted in higher values than previously published values. These discrepancies in relaxivity constants may be due to differences in the environment and the way the samples were measured. Differences in temperature, magnetic strength, and the medium that the contrast agent is in have been known to alter relaxivity constants. In the current study, differences in room temperatures, and difference in measuring method (MR spectroscopy versus MR Imaging) may have been the cause of the discrepancy in the relaxivity constant.

Regardless of which relaxivity value is used to calculate Gd-DTPA\(^2^\) concentration in plasma (3.98 or 5.183), the important thing is to be consistent. Relaxivity is inversely proportional to Gd-DTPA\(^2^\) concentration. Since the current study is comparing relative Gd-DTPA\(^2^\) concentration between subjects, as long as a consistent relaxivity constant is used for all conversions, the relative Gd-DTPA\(^2^\) concentrations should scale accordingly.

**E. \([\text{Gd-DTPA}^2^-] \text{ Calculation from Relaxivity and } T_1^0 \text{ Constants}\)**

Gd-DTPA\(^2^-\) concentrations were calculated for each of the plasma samples using relaxivity and \(T_1^0\) constants. Relaxivity of 5.183 was used consistently for all samples. The subset of plasma samples that were analyzed both with ICP and MR spectroscopy analysis indicated similar results within 3.6%. Therefore, it can be concluded that the MR spectroscopy is a reasonable method for indirectly measuring Gd-DTPA\(^2^-\) concentration in plasma samples. MR spectroscopy is a more cost-effective, accessible method than ICP in the current study, and could be used in future pharmacokinetic studies of Gd-DTPA\(^2^-\). It should be noted that the appropriate relaxivity constant and \(T_1^0\) may need to be measured for the magnetic strength and temperature, as the current study indicated different values than previously published numbers.
F. [Gd-DTPA$^{2-}$] vs. Time

In Weinmann’s study of Gd-DTPA$^{2-}$ pharmacokinetics, healthy male subjects were administered 0.1mmol and 0.25mmol of Gd-DTPA$^{2-}$ per kg body weight [6]. Blood was drawn periodically thereafter and the plasma Gd concentration was measured using ICP analysis. The graph below includes Weinmann’s results from the 0.1mmol and 0.25mmol dose superimposed over the mean values of the current study’s results (Figure 12).

**Figure 12**

[Gd-DTPA$^{2-}$] vs Time

Generally, the results from the current study follow a similar shape as Weinmann’s results. The Gd-DTPA$^{2-}$ concentration decays bi-exponentially through the fast initial distribution and slower elimination phases. From inspection, the current study’s results seem to have a sharper initial
distribution time constant compared to Weinmann’s results. Actual time constants could not be calculated due to the lack of sufficient number of data points for a bi-exponential decay curve fit. The sharper initial distribution time constant could be an influence from the unusually high 15 minutes post-injection samples that may have been caused by contamination from improperly flushed IV line as discussed earlier.

To observe the effect of obesity on blood Gd-DTPA$^{2-}$ levels, the 17 subjects were divided into two populations. There were 5 obese subjects (BMI >30) with mean BMI of 35.4 +/- 3.18. There were 12 non-obese subjects (BMI<30) with mean BMI of 25.1 +/- 2.8. The Gd-DTPA$^{2-}$ concentrations within each population were averaged and plotted as a function of time, as shown in the graph below (Figure 13). The plot also includes Weinmann’s results from the 0.25mmol/kg dose study.
By inspection, the obese population indicated higher levels of Gd-DTPA$^{2-}$ compared to the non-obese population. The following table summarizes the % difference between the obese and non-obese population [Gd-DTPA$^{2-}$] averages at each time point (Table 8):

<table>
<thead>
<tr>
<th>Post-Injection Time</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Difference</td>
<td>394.6</td>
<td>18.1</td>
<td>21.9</td>
<td>13.1</td>
<td>17.3</td>
<td>29.9</td>
</tr>
</tbody>
</table>

Table 8
Again, the elevated values from the earlier time points (15 minutes post-injection) may be affected by contaminated samples.

