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Self-identified gadolinium toxicity: comparison of gadolinium in bone and urine to healthy gadolinium-based contrast agent exposed volunteers

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Abstract

Objective: To report additional gadolinium bone and urine data that can contribute to gaps in knowledge with respect to gadolinium uptake and retention in the body. *Approach*: *In vivo* measurements of gadolinium retention in the tibia bone were performed on individuals self-identified as exhibiting symptoms of gadolinium toxicity as a result of receiving GBCA, as well as on control individuals. Gadolinium urine measurements for controls, symptomatic exposed, and non-symptomatic exposed were conducted through Mayo Medical Laboratories. *Main results*: Gadolinium bone concentration in the exposed group is significantly higher than the control group ($p < 0.01$), with a significant difference between symptomatic and non-symptomatic ($p < 0.01$), using a one-tailed t test on variance-weighted means. Gadolinium urine levels in both control subjects and non-symptomatic exposed subjects are significantly lower than symptomatic exposed subjects ($p \leq 0.05$). A linear regression analysis for gadolinium urine levels and GBCA dose resulted in a positive linear relationship ($R^2 = 0.91, p < 0.01$). Gadolinium levels in urine and gadolinium concentration in bone were found to have a non-significant relationship ($R^2 = 0.11, p = 0.3$). *Significance*: Significant differences in gadolinium levels in bone and urine are observed between individuals experiencing symptoms of gadolinium toxicity and for those who are not exhibiting symptoms. No correlation was observed between gadolinium in bone and gadolinium excreted in urine, suggesting that the retention of gadolinium in the body is complicated, involving multiple long-term storage sites.

1. Introduction

Gadolinium-based contrast agents (GBCAs) are incredibly effective for diagnostic MR imaging, and as such are commonly used in imaging centers worldwide. Developed in the 1980s, GBCAs were thought to be completely safe and excreted from the body within hours of administration (Carr *et al* 1984, Weinmann *et al* 1984, Caravan *et al* 1999). The advent of adverse effects in individuals with renal disease, known as nephrogenic systemic fibrosis (NSF) (Grobner 2006, Marckmann *et al* 2006, Thomsen *et al* 2006), led to a change in protocol for administration of GBCAs for individuals with impaired renal function, followed by a restored confidence in the use of GBCAs for individuals with normal renal function (Altun *et al* 2009). However, at approximately the same time as the discovery of NSF, evidence of gadolinium retention in bone was found in healthy individuals with normal renal function (Gibby *et al* 2004, White *et al* 2006).

Recently, the issue of gadolinium retention in healthy individuals has sparked a great deal of discussion, as many studies have reported gadolinium retention in brain (Errante *et al* 2014, Kanda *et al* 2014, 2015,

2016, McDonald *et al* 2015, Quattrocchi *et al* 2015, Radbruch *et al* 2015), as well as other organs, such as bone (Darrah *et al* 2009, Murata *et al* 2016). Additionally, several reports have been published presenting individuals with self-reported symptoms of gadolinium toxicity following administration of GBCAs, for both linear and macrocyclic agents (Burke *et al* 2016, Ramalho *et al* 2016, Roberts *et al* 2016, Semelka *et al* 2016a). The retention of gadolinium in healthy individuals with normal renal function, resulting in adverse effects, has been referred to as 'gadolinium deposition disease' in some reports (Semelka *et al* 2016b). The symptoms described by individuals are similar; the most common symptoms are central torso pain, peripheral arm and leg pain, brain fog, and skin thickening (Burke *et al* 2016, Ramalho *et al* 2016, Semelka *et al* 2016a, 2016b).

Unlike other trace elements, which can be acquired from drinking water or work exposures, the predominant source of gadolinium retention in the body is from GBCA administered for MRI. Ingestion of anthropogenic gadolinium through drinking water has recently been investigated as a potential contributor to gadolinium retention in the body (Kulaksız and Bau 2011). However, it was found that gadolinium concentrations in drinking water are 8 orders of magnitude lower than the concentration in a single administration of GBCA, and are therefore unlikely to contribute to any gadolinium retention in the body.

With little known about the clinical repercussions of gadolinium in the body, the radiology community is faced with a conflict between using these effective diagnostic agents or considering their safety, in fear of possible GBCA-related symptoms. There are limited data and many gaps in knowledge with respect to gadolinium uptake and retention in the body. Gadolinium excreted in urine is often used as a biomarker by self-identified symptomatic individuals to monitor their potential gadolinium toxicity (Semelka *et al* 2016b). We propose that another potential marker to correlate gadolinium levels and possible gadolinium-related symptoms could be gadolinium concentration in bone. However, to our knowledge, it is currently unknown what the expected levels of gadolinium in urine and bone at various time points should be for healthy asymptomatic individuals following GBCA administration, and further whether there are differences in self-reported symptomatic individuals.

Our research group previously reported gadolinium concentrations in bones of healthy individuals who had received GBCAs (Lord *et al* 2017a). These measurements were performed with a non-invasive *in vivo* biomedical device using the technique of x-ray fluorescence (XRF) (Lord *et al* 2016, 2017b). A significant difference in gadolinium concentration in bone was observed between the control group and GBCA exposed group, and the biomedical device was shown to measure gadolinium in bone in a small population. The purpose of this study is to report additional gadolinium bone and urine data that can contribute to gaps in knowledge with respect to gadolinium uptake and retention in the body. This article reports the findings of gadolinium in bone and urine for three groups of individuals: control, self-reported symptomatic exposed, and asymptomatic exposed.

