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The U.S. Department of Veterans' Affairs depleted uranium exposed cohort at 25 Years: Longitudinal surveillance results

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ABSTRACT

Background: A small group of Gulf War I veterans wounded in depleted uranium (DU) friendly-fire incidents have been monitored for health changes in a clinical surveillance program at the Veterans Affairs Medical Center, Baltimore since 1994.

Methods: During the spring of 2015, an in-patient clinical surveillance protocol was performed on 36 members of the cohort, including exposure monitoring for total and isotopic uranium concentrations in urine and a comprehensive assessment of health outcomes.

Results: On-going mobilization of U from embedded fragments is evidenced by elevated urine U concentrations. The DU isotopic signature is observed principally in participants possessing embedded fragments. Those with only an inhalation exposure have lower urine U concentration and a natural isotopic signature.

Conclusions: At 25 years since first exposure to DU, an aging cohort of military veterans continues to show no U-related health effects in known target organs of U toxicity. As U body burden continues to accrue from in-situ mobilization from metal fragment depots, and increases with exposure duration, critical tissue-specific U concentration thresholds may be reached, thus recommending on-going surveillance of this veteran cohort.

1. Introduction

In a series of desert combat events in February 1991, U.S. armored tanks and fighting vehicles were mistakenly fired upon by other U.S. forces using depleted uranium (DU) penetrators (Office of the Special Assistant for Gulf War Illnesses (OSAGWI), 2000). As a result of this traumatic event, exposure to DU likely occurred via three routes: (1) DU fragments embedded in tissues, (2) particulate aerosols inhaled and deposited in the lung and (3) (superficial) particulate contamination of the skin. In the twenty-five years since that time, longitudinal surveillance has been performed by the U.S. Department of Veterans Affairs for the group of veteran service members who were victims of these “friendly fire” events.

A finding observed during the first surveillance assessment in 1993, was the apparent systemic absorption of metal ions from soft tissue-embedded DU fragments that act as a metal ‘depot’. This novel exposure ‘mode’ has been observed to this day, as documented by on-going excretion of high concentrations of urinary uranium (uU) in the surviving members of the cohort who continue to retain such metal fragments (McDiarmid et al., 2000, 2013; Squibb and McDiarmid, 2006). With surgical morbidity precluding the complete removal of all embedded fragments in those wounded, exposure has been on-going since its initial occurrence, now almost twenty-five years ago.

Thus, assessing health effects over time in a longitudinal surveillance program was instituted. Such an approach has proven helpful in cases such as these, when long-latency effects are unknown due to incomplete exposure assessment, novel exposure routes or when the exposure is on-going, allowing a toxicologic burden to accumulate (McDiarmid et al., 2009; Squibb et al., 2012).

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2. Uranium toxicity

DU is about 99.8% U^{238} by weight and is a man-made by-product of the U-enrichment process. It is derived when U^{234} and U^{235} are removed from natural U obtained from mined ore, to create a product 'enriched' in specific activity and thus suitable for reactor fuel (Army Environmental Policy Institute (AEPI), 1995). With the removal of most of these two isotopes, the remaining by-product is now 'depleted' of approximately 40% of its specific radioactivity. However, it retains its chemical toxicity due to its 'heavy metal' properties which are exploited for armor-piercing capabilities in military applications (Squibb et. al., 2005; AEPI, 1995; Agency for Toxic Substances and Disease Registry (ATSDR), (2013)).

While DU was only first widely deployed in the 1991 Gulf War, animal and cell culture studies had previously characterized the toxic effects of acute U exposure demonstrating that soluble U primarily targeted the kidney and insoluble forms, from inhalation exposures, targeted the lung (Voegtlin and Hodge, 1949; Morrow et al., 1972; The Royal Society, 2001, 2002; ATSDR, 2013). Epidemiology studies of exposed U workers employed in the defense industry also demonstrated renal effects, although of lesser severity considering the exposure concentrations (Thun et. al., 1985; National Research Council (NRC), 2008; The Institute of Medicine (IOM), 2008; ATSDR, 2013).