**G. BMI vs. [Gd-DTPA\(^2\)]**

The aim of the current study was to determine the effect of BMI on blood levels of Gd-DTPA\(^2\). The blood levels were measured in hopes of estimating the equilibrating bath concentration surrounding cartilage tissue *in vivo*. Gd-DTPA\(^2\)- levels in blood changes over time as it distributes into and eliminated from the body. It is unclear as to when it is best to consider the blood concentration to be equal to the equilibrating bath concentration that is washing into the cartilage tissue. Therefore, the measured [Gd-DTPA\(^2\)-] after injection was plotted as a function of BMI and was repeated for each of the post-injection time points when blood was drawn. Plots for the 15 minutes post-injection most probably are affected greatly by the contamination that was discussed previously. 120 minutes post-injection time point is not within the time of interest for *in vivo* dGEMRIC applications, as imaging occurs around the same time. Therefore 15 and 120 minutes post-injection time points will be excluded from further discussion.

To consider the general trend between BMI and blood levels of Gd-DTPA\(^2\)-, a linear regression line was obtained for each time-point plot, and each indicated a positive slope. However, for all of the plots, the R\(^2\) value are relatively low (between 0.069 to 0.355), and there is a wide scatter in the [Gd-DTPA\(^2\)-] values among the whole population as well as subjects within the same range of BMI. This may be due to any number of reasons.
Normal person-to-person variations can alter blood levels of drugs. In Weinmann’s study of blood levels of Gd-DTPA\(^2\) over time in healthy male subjects, each post-injection time point presented variation with a standard deviation of about 10~15\%. In the current study, the variation for all subjects, as represented by the standard deviation, was about 15~35\%. Although the current study’s standard deviation is larger than Weinmann’s study, part of the variability may be accounted by the normal person-to-person variability.

A reason for the variability in blood Gd-DTPA\(^2\) levels for subjects within the same range of BMI may be due to the limits of using BMI as a predictor of body composition. Cheymol reviewed numerous studies that looked at effect of obesity on pharmacokinetic of various drugs and realized the limitations in many of the studies he investigated [10]. One limitation, he pointed out, was the inter-individual variations in assessing pharmacokinetic study results. It was pointed out that individuals with the same BMI might differ in their body composition and fatness, particularly between ethnic groups. Therefore, future studies may benefit from a study that compares high BMI subjects with matched control subjects. This could eliminate the variation and allow a firm understanding of the effect of BMI on blood levels of Gd-DTPA\(^2\).

Despite the low R\(^2\) values due to the large scatter of data points, BMI versus Gd-DTPA\(^2\) concentration plots for all time points did show a positive trend. Using the regression lines from 30, 45, 60, and 90 minutes post-injection time points, the estimated blood Gd-DTPA\(^2\) levels for BMI of 45 was higher than for BMI of 20 by a factor of 1.28 to 1.51, depending on post-injection time point. As discussed in the hypothesis section of this study, implications from Waki’s study on ECW led to the prediction that Gd-DTPA\(^2\) levels for BMI of 45 will be higher by a factor of
1.35 compared to BMI of 20. Depending on which time point to consider, the results of the current study predict a higher level of Gd-DTPA\(^{2-}\) for BMI of 45 by a factor of 1.28 to 1.51 compared to BMI of 20, which concur relatively closely with predicted values.