2. Methods

2.1. Study population

Our original prospective pilot study measuring gadolinium in bone consisted of 11 healthy control subjects, and 11 healthy subjects who had previously received GBCA, whom were referred to as 'exposed' subjects (Lord *et al* 2017a). For this study extension, permission was granted by the Hamilton integrated research board (HiREB) to measure gadolinium in bone for an additional four control subjects, and four exposed subjects. The four additional exposed subjects were self-identified as exhibiting symptoms of gadolinium toxicity, where the 11 original exposed subjects were healthy individuals who did not report symptoms of gadolinium toxicity. In addition to bone measurements, permission was granted to perform gadolinium urine measurements on four control and four non-symptomatic exposed subjects, and to receive gadolinium urine data from the four self-reported symptomatic exposed subjects, who provided us with their most recent 24 h gadolinium urine levels (as measured by the Mayo Medical Laboratories or Genova Diagnostics). All participant demographics can be found in supplementary tables E1–E3 (stacks.iop.org/PM/39/115008/mmedia).

2.2. XRF bone measurement

XRF bone (tibia) measurements took place between June and August 2017 on the additional four control and four self-reported symptomatic exposed subjects, using an identical measurement and analysis protocol and the same biomedical device presented in the original pilot study (Lord *et al* 2017a). A Cd-109 activation source was mounted to the face of a high-purity germanium detector (GL0210R/S; Canberra Industries, Concord, Ontario, Canada) to create an approximately 180° measurement geometry. Subjects were asked to sit for a period of 30 min, placing their leg directly in front of the Cd-109 source, and remaining still throughout the measurement. The gamma rays emitted by the Cd-109 source excited any present gadolinium in the tibia bone, which in turn produced x-rays characteristic of gadolinium. The characteristic x-rays were measured and analyzed further to calculate the tibial concentration of gadolinium. Subjects received an equivalent dose of 357 μSv to the tibia and an effective dose of 0.13 μSv for a 30 min measurement. Detailed methods regarding the use of the XRF bone system, as well as reproducibility measurements, can be found in the original pilot study

(Lord *et al* 2017a). The results of the XRF bone measurements can be found in supplementary tables E1 and E2. It is important to note that gadolinium measurements reported as negative values do not physically represent negative gadolinium concentrations. Negative values are the result of measuring low levels of gadolinium with statistical variation in the x-ray scatter background. When measuring gadolinium, the x-ray scatter background beneath the gadolinium peaks is estimated by fitting an appropriate mathematical function to an energy region that includes the gadolinium peaks plus 'background' regions of immediately lower and higher energies than the gadolinium peaks. Since we are detecting very low levels of gadolinium, the estimated background subtracted from the gadolinium peaks can sometimes be higher than the peaks themselves, resulting in a negative value for gadolinium concentration.

2.3. 24 h urine measurement

Urine tests on the four control and four asymptomatic exposed subjects were carried out in December 2017 and January 2018 using the standardized Mayo Medical Laboratories (Rochester, MN) 24 h gadolinium urine test (Test ID: GDU), which uses the technique of inductively coupled plasma mass spectrometry to detect gadolinium as described by Leung *et al* (2009). The eight subjects were given containers and 24 h urine collection instructions. Each urine sample was transferred into a 10 ml tube and sent to the Mayo Clinic for analysis. The four symptomatic exposed subjects had been monitoring their gadolinium urine levels over time, and provided us with their most recent test data, all of which were completed through standardized tests at either the Mayo Clinic or Genova Diagnostics (Asheville, NC). Since three of the four self-reported symptomatic exposed subjects had received multiple chelation therapies in attempts to remove gadolinium from the body, we used their 'unprovoked' levels (gadolinium urine levels prior to chelation). Information regarding the date and results of the urine tests can be found in supplementary table E3.

2.4. Half life correction

As with other heavy metal accumulation in bone, such as lead, we assume gadolinium to have a unique half life in bone (Chettle 2005). We tested therefore, a half life correction to determine whether the time between the date of GBCA administration and the date of XRF bone measurements was a factor in differences in bone gadolinium level between subjects. Correcting for the half-life of gadolinium in bone determines the concentration of gadolinium in bone 'shortly' after GBCA administration (on a scale of weeks to months). This is assumed because the timescale of gadolinium accumulation into bone is not clear. Gadolinium concentration in bone shortly after GBCA administration was calculated with a simple exponential model shown in equation (1):

$$[\mathrm{Gd}] = [\mathrm{Gd}_0]e^{-\frac{\ln 2}{t_{1/2}}t} \tag{1}$$

where [Gd] is the gadolinium concentration at the time of the XRF measurement, [Gd_0] is the gadolinium concentration following GBCA administration, $t_{1/2}$ is the half life of gadolinium in bone, and t is the time between GBCA administration and the XRF measurement. Half lives of 2, 3, 4, and 5 years were tested to determine whether time provided additional explanation to gadolinium bone concentrations. Both positive and negative Gd concentrations were corrected for half-life using equation (1).

