Surveillance Results of Depleted Uranium-Exposed Gulf War I Veterans: Sixteen Years of Follow-Up

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Surveillance Results of Depleted Uranium–Exposed Gulf War I Veterans: Sixteen Years of Follow-Up

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As part of a longitudinal surveillance program, 35 members of a larger cohort of 77 Gulf War I veterans who were victims of depleted uranium (DU) “friendly fire” during combat underwent a 3-day clinical assessment at the Baltimore Veterans Administration Medical Center (VAMC). The assessment included a detailed medical history, exposure history, physical examination, and laboratory studies. Spot and 24-h urine collections were obtained for renal function parameters and for urine uranium (U) measures. Blood U measures were also performed. Urine U excretion was significantly associated with DU retained shrapnel burden (8.821 mg U/g creatinine [creat.] vs. 0.005 mg U/g creat., p = .04). Blood as a U sampling matrix revealed satisfactory results for measures of total U with a high correlation with urine U results (r = .84) when urine U concentrations were >0.1 mg/g creatinine. However, isotopic results in blood detected DU in only half of the subcohort who had isotopic signatures for DU detectable in urine. After stratifying the cohort based on urine U concentration, the high-U group showed a trend toward higher concentrations of urine β2 microglobulin compared to the low-U group (81.7 vs. 69.0 μg/g creat.; p = .11 respectively) and retinol binding protein (48.1 vs. 31.0 μg/g creat.; p = .07 respectively). Bone metabolism parameters showed only subtle differences between groups. Sixteen years after first exposure, this cohort continues to excrete elevated concentrations of urine U as a function of DU shrapnel burden. Although subtle trends emerge in renal proximal tubular function and bone formation, the cohort exhibits few clinically significant U-related health effects.

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Longitudinal surveillance of toxicant-exposed populations permits assessment of health effects as a function of exposure dose over time. When exposures are ongoing, such as those encountered in the workplace, and when exposures have ceased, such follow-up proves valuable. In the latter instance, prospective surveillance allows a protracted sampling window of time to observe potential adverse health effects of long latency, including cancers that may appear years after first exposure (Consonni et al., 2008). In the case of ongoing exposure, such as in occupational settings, surveillance is one method used to track and prevent potential adverse health effects that may occur over time in exposed workers.

One cohort experiencing an ongoing exposure to a toxicant that likely accumulates in target tissues over time is the group of Gulf War I veterans exposed to depleted uranium (DU). In February 1991, approximately 115 U.S. service members in armored tanks and fighting vehicles were mistakenly fired upon by other U.S. forces using DU penetrators (OSAGWI, 2000). Uranium’s high density provides armor-piercing capacity and its pyrophoric character at high temperature allows small particulates to ignite. At impact, small shards (spall) of the target’s surface are also formed such that an aerosol with a particulate component is produced. (Army Environmental Policy Institute, 1995; Toohey, 2003).

More than 10 fatalities occurred and an additional 50 casualties required medical care. The principal injuries were traumatic, but inhalation and wound contamination with DU
Uranium Toxicity

Uranium, a naturally occurring, radioactive heavy metal found in the earth’s crust, is composed of three isotopes, \(^{238}\text{U}\), \(^{235}\text{U}\), and \(^{234}\text{U}\) (McDiarmid & Squibb, 2001). DU is a man-made form of U, created during the U enrichment process during which the more highly radioactive isotopes, \(^{235}\text{U}\) and \(^{234}\text{U}\), are removed from natural U, leaving a U metal with a lower \(^{235}\text{U}/^{238}\text{U}\) isotopic ratio. This depleted U (DU) poses a lower radiological hazard than natural U since its specific activity is approximately 60% that of natural U (Army Environmental Policy Institute, 1995), but poses a similar chemical hazard to human health (The Royal Society, 2001, 2002).

The kidney has long been recognized to be the most sensitive organ to U’s bioeffects (Leggett, 1989; Hodge et al., 1973; Tannenbaum, 1951) and is considered to be the primary target organ following acute and chronic exposures to soluble U compounds (McDiarmid & Squibb, 2001; Parkhurst, 2003; Cross et al., 1981; Leach et al., 1970, 1973; Mitchel et al., 1999). U accumulation in the kidneys with chronic exposure depends on the relative rates of exposure and elimination of the metal (Squibb et al., 2005; The Royal Society, 2001, 2002). A recent review of the aerosol exposures modeled by the Capstone study (Parkhurst et al., 2004), which used data from simulated DU munition/tank explosions to calculate theoretical exposure concentrations, concurred with the determination that the kidney is the “critical organ” of toxicologic concern for this exposure scenario (NRC, 2008).

There is some evidence from animal studies that other organ systems, including the skeleton and the reproductive and central nervous systems, may also be affected by U exposure (NRC, 2008); thus evaluations of these organ systems were also included in this surveillance study. As with the kidney, U is stored in the skeleton of occupationally exposed individuals (Singh et al., 1987; Kathren et al., 1989; Donoghue et al., 1972) at higher concentrations than those measured in humans with ambient exposure (Harley et al., 1999). Kinetic studies indicate that U initially deposits on bone surfaces and slowly redistributes to the volume of the bone (ICRP, 1995; Guilmette et al., 2004). Limited data suggest that chronic exposure to U in drinking water alters bone metabolism (Kurttio et al., 2005); however, effects of chronic U exposure on bone have not been well studied.

The carcinogenicity attributed to U and, more specifically, DU is also a concern in exposed populations. In vitro and in vivo studies showed that natural U and DU may be genotoxic; however, these forms of U do not appear to be highly carcinogenic (NRC, 2008). Epidemiological studies involving occupationally exposed cohorts show poor evidence for an excess cancer risk in lung, bone or kidney (the most likely targets based on U tissue accumulation long term) (Institute of Medicine, 2000; NRC, 2008).

Based on this knowledge of potential adverse health effects of chronic U exposure, results of the 2007 clinical assessment of the Gulf War DU-exposed cohort, first exposed to DU in 1991, are reported here. Veterans within this group that have retained embedded fragments of DU continue to demonstrate elevated systemic exposure to DU released from the oxidation of their metal fragments in situ. An assessment of the relationships between both cumulative and current U exposure doses and health outcomes is provided. Blood U concentrations were also examined, as a second biomarker of U body burden and exposure. Measures of possible bone effects due to U storage and flux are also presented.