**H. Summary of Results**

The following summarizes the main conclusions from the results of the experiments:

1. To avoid contamination, blood should be drawn from the opposite arm from where the contrast agent was injected.
2. MR spectroscopy is a reasonable method for measuring Gd-DTPA\(^{2-}\) concentration from plasma samples, as validated by ICP analysis.
3. Relaxivity constant for plasma at room temperature for 8.5T magnet using MR spectroscopy was determined to be 5.183. \(T_1\) for the same conditions is 2.333 seconds.
4. Current study’s results indicated a higher level of Gd-DTPA\(^{2-}\) in plasma than expected from Weinmann’s previously published pharmacokinetic studies [6].
5. When mean Gd-DTPA\(^{2-}\) concentrations from subjects were divided into two groups and plotted as a function of post-injection time, the obese population (BMI>30) indicated higher levels compared to the non-obese (BMI<30) population.
6. When analyzed at each post-injection time point, plasma [Gd-DTPA\(^{2-}\)] as a function of BMI resulted in a positive slope regression line.
7. There is wide scatter in the [Gd-DTPA\(^{2-}\)] values among the subjects, both within all of the subjects, and also within subjects who are in the same range of BMI.
8. Using the regression lines from BMI versus [Gd-DTPA$^2$], [Gd-DTPA$^2$] in blood for subjects with BMI of 45 were higher than subjects with BMI of 20 by a factor of 1.28 to 1.51.
VII. Further Analysis and Discussion

For each post-injection time point, plasma [Gd-DTPA\(^2\)] as a function of BMI resulted in a positive slope regression line, indicating that subjects with higher BMI may be effectively dosed higher than subjects with lower BMI. However, there is wide scatter in the [Gd-DTPA\(^2\)] values among subjects within the same range of BMI, and the regression line may be of little statistical significance due to low R\(^2\) values. Further studies may be needed to understand this variability in blood levels of Gd-DTPA\(^2\).

Nevertheless, BMI plotted as a function of Gd-DTPA\(^2\) concentration did show a positive trend despite the large scatter. In the mean time, it would be beneficial to understand the possible effects of BMI on blood Gd-DTPA\(^2\) levels and consequently, on dGEMRIC. To appreciate these results in order to better understand the current dGEMRIC dosing method, further analysis was done.

A. T\(_1\) dGEMRIC Index Simulator

To understand how varying blood [Gd-DTPA\(^2\)] levels could potentially affect dGEMRIC results, a “T\(_1\) dGEMRIC Index Simulator” was created. This simulator was designed to examine how two subjects with the same level of OA severity (i.e. same [GAG] concentration values) but with different bath [Gd-DTPA\(^2\)] levels could indicate different T\(_1\) values measured by dGEMRIC (T\(_1\) dGEMRIC Index). The bases of the simulator calculations were derived from the three governing equations of dGEMRIC, Equation 1 ~ Equation 3. The input to the simulator is the [Gd-DTPA\(^2\)]\(_{\text{bath}}\), and the output is the T\(_1\) dGEMRIC Index, or simply, T\(_1\) from Equation 1. The other variables in the simulator are [Na\(^+\)]\(_{\text{bath}}\), T\(_1\)^0, r, and [GAG]. In the current clinical
dGEMRIC protocol, 1.5T magnets are used. In Bashir’s paper, the following typical physiological values are suggested: [Na+]_bath=150mmol/L, r=3.5 at 1.5T and body temperature in tissue, and T_1^0=0.93 [3]. Typical values of [GAG] can be anywhere from 0mg/mL to 60mg/mL, depending on OA severity. With this simulator and appropriate physiological values, one can determine expected T_1 dGEMRIC Index from a range of [Gd-DTPA^2^-]_bath levels.

B. Effects of BMI on T_1 dGEMRIC Index

Results from the blood [Gd-DTPA^2^-] analysis have indicated that when subjects are dosed solely according to their body weight, subjects with higher BMI result in higher blood [Gd-DTPA^2^-]. In another words, obese individuals may be subjected to higher effective doses of Gd-DTPA^2^- when they are administered Gd-DTPA^2^- by their body weight. However, these results have low statistical significance; R^2 values are low, and there is much scatter in the data points. If the study could somehow be repeated to show similar results but with better statistical significance, how might the effective higher doses in obese subjects affect the dGEMRIC outcome?