2.5. Statistical analysis

A one-tailed t test was used to test gadolinium concentration in the exposed group against the control group. In the previous study measuring gadolinium in bone, both control and exposed groups were not found to deviate significantly from a normal distribution (Lord *et al* 2017a). Thus, normal statistics were used to test for differences in gadolinium concentration between the groups. One-tailed t tests were performed on arithmetic means, as well as inverse variance-weighted means, since inverse variance weighting the data accounts for individual measurement uncertainty and weights the mean towards the more precise measurement values (Chamberlain *et al* 2012). To test for any differences in gadolinium concentration between GBCA exposed groups, a one-tailed t test was performed on the self-reported symptomatic subjects against the asymptomatic subjects, as well as on the variance-weighted means.

A linear regression was performed for gadolinium bone measurements of 15 control subjects, four self-identified symptomatic exposed subjects, and nine non-symptomatic exposed subjects, to test for a possible relationship between gadolinium concentration in bone and cumulative GBCA dose. For all linear regression analyses in this study, Matlab software (MathWorks, Natick, Mass) was used to carry out a linear least-squares regression, the p-value of the slope was used to assess the significance of the regression, and an R^2 value was used to evaluate correlation. In our original pilot study, two individuals could not provide an estimated GBCA dose and were not included in the regression analysis (exposed subjects 9 and 11). Exposed subject 2 was excluded from the linear regression in the original pilot study due to lack of information regarding GBCA dose. Since publication, the individual was able to provide data on approximate doses for previous GBCA administrations and is

now included in the regression analysis. Linear regressions were tested for each half life correction of 2, 3, 4, and 5 years.

Gadolinium urine levels were compared between the four control, four self-reported symptomatic exposed, and four asymptomatic exposed subjects using a one-tailed t test on the groups. Some 24 h urine tests resulted in a non-detectable amount of gadolinium, which the Mayo Clinic defines as having urine levels less than 0.1 μg/24 h. For these individuals, their levels were set as 0.05 μg/24 h for the one-tailed t test. One-tailed t tests with variance weighting could not be carried out for urine analysis since measurement uncertainties were not reported by the Mayo Clinic.

Linear regression analysis was performed on urinary gadolinium data from the four control, four symptomatic exposed, and four non-symptomatic exposed subjects to test for a relationship between gadolinium levels in urine and cumulative GBCA dose. Lastly, linear regression was performed on gadolinium bone concentrations and urinary gadolinium levels to test for a possible relationship between the amount of gadolinium stored in bone and the amount excreted in urine.

3. Results

3.1. Gadolinium concentration in bone of control, symptomatic exposed, and non-symptomatic exposed groups

As shown in figure 1, the arithmetic mean of gadolinium concentration in the exposed group was calculated to be 1.66 ± 1.25 μg Gd g^{-1} bone mineral, which proves to be significantly higher than the arithmetic mean of the control group, -1.20 ± 0.85 μg Gd g^{-1} bone mineral ($p = 0.03$). The variance-weighted means for the exposed and control group were found to be 2.33 ± 0.59 and -0.97 ± 0.57 μg Gd g^{-1} bone mineral, respectively, resulting in a significant difference between concentrations in the two groups with greater confidence ($p = 0.000\,027$). A more detailed assessment within the exposed group showed a non-significant difference between the arithmetic means for the symptomatic and non-symptomatic subgroups ($p = 0.40$). However, a significant difference was found when performing a one-tailed t test on variance-weighted means of the symptomatic and non-symptomatic subgroups ($p = 0.0042$). All arithmetic and variance-weighted means are summarized in table 1.

3.2. Linear regression for gadolinium concentration in bone and cumulative GBCA dose

Linear regression of gadolinium concentration in bone and cumulative GBCA dose for the 15 control and 13 exposed subjects showed that bone gadolinium concentration increases by 0.042 ± 0.020 μg Gd g^{-1} bone mineral per 1 ml of GBCA administered ($p = 0.046$, $R^2 = 0.14$), with a y-intercept of -0.43 ± 0.83 μg Gd g^{-1} bone mineral (figure 2). A half life correction of 3 years for gadolinium in bone results in the highest correlation coefficient ($R^2 = 0.57$) compared to the other half life corrections of 2, 4, and 5 years ($R^2 = 0.52$, $R^2 = 0.53$, $R^2 = 0.46$, respectively). The linear regression of predicted gadolinium concentration in bone shortly after GBCA administration and cumulative GBCA dose, assuming a half life of 3 years, resulted in a positive slope, suggesting that gadolinium concentration in bone (at a short time after administration) increases by 0.49 ± 0.09 μg Gd g^{-1} bone mineral per 1 ml of GBCA administered ($p = 0.000\,0078$), with a y-intercept of -3.53 ± 3.49 μg Gd g^{-1} bone mineral (figure 3).

3.3. Gadolinium in urine of control, symptomatic exposed, and non-symptomatic exposed groups

Gadolinium levels in urine for the symptomatic exposed group were visibly higher compared to the non-symptomatic exposed and control group (figure 4). Mean urinary gadolinium levels for control, symptomatic exposed, and non-symptomatic exposed subjects were 0.05 ± 0.0, 0.45 ± 0.26, and 0.09 ± 0.07 μg/24 h, respectively. Urinary levels between control subjects and non-symptomatic exposed subjects were not significantly different ($p = 0.20$). Urinary gadolinium levels in both control and non-symptomatic exposed subjects were significantly lower than symptomatic exposed subjects ($p = 0.02$ and $p = 0.05$, respectively).