MATERIALS AND METHODS

A subset of 35 members of a larger cohort, which currently numbers 77 DU-exposed Gulf War I veterans who were victims of “friendly fire,” underwent medical surveillance at the Baltimore Veterans Administration (VA) Medical Center between April and June 2007. Three participants in this group were assessed for the first time; the others were seen on several previous biennial surveillance visits (McDiarmid et al., 2001, 2004, 2006, 2007).

Uranium Exposure Assessment

Data analysis for this surveillance visit utilized two types of U exposure metrics as reported previously (McDiarmid et al., 2007). The first method uses the urine U concentration measured at the time of the surveillance visit (current exposure) as the exposure metric which was used for all past surveillance visits (24-h urine U concentration). The second is a cumulative U exposure metric that takes into account the duration of exposure as well as exposure intensity. This metric was used in one previous cohort assessment (McDiarmid et al., 2007).

**Current Urine U Determination.** U concentration and isotopic analysis of 24-h urine samples collected at the surveillance visit were measured by the Armed Forces Institute of Pathology (AFIP) Department of Environmental Toxicologic Pathology (Washington, DC) as previously described (McDiarmid et al., 2007) using an inductively coupled plasma–dynamic reaction cell–mass spectrometer (ICP-DRC-MS) method developed by Ejnik et al. (2005). Urine U concentrations were standardized on the basis of urine creatinine concentrations to account for urine dilution to obtain micrograms U per gram creatinine (Karpas et al., 1998; McDiarmid et al., 2000).

**Cumulative U exposure.** An integrated metric for cumulative U exposure was calculated using the participant’s urine U value at each of the surveillance visits in which they participated.
and the time interval between each of the urine U concentration measurements. An area under the curve (AUC) calculation was derived by modifying the method reported by Chia et al. (1997), who utilized a linear formula for determining cumulative lead exposure. This mathematical operation yielded a time-integrated metric of U burden, the cumulative U index (CumU), based on the periodic urine U concentrations measured at each surveillance visit. (UUr). The calculation of the Cum U is described in detail in a previous paper (McDiarmid et al., 2007).

**Blood U Analysis.** Blood samples were digested using a closed vessel microwave digestion system (CEM MARS Express, Mathews, NC). For quantitative total U analysis, 0.2 ml blood was digested in 2 ml concentrated HNO₃ and 1 ml H₂O₂, with 500 ng/L ²³³U as an internal standard. For the isotopic determinations, 0.5 ml blood was digested in the presence of 2 ml concentrated HNO₃ and 1 ml H₂O₂. All samples were heated to 180°C for 5 min, after which the temperature was maintained at 180°C for additional 5 min to ensure complete digestion. The quantitative analysis of U in blood samples was performed using ICP-DRC-MS (Elan DRC II, manufactured by Perkin-Elmer, Norwalk, CT). The concentration of U in blood was determined using an external calibration curve and ²³³U as an internal standard. The isotopic composition of U was determined using a Thermo Finnigan Element2 sector field ICP-MS (Thermo Electron Corp., Bremen, Germany), equipped with a desolvation system (APEX Q, Elemental Scientific, Omaha, Nebraska). Mass discrimination was corrected using the IRMM interlaboratory comparison materials REIMEP 18A and REIMEP 18C (IRMM, JRC, Geel, Belgium) certified for ²³⁴U, ²³⁵U, ²³⁶U, and ²³⁸U isotope composition. The limit of detection for total U analysis was 4 ng/L using 0.2 ml blood sample. The detection limit for isotopic analysis was 60 ng/L. An isotopic ratio (²³³U/²³⁸U) greater than 0.006 indicated the presence of only natural U in the sample. An isotopic ratio less than 0.003 indicated the presence of DU (Todorov et al., 2007).

**Clinical Assessment**

A 3-day, in-patient hospital clinical assessment included a detailed medical history, an extensive exposure history, a thorough physical examination, and laboratory studies. The laboratory battery included hematological and blood clinical chemistry measures, neuroendocrine and genotoxicological parameters, and semen quality measures. Spot and 24-h urine samples were obtained for measurement of clinical chemistry parameters related to renal function and for urine U determinations. Blood was also collected for a blood U determination. A battery of neurocognitive tests was also included in the protocol.

**Hematological, Renal, and Bone Toxicity Measures**

Hematological parameters, serum and urine creatinine, Ca and PO₄, and serum uric acid measures were evaluated by the VA clinical laboratory using standard methodologies. Aliquots of urine were taken and immediately neutralized using 0.5 N NaOH for β₂-microglobulin analysis. They were analyzed by latex-enhanced nephelometry by Quest Diagnostics Incorporated (San Juan Capistrano, CA). Total protein was measured by the Baltimore VA Clinical Lab using the M-TP microprotein assay from Beckman Coulter that uses pyrogallol red for detection. (Watanabe et al., 1986). The 24-h urine samples were collected in multiple containers per study participant and each container was analyzed separately for total protein and creatinine (McDiarmid et al., 2007). Markers of nephrotoxicity (urine retinol binding protein [RBP], microalbumin [mAlb]), urine intestinal alkaline phosphatase (IAP), and N-acetyl-D-glucosaminidase (NAG) were measured by the Department of Nephrology–Hypertension, University of Antwerp, (Edegem-Antwerp, Belgium), as previously described (McDiarmid et al., 2007).

**Markers of Bone Metabolism**

Bone metabolism was assessed using both urine and serum markers. Bone resorption was evaluated by measuring urine N-telopeptide (NTX) levels in the second morning void specimen. The samples were analyzed by enhanced chemiluminescence at Quest Diagnostic, Incorporated (San Juan Capistrano, CA). Bone formation was assessed by serum bone-specific alkaline phosphatase using the immunoenzymatic methodology by Quest Diagnostic, Incorporated (San Juan Capistrano, CA). Intact parathyroid hormone (PTH) in serum was determined by chemiluminescence immunoassay at Quest Diagnostics, Incorporated (Arbutus, MD). Serum 25-(OH) vitamin D and 1,25-(OH)₂ vitamin D were measured at Quest Diagnostics, Incorporated (Chantilly, VA) by liquid chromatography/mass spectrometry (LC/MS) and radioimmunoassay, respectively. Serum estradiol was measured by Bayer Centaur chemiluminescence at Quest Diagnostics, Incorporated (Arbutus, MD). Twenty-four-hour urine calcium and sodium were assayed using indirect potentiometry, and 24-h urine phosphorus was measured using the phosphomolybdate method at the Baltimore VAMC.