Tiderius et al studied the effects of varying Gd-DTPA^2^- doses on the resultant T_1 dGEMRIC Index [11]. Healthy subjects were injected Gd-DTPA^2^- at a dose of 0.3mmol per kg body weight and the T_1 dGEMRIC Index of the knee cartilage was obtained. A week later, the same experiment was repeated, but with some subjects receiving 0.2mmol per kg body weight, and some subjects receiving 0.1mmol per kg body weight. They found that there was a linear relationship between the injected dose of Gd-DTPA^2^- and the resultant T_1 dGEMRIC Index - higher dose of Gd-DTPA^2^- resulted in proportionally smaller T_1 dGEMRIC Index. They showed a direct correlation between the injected doses with the resultant clinical T_1 dGEMRIC Index.
Although all of the subjects in the current study were injected a consistent dose of 0.2mmol/kg, the effective dose as indicated by their blood levels showed higher values for subjects with higher BMI. To determine the effects of the effective BMI-dependent Gd-DTPA$^{2-}$ dose on the $T_1$ dGEMRIC Index, the following sequence of calculations were carried out:

1. Expected blood [Gd-DTPA$^{2-}$] for a range of BMI values were extrapolated from the linear regression equation obtained from the BMI vs. [Gd-DTPA$^{2-}$] plots.

2. With the assumption that [Gd-DTPA$^{2-}$] in the blood is analogous to [Gd-DTPA$^{2-}$]$_{\text{bath}}$, theoretical values of $T_1$ for the range of [Gd-DTPA$^{2-}$] were calculated using the “$T_1$ dGEMRIC Index Simulator.” In this calculation, the following values were used: $[\text{Na}^+]_{\text{bath}}=150\text{mmol/L}$, $r=3.5$, $T_1^0=0.93$, $[\text{GAG}]=60\text{mg/mL}$.

3. The $T_1$ dGEMRIC Index simulated for each BMI was compared to that at BMI of 30, and the percent difference was calculated.

4. Because it is unclear as to when the blood [Gd-DTPA$^{2-}$] is most analogous to the equilibrating bath concentration, steps 1 through 3 were repeated for each of the post-injection time points.

5. To see how the effects differ for varying levels of OA, steps 2 through 4 were repeated for [GAG] of 20 and 0 mg/mL.

Figure 14 ~ Figure 16 show the % differences between the $T_1$ dGEMRIC Index at BMI of 30 and the $T_1$ dGEMRIC Index for all other BMI for 30, 45, 60, 90, and 120 minutes post-injection time points, when [GAG]=60, 20, and 0mg/mL.
Figure 14

$T_1$ dGEMRIC Index % Difference (BMI30 Centered)

$[GAG]=60, r=3.5, T_{1o}=0.93, [Na^+] = 150$

Figure 15

$T_1$ dGEMRIC Index % Difference (BMI30 Centered)

$[GAG]=20, r=3.5, T_{1o}=0.93, [Na^+] = 150$
Figure 16

T₁ dGEMRIC Index % Difference (BMI30 Centered)

[SiO=0, r=3.5, T1o=0.93, [Na+]=150

% Difference

It is unclear as to which time points best represent the equilibrating [Gd-DTPA$^{2-}$]. The worst-case scenario from BMI-related higher effective doses in the obese seemed to occur if equilibrating [Gd-DTPA$^{2-}$] was best represented by the 30 minutes post-injection blood Gd-DTPA$^{2-}$ levels. The % difference in the T₁ dGEMRIC Index between BMI of 20 and BMI of 45 using blood levels at 30 minutes post-injection indicated the following values:

- [GAG]=60mg/mL  ➔ 22% difference in T₁ between BMI 20 and 45
- [GAG]=20mg/mL  ➔ 27% difference in T₁ between BMI 20 and 45
- [GAG]=0mg/mL  ➔ 29% difference in T₁ between BMI 20 and 45
In another words, two subjects who have the same level of OA severity but one having a BMI of 20 and another having a BMI of 45 could end up having \( T_1 \) dGEMRIC Index differences of up to 22, 27, and 29%, due to differences in *effective* doses. Comparing the % differences for [GAG] of 60mg/mL, 20mg/mL, and 0mg/mL, it can be seen that the effects of dosing become more pronounced for lower GAG content. Therefore, the consequence of differences in effective dosing would be maximal for two patients of BMI of 20 and 45 with severe GAG depletion.
VIII. Conclusion and Further Studies