3.4. Linear regression for gadolinium in urine and cumulative GBCA dose

Linear regression analysis for urinary gadolinium levels and GBCA dose in 12 subjects (figure 5) resulted in a positive linear relationship of 0.0047 ± 0.0004 μg/24 h per 1 ml of GBCA administered ($R^2 = 0.91$, $p = 0.000\,000\,98$), with a y-intercept of 0.0158 ± 0.0635 μg/24 h.

3.5. Relationship between gadolinium in urine and gadolinium in bone

Gadolinium levels in urine and current bone gadolinium concentration had a non-significant relationship of 6.5 ± 5.9 μg/24 h per μg Gd g^{-1} bone mineral through a linear regression analysis ($R^2 = 0.11$, $p = 0.3$).

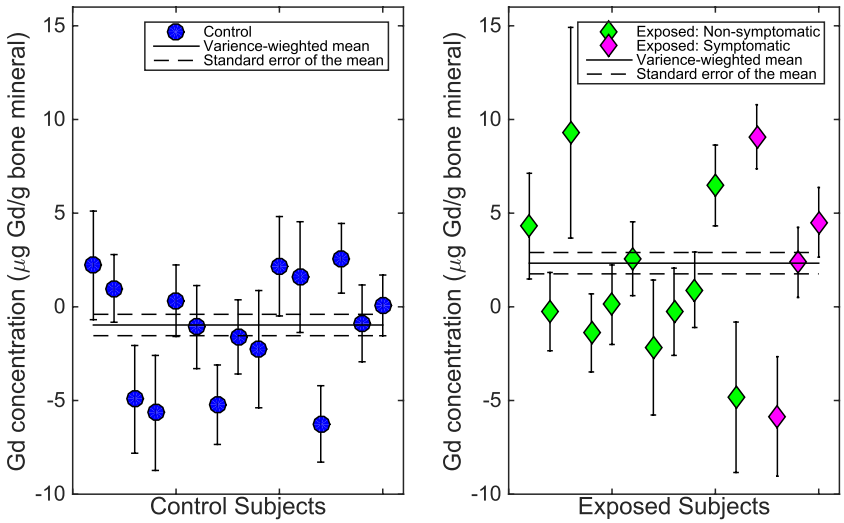

Figure 1. Gadolinium concentration in bone for control and exposed groups: 15 control subjects, 11 non-symptomatic exposed subjects, and four symptomatic exposed subjects. A one-tailed *t* test on the arithmetic means confirms gadolinium concentration in the exposed group is significantly higher than the control group ($p = 0.03$), with inverse variance weighting the data resulting in the same outcome with superior confidence ($p = 0.000027$). A one-tailed *t* test on the arithmetic means of gadolinium concentration for the symptomatic and non-symptomatic exposed subgroups showed an insignificant difference between groups ($p = 0.40$). Inverse variance weighting the data resulted in a significantly higher gadolinium concentration in symptomatic exposed subjects ($p = 0.0042$). Error bars represent individual Gd measurement uncertainties. The variance-weighted means for control and exposed groups are represented by a solid black line, with the corresponding standard error of the mean represented by a black dashed line.

Table 1. Summary of arithmetic and variance-weight means for control and exposed groups, as well as non-symptomatic and symptomatic exposed subgroups. *P*-values from one tailed *t* tests are included to show the significance between the control and exposed groups, as well as the non-symptomatic and symptomatic exposed subgroups.

	Control (μg Gd g^{-1} bone mineral)	Exposed (μg Gd g^{-1} bone mineral)	*p*-value
Arithmetic mean	-1.20 ± 0.85	1.66 ± 1.25	0.03
Variance-weighted mean	-0.97 ± 0.57	2.33 ± 0.59	0.000 027
	Exposed: non-symptomatic (μg Gd g^{-1} bone mineral)	Exposed: symptomatic (μg Gd g^{-1} bone mineral)	*p*-value
Arithmetic mean	1.34 ± 1.35	2.53 ± 4.42	0.40
Variance-weighted mean	1.19 ± 0.73	4.44 ± 0.99	0.0042

4. Discussion

4.1. Gadolinium in bone

Data in figure 1 visually display a difference in present day gadolinium bone concentration between control and exposed population groups, which was previously seen by Lord *et al* in the original pilot study with 11 control and 11 exposed subjects (Lord *et al* 2017a). The addition of four control and 4 symptomatic exposed subjects to the original study population distinguishes the difference in gadolinium concentrations between the exposed and control groups with greater confidence (from $p = .01$ to $p < 0.0001$ for a one-tailed *t* test using variance-weighted means). Additional data increases confidence in the reporting that gadolinium is retained in bone following exposure to GBCAs. There was no statistically significant difference when comparing bone gadolinium concentration between symptomatic and non-symptomatic subjects, based on a one-tailed *t* test ($p = 0.40$). However, when performing a one-tailed *t* test on variance-weighted means, bone gadolinium concentration in symptomatic exposed subjects was significantly higher than non-symptomatic exposed subjects ($p = 0.0042$). Our research group prefers to use variance-weighted means when comparing two populations since bone gadolinium measurements have variable measurement uncertainty that depends on factors such as leg shape, size, mass and subject motion. The use of variance-weighted means places more value on the more precise measurements taken during an experiment (Chamberlain *et al* 2012).