**Neurocognitive/Psychiatric Assessment**

Four neurocognitive and psychiatric impairment indices were constructed from a battery of neurocognitive tests described previously (McDiarmid et al., 2004, 2007). Three impairment indices (accuracy, speed, and throughput) were derived from selected measures of the Automated Neuropsychological Assessment Metrics (ANAM) test system. The Accuracy Index (percent correct), Speed Index (median response time for correct responses), and Throughput Index (a computed score combining speed and accuracy) were derived from the ANAM test system (Reeves et al., 2007). Impairment indices are a common method used to summarize performance on a battery of neurocognitive measures (Heaton
et al., 2004). An additional Index of Cognitive Efficiency (ICE) derived largely from the throughput scores and described previously determined was also used (McDiarmid et al., 2007).

Reproductive Health Measures

Neuroendocrine parameters. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, thyroid-stimulating hormone (TSH), free thyroxine, and total testosterone were analyzed at the Baltimore VA clinical laboratory by enzyme immunoassay using a Beckman Coulter Testosterone were analyzed at the Baltimore VA clinical laboratory by enzyme immunoassay using a Beckman Coulter Access 2 Analyzer.

Semen characteristics. Semen was collected from study participants who agreed to participate in the semen analysis portion of the 2007 assessment (n = 17). Analysis of semen volume, sperm concentration, total sperm count, and functional parameters of sperm motility was conducted as previously described (McDiarmid et al., 2004).

Genotoxicity Measures

Chromosomal aberrations (CAs). Peripheral blood lymphocytes were cultured for the examination of background frequencies of chromosomal aberrations (CA) using standard methods (Evans & O’Riordan, 1975; Swierenga et al., 1991). Briefly, cells were cultured for 48 h. After staining, 200 cells were examined from each sample for CA.

Hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutation assay. Venous blood samples (30 ml) were obtained in heparinized vacuum tubes in Baltimore and sent at ambient temperature by overnight airmail to the BioMosaics laboratory in Burlington, VT. On receipt, blood samples were centrifuged and the mononuclear cell fractions (containing the lymphocytes) were separated, washed, counted, and cryopreserved in liquid nitrogen. Samples were analyzed as described previously (McDiarmid et al., 2004). The ratio of cloning efficiency (CE) in the presence of 6-thioguanine to the CE in the absence of 6-thioguanine selection defined the mutation frequency (MF).

Fluorescent in situ hybridization (FISH) assay. FISH analysis of metaphase cells for the detection of low-level chromosome abnormalities involving targeted chromosomes was conducted by the University of Maryland School of Medicine Cytogenetics Laboratory (Baltimore, MD) using the method described by Zhang et al. (1998, 1999). In this study, metaphase cells from peripheral blood specimens were prepared using standard cytogenetic procedures. FISH was performed using a D5S721(5p15.2)/EGR1(5q31) probe set, an ELN(7q11.23)/D7S486(7q31) probe set, an MLL probe set at 11q23, and a D13S319 probe at 13q14 to detect abnormalities involving chromosomes 5, 7, 11, and 13, respectively. Two hundred metaphase cells from each subject were examined with each probe set. These probes were obtained from Vysis (Downers Grove, IL) and were validated in accordance with the recommendations of the American College of Medical Genetics. The hybridization procedures were performed following the manufacturer’s instructions. The images were acquired using an Olympus Provis fluorescence microscope and an Applied Imaging system and its software (Applied Imaging International Ltd., Newcastle Upon Tyne, UK).

Statistical Data Analysis

Urine uranium as a binary variable. Two exposure groups, high (n = 10) versus low (n = 25), were determined based on each individual participant’s current (2007) urine U results or their cumulative urine U exposure metric. As in previous years (McDiarmid et al., 2001, 2004, 2006, 2007), high exposure was defined as current urine U concentrations greater than or equal to 0.1 μg U/g creatinine, a value between 0.043 (the 95% percentile reported for creatinine-adjusted urine U concentration in non-exposed populations in the United States (NHANES, 2003) and 0.35 μg U/L reported as a urine U upper limit that occurs naturally in areas with elevated U in water and food (ICRP, 1974). Data were also analyzed using the cumulative U exposure metric using a cut point of 10 μg U/g creatinine years. This cut point was chosen based on the distribution of the data, which showed a natural break at this cumulative dose. This cut point gave the same n value for the high-U group as the 0.1 μg U/g creatinine current U cut point, but the individual participants in each group differed by one participant.

Tests of differences in high versus low urine U groups. For each outcome, differences in outcome measures of distribution location (e.g., median) between high and low urine U groups were examined using the Mann–Whitney U test (Wilcoxon rank sum test), which assumes equally shaped distributions (Woolson, 1987), although they can differ in their means. SPSS 12.0 (Statistical Products and Service Solutions, 2003) was used for these tests. To test the assumption of equally shaped distributions, the two-sample version of the Kolmogorov–Smirnov test was used to compare the shapes of the distributions. In none of the comparisons did one detect significantly unequal distribution shapes. Hence, the Mann–Whitney exact test was used for all comparisons of high- versus low-U groups. Mean differences were considered statistically significant when p < .05. However, attention was paid to differences with p values of .2 or less because this is a surveillance program and it is important to look for sentinels of effect and trends in data from year to year.

HPRT MF means were adjusted for cloning efficiency (CE) and age. Methods for cloning efficiency and age adjustment were derived by combining the data from three previous time points and regressing the natural log of mutation frequency (lnMF) on the age at that time point, CE and time point (a categorical variable). Subject identification (ID) was included as a categorical random effect to take into account the correlation between multiple observations on the same person (some of which is due to exposure).
The coefficients for CE and age were then used to adjust the lnMF values to the average CE (.28) and age (39). The coefficients were similar to those previously obtained for healthy individuals (Finette et al., 1994). The adjusted values were computed as follows:

\[ \ln MF_{a1} = \ln(MF) - 1.648(0.28 - CE) \]

\[ \ln MF_{a2} = \ln(MF) - 1.620(0.28 - CE) + 0.0116(39 - age) \]

The antilog was then computed to produce mean values of mutation frequency adjusted for (a) CE and (b) CE and age.

**Association between natural logarithm of urine U (ln[urine U]) and health outcome measures.** Because of the possible presence of outliers, robust regression, which down-weights outliers, was used to study the association between the neurocognitive indices and the ln[continuous urinary U]. Because any nonlinearity of continuous urinary U in the linear regression model for the outcome measures might produce apparent outliers, the association between ln[continuous urinary U] and HPRT and neurocognitive endpoints was also studied using fractional polynomial transformations of ln[continuous urinary U]. The relative contributions of covariates were tested to the fit by the process of backward elimination to determine whether any covariate would become significant, or by testing the linear association between each potential covariate and each outcome. Fractional polynomial transformations were done using STATA 2003 (StataCorp, 2003).