The goal of this study was to examine the effect of BMI on blood level of Gd-DTPA\textsuperscript{2-}. It was hypothesized that if subjects were dosed according to their body weight, then higher BMI subjects will be effectively dosed higher compared to lower BMI subjects. In this study, 17 subjects of varying BMI were injected with Gd-DTPA\textsuperscript{2-} with a dose proportional to their body weight. Blood levels of Gd-DTPA\textsuperscript{2-} were measured at several time points after injection. When these values were examined as a function of BMI, there was variability among the subjects, both among all the subjects and also within subjects in the same range of BMI. Nevertheless, each time point represented a positive trend between BMI and blood Gd-DTPA\textsuperscript{2-}, as predicted by the hypothesis. If this trend were statistically significant and that subjects with higher BMI were being effectively dosed higher, then it could represent an error of up to 29\% difference in clinical T\textsubscript{1} dGEMRIC Index between high and low BMI subjects (45 versus 20) with the same level of OA severity. In another words, subjects with higher BMI could be receiving higher effective doses of Gd-DTPA\textsuperscript{2-} and indicate a lower T\textsubscript{1} dGEMRIC Index as a result. Lower T\textsubscript{1} dGEMRIC Index is an indicator of a lower GAG concentration and an increased OA severity. Therefore, the effective higher doses in high BMI subjects could lead to misrepresentative exaggeration of OA severity.

However, before any firm conclusion about the relationship between BMI and Gd-DTPA\textsuperscript{2-} can be made, several issues need to be examined further. For example, the variability among the subjects in blood Gd-DTPA\textsuperscript{2-} needs to be investigated. Although there was a positive trend in BMI versus Gd-DTPA\textsuperscript{2-} levels as hypothesized, there was much variability in the resulting data. This could imply that there may be other factors that influence blood Gd-DTPA\textsuperscript{2-} levels in blood.
other than BMI. Relating to the issue of variability, it would also be interesting to see the intra-variability of the concentration curve for a given subject. This could be accomplished by repeating the experiment on the same subject on different days could do this.

Different pharmacokinetic analysis could be conducted as well. In the current study, Gd-DTPA$^{2-}$ was plotted as a function of BMI for each of the time points. However, this kind of analysis is limited in that, we are only looking at Gd-DTPA$^{2-}$ concentrations at instantaneous time points. Information regarding the overall exposure of Gd-DTPA$^{2-}$ over a period of time may give better indication of the effect of BMI on dGEMRIC dosing. In pharmacokinetic studies of various other drugs, the area under the concentration vs. time curve is often calculated to indicate drug exposure in a subject [12]. This kind of analysis would require more data points than were taken in the current study, especially in the earlier time points during the distribution phase. Blood draws at 1, 3, 5, 10, 15, 20, 30, 45, 60, and 90 minutes post-injection would provide sufficient data to give an adequate bi-exponential curve fit to calculate the area under the curve.

The current study looked at Gd-DTPA$^{2-}$ levels in a range of BMI from around 20 to 45. In future studies, volunteer subjects could be grouped into a low-BMI group and high-BMI group, with narrow ranges, to see a more pronounced effect of BMI. For example, one population could have a BMI range of 20–22, and another could have a BMI range of 35–37.

In conclusion, if the dGEMRIC method were to be used to compare OA severity among multiple subjects of varying BMI, then the dosing method needs to be re-evaluated. Alternatively, it may
be necessary for clinicians to consider the effect of BMI on dosing when evaluating OA severity in subjects using the dGEMRIC method.
IX. References