In the original pilot study, linear regression analysis of gadolinium concentration in bone and GBCA dose showed a significant positive correlation ($R^2 = 0.42$, $p = 0.01$). The addition of four control and four symptomatic exposed subjects to this study decreased the significance of the relationship between gadolinium concentration in bone and GBCA dose ($R^2 = 0.14$, $p = 0.046$). Applying a half life correction accounts for the varying

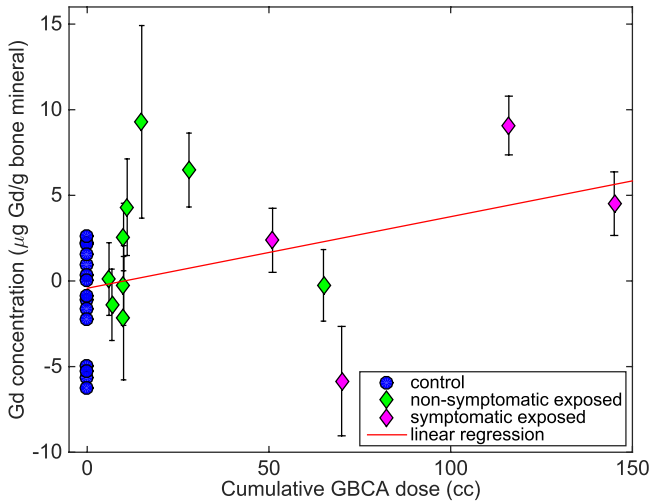

Figure 2. Gadolinium concentration in bone versus cumulative GBCA dose (various brands) for control and exposed groups: 15 control subjects, four symptomatic exposed subjects, and nine non-symptomatic exposed subjects. A positive relationship of 0.042 ± 0.020 μg Gd g^{-1} bone mineral per 1 ml of GBCA administered is shown ($p = 0.046$, $R^2 = 0.14$), with a y-intercept of -0.43 ± 0.83 μg Gd g^{-1} bone mineral. Error bars represent the standard deviation of the calculated gadolinium concentration.

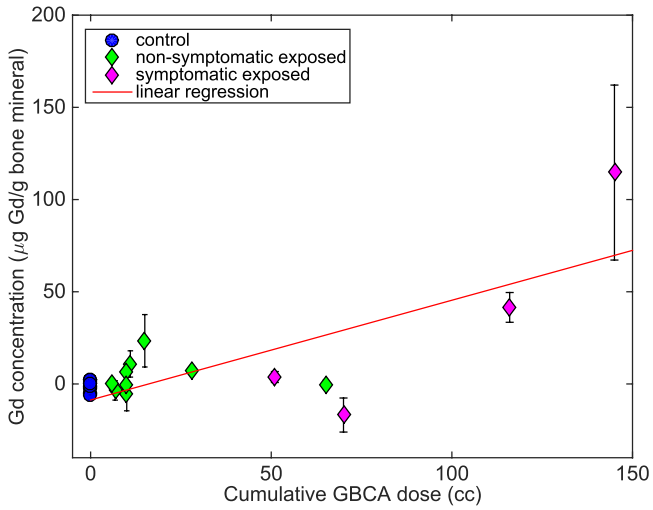

Figure 3. Linear regression analysis repeated for gadolinium concentration in bone shortly after GBCA administration versus cumulative GBCA dose for control and exposed groups, assuming a 3-year half life for gadolinium in bone. A positive relationship of 0.49 ± 0.09 μg Gd g^{-1} bone mineral per 1 ml of GBCA administered is shown ($p = 0.000\,0078$, $R^2 = 0.57$), with a y-intercept of -3.53 ± 3.49 μg Gd g^{-1} bone mineral. Error bars represent the standard deviation of the calculated gadolinium concentration.

amount of time between the most recent GBCA administration and the XRF bone measurement between subjects. Time between bone Gd measurement and GBCA administration in this group of subjects varied from 5 to 15 years, whereas in the initially reported study the variability in time was significantly less. For example, one additional self-reported symptomatic exposed subject (number 12) had not received any contrast agent since 2002, and a substantial amount of gadolinium initially accumulated in the bone after receiving GBCA could have depleted during the time between administration and the XRF bone measurement. A half life correction of 3 years resulted in the strongest correlation between gadolinium concentration in bone and cumulative GBCA dose, increasing the correlation coefficient from $R^2 = 0.14$ to $R^2 = 0.57$, suggesting a relatively short half-life of a few years. While this correction gives a significant improvement in the regression analysis, it is only a first order correction. It may not be true that the same half life applies to each subject in this study, given the wide range of GBCAs used and the variation in age and sex of the participants. In addition, the improved significance in the regression analysis heavily relies on the two highest data points, one of which has the largest uncertainty. A half life of 3 years is a suggested starting point for further assessment of gadolinium in bone. This, however, deserves further study to see how bone gadolinium half life may drive urinary and blood levels.