Other health risks relate to local tissue reactions and foreign body effects from prolonged contact with metal fragments (Hahn et al., 2002). These fragments have also been seen to oxidize in situ, thus enabling metal ion mobilization to the systemic circulation (as described above) and permitting metal accumulation in tissues remote from the metal depot location (Hooper et al., 1999; Pellmar et al., 1999; Squibb et al., 2005).

3. Surveillance for other metals in fragment alloys

Importantly, the metal fragments retained in soft tissue by our cohort members, are not exclusively DU, but contain other alloy constituents including titanium (Parkhurst et al., 2005) and may include material carried into the patient's wound from a secondary impaction of other material in the trajectory of the projectile.

Evidence from other patient cohorts of metal absorption suggest that other metals in embedded materials may mobilize from in situ tissue depots. These include reports of elevated cobalt and chromium levels in the circulation of hip implant patients (Machle, 1940; Dillman et al., 1979; Sunderman et al., 1989; International Agency for Research on Cancer (IARC), 1999; Jacobs et al., 1998; Keegan et al., 2007).

The chemical analysis of other surgically removed, combat-related fragments such as those from improvised explosive devices (IED) used during the recent Iraq and Afghanistan conflicts has further informed our exposure assessment protocol. These analyses have yielded a suite of 14 metals identified as the most commonly observed metal components of surgically removed fragments from IED injured service members (Centeno et al., 2014). Due to concern about retained fragments from other metal sources, the bio-monitoring battery of the DU cohort has been enlarged to include this broader suite of metals encountered in IEDs, to assess the potential presence and toxicity of non-DU retained metals.

To characterize the long-term health consequences of exposure to DU and possibly other metals and to inform medical management of this cohort, biennial surveillance has been conducted for these veterans since 1993. We report here clinical outcomes of interest from the 2015 surveillance assessment and describe the health implications for and medical management challenges of these patients.

4. Materials and methods

Between April and June of 2015, 36 members of a larger dynamic cohort, currently numbering 80 Gulf War I veterans who were victims of 'friendly fire' involving DU munitions and DU-armored tanks, participated in medical surveillance at the Baltimore, Maryland Veterans Affairs Medical Center (VAMC). Although all members of this cohort were invited to participate and travel and per diem costs were supplied, on-going military deployments and personal obligations typically limit participation to about half of the total cohort. All participants have been seen on previous biennial surveillance visits, with the number of visits per participant averaging more than seven. The surveillance protocol used in this study was approved by the University of Maryland School of Medicine's and the Baltimore VAMC's IRB programs. All participants signed a written informed consent document.

4.1. Exposure assessment for uranium and other metals of concern

Total U concentration and the $^{235}U/\ ^{238}U$ isotopic ratio of 24-hr urine samples collected during the 2015 surveillance visit were measured by the Joint Pathology Center (JPC) Biophysical Toxicology Laboratory (Silver Spring, MD) (previously the Armed Forces Institute of Pathology's Department of Environmental and Toxicologic Pathology [Washington, DC]). Prior to the metal analysis, urine aliquots were slightly acidified with 1% nitric acid (highly-pure Ultrex tarce metal-free HNO3) in acid-washed polypropylene 15 ml plastic tubes. Acidified uranium aliquots were analyzed for total U using a quadrupole-based Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (ELAN 6100; Perkin-Elmer Corporation, Connecticut, USA) (Ejnik et al., 2005; Gray et al., 2012), while uranium isotopic ratios ($^{235}U/\ ^{238}U$) were determined employing a high-resolution magnetic sector ICP-MS (HR-ICP-MS) (Thermo Finnigan Element-2; ThermoFisher Scientific, Connecticut, USA) (Gray et al., 2012). Urine U concentrations were standardized on the basis of urine creatinine concentrations to account for urine dilution to obtain μg U/g creatinine (McDiarmid et al., 2000; Karpas et al., 1998).