**RESULTS**

Thirty-five members of a cohort of 77 veterans of the 1991 Gulf War involved in DU “friendly-fire” incidents participated in this seventh round of surveillance at the Baltimore VA Medical Center in the spring of 2007. As seen in Table 1, the demographics of this subgroup are similar to the full cohort with respect to age and race. In addition, note that three members of this subcohort participated in the assessment for the first time.

**Urine and Blood U as Measures of Depleted Uranium Exposure**

Urine U excretion is a reliable measure of ongoing systemic exposure to U in the DU-exposed Gulf War cohort. Figure 1 shows the distribution of urinary U concentrations (from low to high) in the cohort of 35 veterans who participated in the 2007 surveillance visit. Approximately 40% (n = 14) of these individuals showed evidence of retained shrapnel when evaluated by an x-ray plain film skeletal survey (Hooper et al., 1999; Squibb & McDIarmid, 2006). The urine U concentrations range from approximately 0.001 to 60 μg/g creatinine. This range is similar to that observed in past surveillance visits. U excretion is above the NHANES 95th percentile of the distribution of values of 0.034 μg/g creatinine for veterans with DU detected in their urine, and highest in veterans known to have embedded fragments (mean = 8.821 μg U/g creat. vs. 0.005 μg U/g creat., for veterans with no embedded fragments, p=.04). The presence of DU in the urine was determined by isotopic analysis, using a 235U/238U ratio of 0.003 or less as an indication that DU is present in the urine sample. Twelve of the 35 individuals in this group showed evidence of DU, as indicated by the DU isotopic ratio.

Blood as a sampling matrix is not better than urine for detecting DU exposure in this group of veterans. As shown in Figure 2, blood total U concentrations ranged from approximately 0.001 to 0.8 μg/L. Veterans known to have DU embedded fragments had higher blood U concentrations than those without DU fragments; however, DU (based on 235U/238U ratio analysis) was detected in only 6 of the 35 individuals in the cohort. Current analysis methods for U isotopes in blood are not as sensitive as methods recently developed for urine samples. Blood and urine total U concentrations in this cohort were closely associated, however, particularly in the veterans with DU shrapnel (Figure 3). Regression analysis gave an r value of .84 for paired blood/urine samples when urine U concentrations were above 0.1 μg/g creatinine. For urine U concentrations less than 0.1 μg/g creatinine, the r value dropped to .07, due most likely to a lack of equilibrium between U from recent dietary exposure and body stores of U.

**Clinical Findings**

There were no statistically significant differences observed between the high and low urine U groups in the hematology

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
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<tbody>
<tr>
<td>Demographic Characteristics of the 2007 Participantsa Compared to All Participantsb</td>
</tr>
<tr>
<td>2007 Cohort (n = 35)</td>
</tr>
<tr>
<td>Race</td>
</tr>
<tr>
<td>Caucasian</td>
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<tr>
<td>African American</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
<tr>
<td>Asian American</td>
</tr>
<tr>
<td>Native American</td>
</tr>
<tr>
<td>Agec</td>
</tr>
</tbody>
</table>

aIncludes three veterans seen for the first time (Gulf War I only). bAll participants enrolled in DU Follow-up Program (Gulf War I only). cMean age at time of 2007 evaluation (± standard deviation).
parameters and all results were within the normal range. The same was true for the clinical chemistry results with the exception of a marginally elevated mean bilirubin in the low-U group compared to the high-U group (0.74 mg/dl vs. 0.5 mg/dl; \( p < .01 \); normal (0–1.3 mg/dl). Triglycerides were markedly elevated in the low-U group, and outside the normal range, but not statistically different from the high-U group (all data not shown).

**Neuroendocrine measures.** Neuroendocrine measures showed no difference in mean results when comparing low versus high urine U groups. These mean values were also all within the normal range with the exception of prolactin for which both the low- and high-U group means were slightly (less than 10%) above the upper limit of normal and have been observed in prior years (data not shown). The effects of current medications were also considered in the neuroendocrine measure comparisons and found not to influence any of the outcomes.

**Biomarkers of renal effects.** Table 2 presents the results of urine and serum measurements that provide an assessment of renal function. A comparison of the means between the low- and the high-U groups indicates that there is no evidence of clinically or statistically significant changes in renal function resulting from the U exposure experienced by the veterans with embedded DU fragments. Differences between the means of only five parameters approach statistical significance, with \( p \) values of \( \leq .11 \), for glomerular filtration rate, serum glucose, serum creatinine, and urine beta-2 microglobulin and retinol binding protein concentrations. However, the mean of the high group is lower than that of the low group for the blood glucose and the serum creatinine concentrations, which is not the direction of change one would expect to see for either of these if renal function were impaired. These differences do not appear to be related to differences in body mass index (BMI) between the two groups since the mean BMI for the low versus high...
FIG. 2. Distribution of blood uranium concentrations (from low to high) in Gulf War I DU-exposed soldiers who participated in the 2007 health surveillance visit at the Baltimore VA Medical Center. The solid line drawn at 0.14 μg U/L is a blood U concentration value reported by Fisene and Perry (1985) for New York City residents with no known occupational exposure to U. DU was detected by isotopic analysis in the blood of 6 soldiers (solid diamonds). Analysis of one sample (solid square) identified an isotopic ratio consistent with natural U. Open diamonds indicate samples for which the total U concentration was too low to accurately measure the ratio of the $^{235}U/^{238}U$ isotopes. The mean blood U concentration in soldiers with urine U concentrations less than 0.1 μg/g creatinine was 0.02 ± 0.004 μg/L, while the concentration in soldiers with urine U concentrations ≥0.1 μg/g creatinine was 0.22 ± 0.092 μg/L.