All linear regression analyses performed in this study include control data to avoid any potential bias. However, the inclusion of 11 control data points with zero dose has the potential to give an uneven weight to the

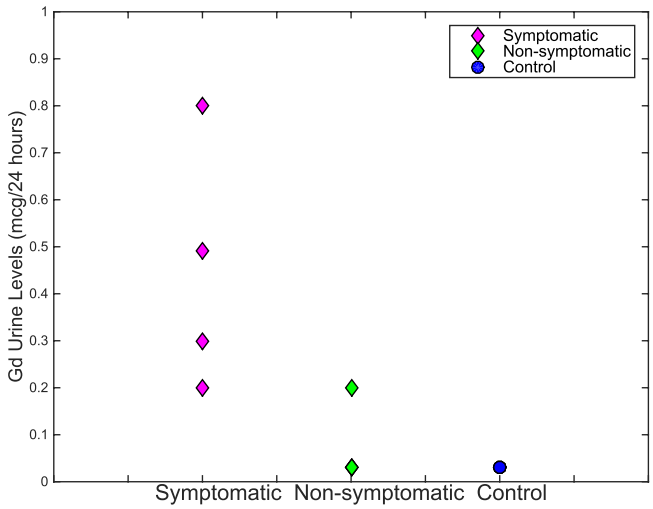

Figure 4. Gadolinium urine levels for control and exposed groups: four control subjects, four self-identified symptomatic exposed subjects, and four non-symptomatic exposed subjects; with mean gadolinium urine levels being 0.05, 0.45, and 0.09 μg/24 h, respectively. Urine levels for symptomatic exposed subjects are significantly higher than both control and non-symptomatic subjects ($p = 0.02$ and $p = 0.05$, respectively).

regression. For this reason, we repeated the linear regression analyses of gadolinium concentration in bone and GBCA dose, excluding the control data. Repeating the regression analysis for the relationship in figure 2 results in an insignificant relationship between gadolinium concentration in bone and GBCA dose ($p = 0.45$). Therefore, the addition of the control data to the regression analysis causes the significance of this relationship. However, repeating the regression analysis without control data for the relationship in figure 3 results in the relationship between gadolinium concentration in bone and GBCA dose with the 3-year half-life correction remaining highly significant ($p < 0.01$).

Due to limitations with our study samples groups (discussed below), further investigation with fewer variables is required to better understand the relationship between bone gadolinium, GBCA dose, and time since administration.

4.2. Gadolinium in urine

The symptomatic exposed group demonstrated the highest mean urinary gadolinium level, being significantly higher than both control and non-symptomatic exposed groups. This suggests either (a) there is a difference in gadolinium excretion between symptomatic and asymptomatic individuals, or (b) self-reported symptomatic individuals received a higher initial GBCA dose which has been retained in a long-term body compartment that does not appear to be bone. The only subjects to have detectable levels of gadolinium in their urine are those who have received multiple doses of GBCAs. Subjects who had only received a single dose of GBCA have an undetectable amount of gadolinium in their urine. A noteworthy discrepancy in this data is the range of time elapsed between the last GBCA administration and the date of urine collection in the subjects, which ranges from 1.5 to 14 years. The presence of gadolinium in urine is assumed to come from various long term storage sites in which gadolinium is distributed throughout the body.

The biodistribution of gadolinium in the body is complex, with little known about storage compartments in humans. Since the majority of administered GBCA is excreted within the first 24 h, the long term excretion of gadolinium in urine suggests long term storage sites in the body.

Over the past 5 years there has been a large focus on gadolinium retention in the brain, as multiple studies have detected gadolinium in the brain, either through high T1-weighted signal intensities or inductively coupled plasma mass spectrometry of autopsy samples (Errante *et al* 2014, Kanda *et al* 2014, 2015, 2016, McDonald *et al* 2015, Quattrocchi *et al* 2015, Radbruch *et al* 2015, Murata *et al* 2016). Robert *et al* compared long-term brain elimination kinetics after repeated injections of GBCAs, and found a large fraction of administered gadolinium retained in the brain after one year for linear GBCAs (Robert *et al* 2018). However, in a set of autopsy samples, Murata *et al* found gadolinium concentration in bone samples to be 23 times larger than gadolinium concentration in brain, suggesting that brain is not the main storage site for gadolinium in the body (Murata *et al* 2016).

As briefly mentioned in the introduction, bone is another organ in which gadolinium has been found to accumulate in higher concentrations (Gibby *et al* 2004, White *et al* 2006, Darrah *et al* 2009, Murata *et al* 2016). In our pilot study, which used XRF to measure gadolinium in bone, we were able to detect gadolinium up to 5 years following the administration of GBCA (Lord *et al* 2017a). In this study, we were able to detect gadolinium

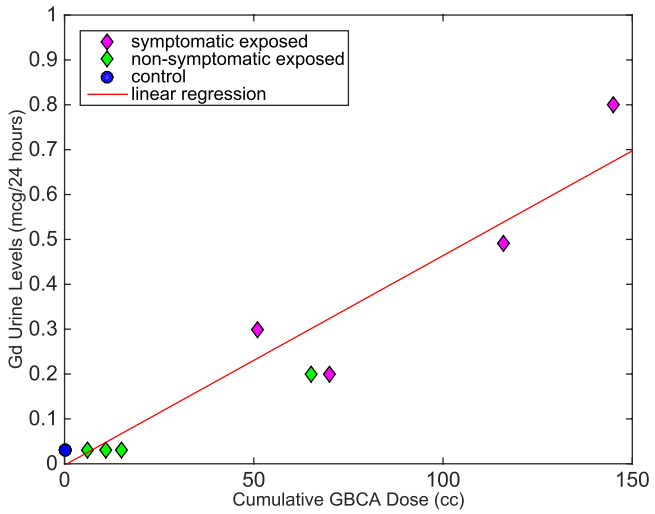

Figure 5. Gadolinium levels in urine versus cumulative GBCA dose (various brands) for control and exposed groups: four control subjects, four symptomatic exposed subjects, and four non-symptomatic exposed subjects. A positive relationship of 0.0047 ± 0.0004 μg/24 h per 1 ml of GBCA administered is shown ($R^2 = 0.91$, $p = 0.000\,000\,98$), with a y-intercept of 0.0158 ± 0.0635 μg/24 h.