The 24 h urine specimens were also analyzed for 13 additional metals including: aluminum, arsenic, cadmium, cobalt, chromium, copper, iron, lead, manganese, molybdenum, nickel, tungsten and zinc. These metals were chosen based on their presence in analyzed embedded fragments (Centeno et al., 2014) and their potential toxicity and carcinogenicity (Gaitens et al., 2010). This panel of 13 additional metals was simultaneously analyzed employing HR-ICP-MS. Preparation of urine samples and metal analysis by sector field high resolution ICP-MS were conducted as described by Gray et al. (2012). Detection limits (DL) for most of the metals ranged from of 0.1–1 ppb (=ng/ml).

Urine arsenic species, including arsenobetaine (AsB), arsenocholine (AsCh), trimethylarsine Oxide (TMO), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenous (III) acid, arsenic (V) acid, were measured using high performance liquid chromatography (HPLC) are inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). The sum of AsIII, AsV, DMA and MMA was used to determine inorganic arsenic exposure (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (USDHHS CDC), 2014).

4.2. Clinical assessment

A three-day, in-patient clinical assessment has been performed since the mid-1990s and consists of a detailed medical history, an extensive exposure history, a thorough physical examination, and laboratory studies. The laboratory battery includes hematological blood clinical chemistry and bone metabolism measures. Hematological and neuroendocrine parameters, serum and urine creatinine, calcium and phosphate, serum uric acid measures and urine glucose and total protein were evaluated by the Baltimore, MD VAMC clinical laboratory using standard methodologies. Spot and 24-hr urine samples were

obtained for measurement of clinical chemistry parameters related to renal function and bone metabolism as described below. Neurocognitive performance was again assessed as a measure of central nervous system (CNS) insult as per our previous protocols. Specifically, participants completed the Automated Neuropsychological Assessment Metrics, a computerized neurocognitive battery originally developed by the Department of Defense. Four neurocognitive indices of accuracy, speed, throughput (i.e., speed/accuracy) and cognitive efficiency were computed (McDiarmid et al., 2004, 2007, 2009).

4.3. Renal assessment

Markers of nephrotoxicity in urine [retinol binding protein (RBP), microalbumin (mAlb), intestinal alkaline phosphatase (IAP), N-acetyl-D-glucosaminidase (NAG), Kidney Injury Marker -1 (KIM-1), Neutro Gelatinase-Associated Lipocalin (NGAL) and IL-18 and β_2 microglobulin] were measured and analyzed as previously described (McDiarmid et al., 2013).

4.4. Pulmonary functional assessment

Spirometry was performed using a Sensormedics Carefusion Vmax system (Yorba Linda, CA) according to American Thoracic Society guidelines by Registered Respiratory Therapists certified by the National Board for Respiratory Care (Miller et al., 2005). Pulmonary function testing was performed on a flow-sensor spirometer. Predicted normal values for spirometry were obtained from Morris et al. (1971). Spirometry values included forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and the ratio of FEV1 to FVC.

5. Statistical analysis

5.1. Urine uranium as a binary variable

As in previous years, the cohort was sorted into two (categorical) exposure groups (low-high) based on each individual participant's current (2015) uU result (low n=26; high n=10). As in previous years (McDiarmid et al., 2000, 2001, 2004, 2006, 2007, 2009, 2011, 2013, 2015), the low uU group is defined as those having uU < 0.1 μg U/g creatinine. High exposure was defined as current uU concentrations ≥0.1 μg U/g creatinine, a value between 0.034 μg/g (the 95% percentile reported for creatinine-adjusted uU concentrations in non-exposed populations in the U.S. (USDHHS CDC, 2003)) and 0.35 μg/g U/L which is reported as a uU upper limit that occurs naturally in areas with elevated U in water and food (International Commission on Radiological Protection (ICRP), 2002).