FIG. 3. Blood and urine U concentrations for the Gulf War I DU-exposed soldiers participating in the 2007 surveillance visit were strongly correlated with each other when compared for all participants ($r = .87$) and for the more highly exposed participants (urine U concentrations ≥0.1 μg/g creatinine) ($r = .84$). In the group of soldiers with urine U concentrations <0.1 μg/g creatinine, the correlation between blood and urine U concentrations dropped to $r = .07$. 
<table>
<thead>
<tr>
<th>Laboratory test (normal range)</th>
<th>Low U group(^a)</th>
<th>High U group(^b)</th>
<th>Mann–Whitney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean ± SE)</td>
<td>(mean ± SE)</td>
<td>(p)</td>
</tr>
<tr>
<td>Urine creatinine (1.5–2.6 g/24 h)</td>
<td>1.56 ± 0.14</td>
<td>1.69 ± 0.14</td>
<td>.43</td>
</tr>
<tr>
<td>Creatinine clearance (97–137 ml/min)</td>
<td>122.60 ± 6.08</td>
<td>150.81 ± 20.09</td>
<td>.44</td>
</tr>
<tr>
<td>Glomerular filtration rate (&gt;90 ml/min)</td>
<td>87.14 ± 3.28</td>
<td>104.94 ± 8.88</td>
<td>.06</td>
</tr>
<tr>
<td>Urine glucose (0.0–0.5 g/24 h)</td>
<td>6.30 ± 4.38</td>
<td>0.12 ± 0.01</td>
<td>.17</td>
</tr>
<tr>
<td>Urine calcium (100–300 mg/24 hr)</td>
<td>127.42 ±15.77</td>
<td>175.66 ±22.80</td>
<td>.14</td>
</tr>
<tr>
<td>Urine PO(_4) (0.4–1.3 g/24 h)</td>
<td>0.83 ± 0.08</td>
<td>0.95 ± 0.08</td>
<td>.57</td>
</tr>
<tr>
<td>Urine (\beta)-microglobulin (0–160 μg/g creatinine)</td>
<td>59.08 ± 7.48</td>
<td>81.72 ± 13.28</td>
<td>.11</td>
</tr>
<tr>
<td>Urine intestinal alkaline phosphatase (IAP) (&lt;2 U/g creatinine)</td>
<td>0.66 ± 0.36</td>
<td>0.57 ± 0.23</td>
<td>.38</td>
</tr>
<tr>
<td>Urine (N)-acetyl-(\beta)-glucosaminidase (NAG) (&lt;5 U/g creatinine)</td>
<td>1.29 ± 0.25</td>
<td>1.23 ± 0.17</td>
<td>.32</td>
</tr>
<tr>
<td>Urine total protein (1–150 mg/24 h)</td>
<td>135.02 ±45.73</td>
<td>106.81 ±18.44</td>
<td>.49</td>
</tr>
<tr>
<td>Urine micro-albumin (&lt;25 mg/g cre)</td>
<td>24.84 ± 22.11</td>
<td>15.62 ± 11.01</td>
<td>.17</td>
</tr>
<tr>
<td>Urine retinol binding protein (&lt;610μg/g cre)</td>
<td>31.00 ± 4.73</td>
<td>48.11 ± 9.73</td>
<td>.07</td>
</tr>
<tr>
<td>Serum glucose (70–105 mg/dl)</td>
<td>111.32 ± 8.29</td>
<td>89.80 ± 3.39</td>
<td>.07</td>
</tr>
<tr>
<td>Serum creatinine (0–1.4 mg/dl)</td>
<td>1.08 ± 0.04</td>
<td>0.95 ± 0.06</td>
<td>.11</td>
</tr>
<tr>
<td>Serum calcium (8.4–10.2 mg/dl)</td>
<td>9.25 ± 0.06</td>
<td>9.37 ± 0.07</td>
<td>.26</td>
</tr>
<tr>
<td>Serum PO(_4) (2.7–4.5 mg/dl)</td>
<td>0.83 ± 0.08</td>
<td>0.95 ± 0.08</td>
<td>.57</td>
</tr>
<tr>
<td>Serum uric acid (3.4–7 mg/dl)</td>
<td>0.52 ± 0.28</td>
<td>0.62 ± 0.19</td>
<td>.17</td>
</tr>
</tbody>
</table>

\(^a\)For <0.10 μg U/g creatinine \((n = 25)\).

\(^b\)For ≥0.10 μg U/g creatinine \((n = 10)\).
Bone metabolism markers. Bone turnover was evaluated by measuring markers of both osteoblast (bone formation) and osteoclast (bone resorption) function. During normal bone turnover, as occurs during bone remodeling in adults, these processes are coupled and highly balanced (Hadjidakis & Androulakis, 2006). Bone-specific alkaline phosphatase (a measure of osteoblast function) concentrations in serum were slightly decreased in the high-U group as compared to the low-U group, where groupings were determined by either the current urine U (11.48 vs. 14.22 μg/L, p = .18; normal range 5.9–22.9 μg/L) or cumulative U measures (11.55 vs. 14.20 μg/L, p = .21) as seen in Table 3. No age effect was observed and these values are within normal limits. The difference between the groups might suggest that osteoblast activity is decreased in those veterans with higher urine U concentrations. In contrast, there was no difference in urine levels of N-telopeptide, a marker of collagen breakdown and bone resorption by osteoclasts. These findings might suggest a clinically insignificant uncoupling of the bone turnover processes in those with higher urinary U concentrations.

Bone metabolism, as a function of calcium phosphate homeostasis, was evaluated by measuring vitamin D and PTH levels and urine calcium and phosphate excretion. The 1,25-dihydroxy vitamin D level was statistically significantly higher in the high-U group as compared to the low-U group for both the current and cumulative U measure as can be seen in Table 3. However, the actual concentrations of the 1,25-dihydroxy vitamin D for both groups are within normal limits. PTH is typically the primary determinant of 1,25-dihydroxy vitamin D levels; however, it does not explain the findings in this population as there is no difference in the intact PTH levels between the high- and low-U groups (Table 3). The difference between the groups is not altered when considering age but may be related to a slightly lower creatinine clearance in the low-U group. Both U groups had similar 25-hydroxy vitamin D levels below the lower limit of normal. This is reflective of national dietary intake and not unexpected. The high-U group had a slightly higher urinary calcium concentration which may be related to the higher 1,25-dihydroxy vitamin D levels or to an uncoupling of bone turnover versus differences in dietary intake or renal function. Markers of endocrine function (testosterone, LH, and FSH) were not different between the two U groups eliminating endocrine dysfunction as a mechanism for bone demineralization (Amin et al., 2000).

Estradiol was previously shown to correlate with bone mineral density in men. Low estradiol levels are associated with high bone turnover and low bone mineral density, as well as risk of osteoporotic fractures (Kuchuk et al., 2007). Estradiol levels are no different between the low- versus the high-U exposure groups. As seen in Table 3 the mean serum estradiol concentration is above the upper limit of normal for the low-U group. This is driven by one outlier and when the individual is removed from analysis the mean is within normal limits and similar to the mean for the high-U group.