in bone in an individual (exposed subject 12) who last received contrast 8 years prior to the XRF measurement, and an individual (exposed subject 15) who last received contrast 15 years prior to the XRF measurement, suggesting that the retention of gadolinium in bone is long-term. In addition to observation of long-term retention, gadolinium is one of the greatest competitive inhibitors for calcium ions (Ca^{2+}) and is therefore likely to replace calcium in bone (Sherry *et al* 2009).

Since (a) there is a correlation between gadolinium levels in urine and GBCA dose and (b) there is not a correlation between gadolinium in urine and current gadolinium in bone, there is an implication that bone is not the only significant long term storage site for Gd in the body. Other organs that have been reported to retain significant levels of gadolinium are the liver and kidneys (Tweedle *et al* 1995, Aime and Caravan 2009, Maximova *et al* 2016, Bussi *et al* 2018). However, there is limited information on gadolinium storage for these organs in the human body, as the majority of biodistribution studies have been carried out in animal populations. Therefore, the source of gadolinium in urine is complex, and a first order correction cannot be applied to account for the difference in time between GBCA administration and urine collection, as was previously done for gadolinium in bone.

The strong correlation between gadolinium levels in urine and cumulative GBCA dose in 12 subjects (four control, four symptomatic exposed, and four non-symptomatic exposed) indicates that gadolinium content in urine increases linearly as the total dose of GBCA increases. The regression analysis for the relationship between gadolinium levels in urine and GBCA dose was repeated excluding control data to investigate if multiple zero dose data points caused an uneven weight to the regression. Excluding control data from the linear regression did not cause a significant change in the relationship, as $p < 0.01$ with or without the control data.

4.3. Relationship between gadolinium in bone and gadolinium in urine

No apparent relationship was observed between current gadolinium levels in urine and current gadolinium concentration in bone. The fact that urine levels do not correlate with bone concentration could be a result of limitations from the study population, differing biokinetics and/or pharmacokinetics for each group, or the fact that there could be another major storage site for gadolinium other than bone. Further study is required to investigate the long term storage of gadolinium in the body.

4.4. Study limitations

This study has limitations due to both the XRF technology and the study population. As discussed in detail in the previously published pilot study, statistical limitations of the XRF system lead to a minimum detection limit, which is the minimum bone gadolinium concentration that can be detected with the XRF system. Our Cd-109 activation source is constantly decaying with a half-life of 461 d, resulting in larger concentrations of gadolinium in bone being undetectable, over time. Our original pilot study had a minimum detection limit of 2.3 μg Gd g^{-1} bone mineral for the original 11 control and 11 non-symptomatic exposed subjects, which increased to 2.6 μg Gd g^{-1} bone mineral for the additional four control and four symptomatic exposed subjects. Therefore, gadolinium concentrations less than 2.6 μg Gd g^{-1} bone mineral were not detected for the additional measurements completed in this study.

All statistical tests were carried out on a small population: 15 control, four self-reported symptomatic exposed, and 11 non-symptomatic exposed, for gadolinium in bone; four control, four self-reported symptomatic exposed, and four non-symptomatic exposed, for gadolinium in urine. In addition to a small study population, subjects are a mixed sample population having received various brands and doses of GBCA at different times. Some self-reported individuals reported the brand and date of administration for GBCA received, but did not have access to their dose. Since recommended clinical practise is to inject GBCAs on a per weight basis, we estimated dose from these patients based on their mass and recommended dose for the particular GBCA brand used. Obviously, this would assume there was no significant deviation in their weight from the time they were injected to the time we performed our measurements. For the symptomatic-exposed group, there may be a selection bias since the self-identified symptomatic individuals have self-reported themselves as having symptoms corresponding to having received GBCA and potentially may have used observation of Gd in urine in that self assessment. A large population with fewer variables with respect to GBCA brand, dose, and time of administration is required in future studies to draw any significant clinical conclusions about gadolinium retention.

5. Conclusion

In conclusion, additional *in vivo* XRF measurements of bone gadolinium, and urinary gadolinium measurements have been conducted for control, self-identified symptomatic exposed, and non-symptomatic exposed individuals. Although the group sample sizes were small, significant differences in gadolinium levels in bone and urine were observed between individuals who report symptoms of gadolinium toxicity and those who do not. Differences may be attributable to the initial GBCA dose. From these data, there seems to be no relationship between gadolinium in bone and gadolinium excreted in urine, suggesting that the retention of gadolinium in the body is complicated, involving multiple long-term storage sites. To truly understand gadolinium retention in the body, a large scale study with less variability in GBCA dose, brand, and time of administration, is required. We believe *in vivo* XRF measurements of gadolinium in bone, as well as urinary gadolinium measurements, have the potential to provide data to fill important knowledge gaps with respect to the clinical significance of gadolinium uptake and retention.