Neurocognitive Evaluation. The performance of the 2007 subcohort on the neurocognitive indices shown in Table 4 were generally within normal limits regardless of U group. Correlations between age, IQ, and depression and neurocognitive indices were not significant and not included as covariates in between-group comparisons. There was a single trend for poorer performance by the high-U group (current and cumulative urine U) on the accuracy index of the computerized neuropsychological measure (ANAM); however, this result was not significant at the .05 level (p = .14). This trend for poorer performance on only one additional measure on the computerized testing, on average, is not indicative of a clinically significant difference between the two groups (mean accuracy index: low U = 0.25; high U = 0.34). The mean accuracy index values for both groups overall were generally within normal limits. All other between-group comparisons for the neurocognitive indices were not statistically different. Again for this surveillance visit, fractional polynomial analysis of the NP index, the ANAM indices, and the ICE failed to show evidence of a significant relationship between current U or cumulative exposure and neurocognitive outcome.

Genotoxicologic Results. No statistically significant differences were observed between the high- and low-U groups for two genotoxicity measures examined in this surveillance visit (Table 5). For comparison, Table 6 summarizes the various measures of genotoxicity that have been used to assess this population over time. A sensitive measure of point mutation, sister chromatid exchange (SCE), was used in the earlier years of the surveillance and showed inconsistent results regarding genotoxic insult as a function of U burden. This measure, over time, has been replaced by other methods considered more robust. Using another marker of point mutation, the hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutation frequency (MF) has shown a suggestion of an effect, yielding a higher MF in the high-U group, but never reaching statistical significance. Measures of chromosomal aberrations (CA), including traditional G-banding CA and those identified through fluorescent in situ hybridization (FISH), have failed to show a consistent effect due to U burden. The body of evidence in this cohort shows relatively weak genotoxic effects from U exposure.
<table>
<thead>
<tr>
<th>Laboratory test (normal range)</th>
<th>Low U group&lt;sup&gt;a&lt;/sup&gt; (mean ± SE)</th>
<th>High U group&lt;sup&gt;b&lt;/sup&gt; (mean ± SE)</th>
<th>Mann-Whitney&lt;sup&gt;p&lt;/sup&gt;</th>
<th>Low CumU group&lt;sup&gt;c&lt;/sup&gt; (mean ± SE)</th>
<th>High CumU group&lt;sup&gt;d&lt;/sup&gt; (mean ± SE)</th>
<th>Mann–Whitney&lt;sup&gt;p&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (serum) (0–35 pg/ml)</td>
<td>46.30 ± 15.24</td>
<td>33.00 ± 2.67</td>
<td>.56</td>
<td>46.66 ± 15.22</td>
<td>32.10 ± 2.81</td>
<td>.96</td>
</tr>
<tr>
<td>Bone specific alkaline phosphatase (5.9–22.9 μg/L (serum))</td>
<td>14.22 ± 1.27</td>
<td>11.48 ± 1.58</td>
<td>.18</td>
<td>14.20 ± 1.27</td>
<td>11.55 ± 1.58</td>
<td>.21</td>
</tr>
<tr>
<td>1,25-Dihydroxy vitamin D (15–60 pg/ml&lt;sup&gt;e&lt;/sup&gt; (serum))</td>
<td>42.14 ± 2.73</td>
<td>59.13 ± 6.88</td>
<td>.02</td>
<td>42.45 ± 2.72</td>
<td>58.25 ± 7.20</td>
<td>.05</td>
</tr>
<tr>
<td>25-Hydroxy vitamin D (32–100 ng/ml&lt;sup&gt;f&lt;/sup&gt; (serum))</td>
<td>26.08 ± 2.05</td>
<td>25.20 ± 3.55</td>
<td>.64</td>
<td>27.00 ± 2.30</td>
<td>22.90 ± 2.04</td>
<td>.42</td>
</tr>
<tr>
<td>PTH (intact) (10–65 pg/ml&lt;sup&gt;f&lt;/sup&gt; (serum))</td>
<td>34.29 ± 3.78</td>
<td>33.80 ± 5.74</td>
<td>.99</td>
<td>33.71 ± 3.86</td>
<td>35.20 ± 5.40</td>
<td>.72</td>
</tr>
<tr>
<td>Urine N-teleopeptide (NTX) (0–85 nMol BCE/mMol creatinine)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>28.90 ± 5.16</td>
<td>30.88 ± 4.49</td>
<td>.23</td>
<td>29.81 ± 5.18</td>
<td>28.50 ± 4.35</td>
<td>.64</td>
</tr>
</tbody>
</table>

<sup>a</sup>For <0.10 μg/g creatinine (<i>n</i> = 25).
<sup>b</sup>For ≥0.10 μg/g creatinine (<i>n</i> = 10).
<sup>c</sup>For <10.0 μg/g creatinine years (<i>n</i> = 25).
<sup>d</sup>For ≥10.0 μg/g creatinine years (<i>n</i> = 10).
<sup>e</sup>With <i>n</i> in low group = 22, <i>n</i> in high group = 8.
<sup>f</sup>With <i>n</i> in low group = 24, <i>n</i> in high group = 10.
<sup>g</sup>With <i>n</i> in low group = 21, <i>n</i> in high group = 8.
<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Low U group&lt;sup&gt;a&lt;/sup&gt; (mean ± SE)</th>
<th>High U group&lt;sup&gt;b&lt;/sup&gt; (mean ± SE)</th>
<th>Mann–Whitney</th>
<th>Low CumU group&lt;sup&gt;c&lt;/sup&gt; (mean ± SE)</th>
<th>High CumU group&lt;sup&gt;d&lt;/sup&gt; (mean ± SE)</th>
<th>Mann–Whitney</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANAM Accuracy Index</td>
<td>0.255 ± 0.046</td>
<td>0.340 ± 0.052</td>
<td>.094</td>
<td>0.255 ± 0.046</td>
<td>0.340 ± 0.052</td>
<td>.094</td>
</tr>
<tr>
<td>ANAM Speed Index</td>
<td>0.227 ± 0.046</td>
<td>0.200 ± 0.074</td>
<td>.702</td>
<td>0.212 ± 0.047</td>
<td>0.233 ± 0.071</td>
<td>.787</td>
</tr>
<tr>
<td>ANAM Throughput Index</td>
<td>0.148 ± 0.063</td>
<td>0.150 ± 0.100</td>
<td>.940</td>
<td>0.159 ± 0.063</td>
<td>0.125 ± 0.100</td>
<td>.425</td>
</tr>
</tbody>
</table>

<sup>a</sup>For <0.10 μg/g creatinine (n = 22).  
<sup>b</sup>For ≥0.10 μg/g creatinine (n = 10).  
<sup>c</sup>For <10.0 μg U/g creatinine years (n = 22).  
<sup>d</sup>For ≥10.0 μg/g creatinine years (n = 10).
DISCUSSION
The surgical morbidity associated with removal of multiple small shrapnel fragments precludes the elimination of the ongoing U exposure this friendly-fire veteran cohort experiences. The principal finding of this study corroborates that which was reported in each of our previous surveillance study reports (Hooper et al., 1999; McDiarmid et al., 2000, 2001; Squibb & McDiarmid, 2006, 2007), that urine U excretion remains, even after 16 yr, significantly associated qualitatively with retained shrapnel status (yes/no) and quantitatively with that fragment burden.

Longitudinal surveillance activities, similar to this one, often use a time-integrated exposure metric that incorporates duration of exposure, as well as intensity of exposure, allowing a measure of cumulative exposure burden to be derived (Chia

**TABLE 5**
Comparison of FISH and HPRT Parameters Between Low and High Uranium Groups

<table>
<thead>
<tr>
<th>Genotoxicity parameter</th>
<th>Low U group&lt;sup&gt;a&lt;/sup&gt; (mean ± SE)</th>
<th>High U group&lt;sup&gt;b&lt;/sup&gt; (mean ± SE)</th>
<th>Mann–Whitney &lt;sup&gt;p&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPRT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation frequency (MF106)</td>
<td>20.90 ± 1.75</td>
<td>28.82 ± 9.47</td>
<td>.42</td>
</tr>
<tr>
<td>MF adjusted for cloning efficiency</td>
<td>16.45 ± 1.32</td>
<td>25.27 ± 8.39</td>
<td>.72</td>
</tr>
<tr>
<td>MF adjusted for cloning efficiency and age</td>
<td>16.00 ± 1.30</td>
<td>23.96 ± 7.85</td>
<td>.72</td>
</tr>
<tr>
<td>FISH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 5&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>0.20 ± 0.08</td>
<td>0.10 ± 0.10</td>
<td>.49</td>
</tr>
<tr>
<td>Chromosome 7</td>
<td>0.08 ± 0.06</td>
<td>0.10 ± 0.10</td>
<td>.85</td>
</tr>
<tr>
<td>Chromosome 11</td>
<td>0.08 ± 0.04</td>
<td>0.20 ± 0.13</td>
<td>.15</td>
</tr>
<tr>
<td>Chromosome 13</td>
<td>0.28 ± 0.09</td>
<td>0.20 ± 0.13</td>
<td>.76</td>
</tr>
<tr>
<td>Summary findings (FISH)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of subjects with any mutation</td>
<td>0.48 ± 0.10</td>
<td>0.50 ± 0.17</td>
<td>.92</td>
</tr>
<tr>
<td>Mean number of total mutations per subject</td>
<td>0.64 ± 0.15</td>
<td>0.60 ± 0.22</td>
<td>.95</td>
</tr>
</tbody>
</table>

<sup>a</sup>For <0.10 μg U/g creatinine (n=25).
<sup>b</sup>For ≥0.10 μg U/g creatinine (n=10).
<sup>c</sup>Mean abnormal metaphases per uranium group.
<sup>d</sup>For 200 metaphases counted per subject per chromosome.

**TABLE 6**
Summary of Differences in Genotoxicity Parameters across Evaluations

<table>
<thead>
<tr>
<th>Genotoxicity parameter</th>
<th>Evaluation year</th>
<th>1994</th>
<th>1997</th>
<th>1999</th>
<th>2001</th>
<th>2003</th>
<th>2005</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sister chromatid exchange (SCE) (% cells with aberrations)</td>
<td>l&gt;h ns</td>
<td>H&gt;L</td>
<td>l&gt;h</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chromosomal aberrations (CA) (mean aberrations per cell)</td>
<td>ns</td>
<td>ns</td>
<td>H&gt;L</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutation frequency (MF)</td>
<td>h&gt;1</td>
<td>ns</td>
<td>h&gt;1</td>
<td>ns</td>
<td>h&gt;1</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Fluorescent in situ hybridization (FISH); mean number of total mutations per subject in chromosomes 5, 7, 11, and 13</td>
<td>h&gt;1</td>
<td>p = .08</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>Note. L, l=low urine uranium group (U < 0.1 μg/g creatinine); H, h=high urine uranium group (U ≥ 0.1 μg/g creatinine); ns = no significant differences between groups; lower case letters=nonsignificant findings; upper case letters=statistically significant findings.</sup>

Semen Characteristics. No statistically significant differences between urine U groups were observed in any of the World Health Organization (WHO, 1987) criteria semen characteristics measured (data not shown).
et al., 1997; Links et al., 2001). Metal-exposed cohorts, and cohorts exposed to mineral fibers persistent in the body, have most commonly been followed in such surveillance studies in order to avoid missing potential target organ threshold metal concentrations that determine toxicant effects. Thus analysis of data for this study incorporated the use of a cumulative measure of exposure (μg U/g creatinine years), as well as our standard measure of current exposure, which has always been a 24-h urine U concentration measurement standardized per creatinine (μg U/g creatinine). During data analysis, it was hypothesized a priori, which U exposure metric, current or cumulative, would best predict specific outcomes. Data showed little difference in statistical associations when using the two U exposure metrics. This difference in measuring exposure may become important in time, however, as this cohort is followed forward and the duration of exposure continues. Effects observed using the cumulative exposure metric may indicate an important threshold of effect.

Blood as a Biomarker of DU Exposure

Varying the tissue or sampling matrix used to measure DU exposure was also examined by comparing blood with urine U concentration values. Urine has been the biological matrix of choice for measuring U exposure in occupationally exposed cohorts for several decades due to ease of collection (Thun et al., 1985). Protection from renal effects of U exposure in historically exposed workers was achieved by using urine U concentrations to predict renal U concentrations using a systemic biokinetic model for U (ICRP, 1995). Primarily due to analytical difficulties involved in measuring U in blood samples, this matrix has been of limited value for biomonitoring. Using a recently developed ICP/MS assay for measuring total U and the $^{235}$U/$^{238}$U ratio in blood samples (Squibb et al., 2008), the hypothesis tested was that blood may be a preferred matrix for detecting DU in soldiers with suspected DU exposure. Results reported here indicate that urine testing is much more sensitive for detecting exposure to DU, since only half of the DU exposed veterans identified by urine testing were identified by the blood isotopic analysis approach. This confirms that urine rather than blood is the best biological matrix to use for assessing DU exposure.