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Supplementary Material

Table E1

Exposed subject demographics, GBCA dose information, and gadolinium concentration in bone.

Subject	Age	Sex	Mass (kg)	GBCAs received	Cumulative GBCA Dose (cc)	Date of last GBCA	Gd in Bone (µg Gd/g bone mineral)	+/-
E1	66	M	110	Gadobutrol (Gadovist)	11	2012	4.31	2.82
E2†	51	M	72	Gadobutrol (Gadovist) Gadodiamide (Omniscan) Gadopentetic Acid (Magnevist)	65‡	March 2016	-0.26	2.09
E3	51	F	75	Gadobutrol (Gadovist)	15	2012	9.29	5.62
E4	57	F	68	Gadobutrol (Gadovist)	6.8	2012	-1.39	2.08
E5†	30	F	59	Gadobutrol (Gadovist)	6	May 2016	0.11	2.12
E6	55	M	100	Gadobutrol (Gadovist)	10	2012	2.57	1.97
E7	50	F	68	Gadobutrol (Gadovist)	10	2012	-2.17	3.60
E8	31	M	86	Gadobutrol (Gadovist)	10	2012	-0.26	2.33
E9†	53	F	NA	Gadoteridol (Prohance)	NA	June 2016	0.91	2.01
E10†	65	M	68	Gadodiamide (Omniscan)	28	February 2016	6.48	2.16
E11†	32	M	NA	NA	NA	August 2014	-4.83	4.01
E12†♦	72	M	69	Gadodiamide (Omniscan) Gadopentetic Acid (Magnevist)	115‡	October 2009	9.08	1.71
E13†♦	64	F	52	Gadoversetamide (Optimark) Gadobenic Acid (MultiHance)	70	November 2011	-5.85	3.19
E14†♦	32	M	86	Gadopentetic Acid (Magnevist) Gadodiamide (Omniscan) Gadoversetamide (Optimark)	50‡	2014	2.37	1.87
E15†♦	62	M	70	Gadopentetic Acid (Magnevist) Gadodiamide (Omniscan) Gadobenic acid (MultiHance) Gadobutrol (Gadovist)	145	2002	4.51	1.86

Note – F = female, M = male, E = exposed, NA = data not available

† Subject self-reported GBCA administration
‡ Some doses were estimated from subject mass and recommended GBCA dose for the specific brand. This assumes they have the same approximate weight from time of administration to time of measurement
♦ Subject self-identified as having symptoms from receiving GBCAs

Table E2

Control subject demographics, and gadolinium concentration in bone.

Subject	Age	Sex	Gdin Bone (µg Gd/g bone min)	+/-
C1	64	M	2.21	2.90
C2	48	M	0.98	1.81
C3	52	F	-4.94	2.88
C4	60	F	-5.66	3.07
C5	26	F	0.33	1.91
C6	60	M	-1.08	2.22
C7	51	F	-5.22	2.12
C8	26	M	-1.61	1.98
C9	53	F	-2.26	3.13
C10	64	M	2.17	2.65
C11	32	M	1.59	2.96
C12	67	M	-6.25	2.04
C13	61	F	2.59	1.86
C14	38	M	-0.88	2.05
C15	65	M	0.08	1.62

Note – C = control

Table E3

Exposed and control subject demographics, GBCA dose information, and 24-hour urine gadolinium measurement results.

Subject	Age	Sex	Mass (kg)	GBCAs received	Cumulative GBCA Dose (cc)	Date of last GBCA	Urine collection date	Gadolinium urine level (mcg/24hr)
E1	66	M	110	Gadobutrol (Gadovist)	11	2012	December 2017	**<0.1**
E2†	51	M	72	Gadobutrol (Gadovist) Gadodiamide (Omniscan) Gadopentetic Acid (Magnevist)	65‡	Mar-16	December 2017	**0.2**
E3	51	F	75	Gadobutrol (Gadovist)	15	2012	December 2017	**<0.1**
E5†	30	F	59	Gadobutrol (Gadovist)	6	May-16	January 2018	**<0.1**
E12†♦	72	M	69	Gadodiamide (Omniscan) Gadopentetic Acid (Magnevist)	115‡	Oct-09	June 2017	**0.49**
E13†♦	64	F	52	Gadoversetamide (Optimark) Gadobenic acid (MultiHance)	70	Nov-11	April 2017	**0.2**
E14†♦	32	M	86	Gadopentetic Acid (Magnevist) Gadodiamide (Omniscan) Gadoversetamide (Optimark)	50‡	2014	July 2016	**0.3**
E15†♦	62	M	70	Gadopentetic Acid (Magnevist) Gadodiamide (Omniscan) Gadobenic acid (MultiHance) Gadobutrol (Gadovist)	145	2002	December 2015	**0.8**
C1	64	M	NA	NA	NA	NA	January 2018	**<0.1**
C4	60	F	NA	NA	NA	NA	January 2018	**<0.1**
C5	26	F	NA	NA	NA	NA	December 2017	**<0.1**
C14	38	F	NA	NA	NA	NA	December 2017	**<0.1**

Note – F = female, M = male, E = exposed, C = control, NA = data not available

† Subject self-reported GBCA administration

‡ Some doses were estimated from subject mass and recommended GBCA dose for the specific brand

♦ Subject self-identified as having symptoms from receiving GBCAs