Unlike the unsatisfactory isotopic blood U results, the close association between blood and total urine U concentrations indicates that U does accumulate in blood and may provide a potentially satisfactory measure of U body burden. Blood U concentrations measured in the 2003 DU-exposed Gulf War cohort were consistent with those reported here for the 2007 cohort. Concentrations ranged from 0.01 to 0.88 μg/L in the 2003 cohort. Thus, as with the urine concentrations, these indicators of current systemic exposure to U released from the embedded fragments show ongoing, consistently elevated exposure to U for 16 yr since their combat injuries left them with embedded DU fragments. It is important to note that soldiers exposed by inhalation only during the friendly fire incidents do not have elevated urine or blood concentrations. For two-thirds of the cohort, their blood U concentrations are lower than blood U concentrations observed in New York City residents with no known occupational exposure to U (Fisenne & Perry, 1985).

Clinical Findings

There were few clinical findings that suggested a U effect in any organ system of interest. Neurocognitive testing parameters have been followed since this surveillance program’s inception because of the known effects on the central nervous system of other heavy metal exposure and due to the animal evidence that U does cross the blood brain barrier (Pellmar et al., 1999a, 1999b).

Renal Function. Particular attention is paid to biomarkers of renal function in this surveillance program since the kidney is thought to be a primary “target organ” for U under both acute and chronic exposure conditions, as it is for many metals (McDiarmid & Squibb, 2001; Parkhurst, 2003; Cross et al., 1981; Leach et al., 1970; 1973; Mitchel et al., 1999). Although small elevations in the concentrations of filtered proteins in the urine were noted in past surveillance visits, similar to the increase in beta2-microglobulin and retinol binding protein observed in this 2007 surveillance visit, these changes have not been persistent from year to year, and have not increased in magnitude. The observed differences between the low versus high groups did not change significantly when they were analyzed based on cumulative U exposure versus current urine U exposure measurements. This further suggests that U concentrations in the kidneys of these exposed veterans have not increased sufficiently over time to cross a critical threshold for impaired function.

Bone Metabolism Markers. For the first time detailed outcomes of bone metabolism were examined. Bone is one of the primary long-term storage depots for U in the body (McDiarmid & Squibb, 2001) and may be affected by chronic exposure to U. Studies of the effects of U exposure on bone health in humans are limited. A number of animal studies have been completed (Diaz-Sylvester et al., 2002; Giglielmotti et al., 1983, 1985; Pellmar et al., 1999b; Tasat, et al., 2007; Tissandie et al., 2006, 2007; Ubios et al., 1991); however, care must be taken when applying the findings to humans as the majority studied bone ossification rather than bone modeling or remodeling, which are the primary mechanisms of bone turnover in adults.

In this cohort of DU-exposed veterans, some evidence of decreased osteoblast (bone formation) activity was found as determined by a lower (but still normal) bone-specific alkaline phosphatase value in the high-U group as compared to the low. Although, the finding is clinically insignificant, it is consistent with a number of animal and in vitro studies. For example, decreased bone volume and density and abnormal bone structure during ossification and bone healing were observed in rats...
following intraperitoneal injection of U (Guglielmotti et al., 1983, 1985). These changes have been attributed to depletion of osteoblasts (Guglielmotti et al., 1985; Diaz Sylvester et al., 2002). Rats exposed to U during bone modeling or remodeling were found to have an increased number of inactive osteoblasts (Ubios et al., 1991). In vitro studies of human fetal osteoblast cells exposed to U found a concentration-dependent increase in reactive oxygen species that was thought to lead to the abnormal cell architecture and function that was observed (Tasat et al., 2007). Although an increase in osteoclast (bone resorption) activity was not observed in this study, it was previously demonstrated in men with chronic exposure to natural U in drinking water (Kurtto et al., 2005). As with bone formation, U exposure was shown to influence bone resorption in animals. Osteoclast activity was enhanced during bone remodeling in rats following intraperitoneal injection of U (Ubios et al., 1991). This enhancement is thought to be due to the presence of reactive oxygen species (Tasat et al., 2007).

A statistically significant difference in the levels of 1,25-dihydroxy vitamin D levels was observed in the DU cohort with levels higher in the high-U group as compared to the low. This is the opposite of what is expected with U exposure based on the limited number of studies available for comparison. Tissandie et al. (2006, 2007) showed that 1,25-dihydroxy vitamin D levels decrease following both acute and chronic ingestion of DU in rats. As discussed earlier, PTH is the primary determinant of 1,25-dihydroxy vitamin D levels. In this cohort, PTH levels are not different. In addition, age does not modify the relationship. However, levels of 1,25-dihydroxy vitamin D are affected by renal function. Although the difference in the creatinine clearance between the high- and low-U exposure group is not significant (150.81 ml/min vs. 122.60 ml/min), clearance in the low-U group is approximately 20% lower. This may explain the difference observed in the 1,25 dihydroxy vitamin D levels.

In general, the slight decrease in osteoblast or bone formation function with preservation of osteoclast function suggests that there may be a clinically insignificant uncoupling of bone turnover in this DU exposed population. This imbalance during bone remodeling might lead to decreased bone mineral density and early onset osteoporosis. Continued monitoring of this population needs to include markers of bone turnover, monitoring of calcium phosphate homeostasis, and possibly the measurement of bone mineral density with DEXA scans. Changes may be seen with time as the duration of exposure increases and the cumulative DU exposure metric may be a better measure by which to follow these effects in the future, although at this time there is no difference between the findings when the exposure groups are defined by current versus cumulative U metrics.

CONCLUSION

Sixteen years after first exposure, this DU-exposed cohort continues to exhibit few clinically significant U-related health effects. For the first time, bone metabolism was examined and only subtle differences in bone formation activity were found as a function of U concentration, which are of unknown clinical importance. Subtle differences in measures of renal proximal tubular function were observed between the low- and high-U groups. Because exposure is ongoing for the subgroup of this cohort with embedded DU fragments, U tissue burden is increasing with exposure duration. Therefore, continued surveillance for early detection of potential threshold effects that are exposure duration dependent is prudent.

REFERENCES


Army Environmental Policy Institute. 1995. Health and environmental consequences of depleted uranium use in the U.S. Army. Atlanta, GA.