5.2. Tests of differences between low versus high urine U (uU) exposure groups

We present here each outcome by U exposure category (low vs high) in tables using mean values and standard errors. We tested for significance of differences between U groups using the Mann-Whitney *U* test (Wilcoxon Rank sum test). Hence, the Mann-Whitney test was used for all comparisons of the low versus high U groups. Differences were considered statistically significant when $p < 0.05$.

5.3. Analyses of other metal concentrations

For each of the 13 additional metals examined, we determined if a metal concentration was "elevated" by comparing the result to a reference value for unexposed populations. As described in Gaitens et al. (2010), when available, the 2003–2004 National Health and Nutrition Examination Survey (NHANES) 95th percentile creatinine adjusted value was used as the reference value; otherwise, other reported reference values, such as the upper values reported by clinical laboratories, were used. We used the Fisher's Exact test to determine if there was an association between elevated metal concentrations and high exposure to U as measured by uU concentrations.

Table 1
Population characteristics of the 2015 surveillance participants.

	2015 cohort (N=36)		All Gulf War I participants (N=80)	
	N	(%)[a]	N	(%)[a]
Race/Ethnicity				
African American	13	(36)	24	(30)
Asian American	1	(2)	1	(1)
Caucasian	17	(47)	46	(56)
Hispanic	5	(14)	8	(10)
Native American	0	(0)	1	(1)
Age[b]				
Mean (S.D.[c])	50.94 (5.31)		49 (4.76)	
Body mass index (BMI) by U group[d,e]				
Mean (S.E.[f]) Low- 30.83 (1.02) High- 30.37 (3.24)				
Current smoker				
Number (%) Low- 3 (11) High- 1 (10)				

[a] May not add to 100% due to rounding.
[b] Age in 2015.
[c] S.D.=Standard deviation.
[d] Low group- urine uranium (uU) < 0.10 μg/g creatinine (n=24); High group- uU≥0.10 μg/g creatinine (n=10).
[e] BMI and "current smoker" status information not available for the full cohort of 80.
[f] S.E.=Standard error.

6. Results

Between April and June of 2015, 36 members of a larger dynamic cohort, currently numbering 80 Gulf War I veterans, who were victims of 'friendly fire' involving DU munitions and DU-armored tanks, participated in medical surveillance at the Baltimore Veterans Affairs Medical Center (VAMC). Table 1 displays the demographic characteristics of the subgroup of veterans participating in the 2015 surveillance visit and depicts a similar race and age structure to that of the larger complete cohort. All have participated in multiple previous assessments, with the average number of assessments attended by participants being almost eight. Importantly, those with a high uU, who are the most at-risk of U-related health effects, tend to more regularly attend these surveillance visits and thus it is unlikely that we are missing an important health signal contributed from the non-participants.

Urine U concentrations have been used as a marker of systemic exposure to U for the affected cohort since the inception of the surveillance program. Fig. 1 displays the uU distribution from low to high values of the 2015 cohort of participants. In addition to total uU, the figure, using various data point symbols, depicts isotopic U signature and patient fragment status. Because the isotopic signature determination requires a total uU of > 10 μg/g creatinine (which is a limit of detection of 10 μg/g creatinine), the isotopic signature for the lower uU values cannot be determined. The figure includes various uU comparison values reported in other populations which are indicated by horizontal lines on the graph. The top line (65.1 μg/L) represents the mean total urine uranium found in a sub-cohort of uranium fabrication workers in 1975, as reported in a study by (Thun et al., 1985). The U.S. Dietary Limit (0.320 μg/L) is an upper limit for the dietary contribution of uranium in urine for a U.S. general population from drinking water (ICRP, 2002). This value was calculated by dividing the upper limit for 24-h uranium excretion for "reference man" by 1.6L 24 h. It is assumed that corrections per gram creatinine and per liter urine are generally equal for "reference man" and for this group of veterans with normal renal function (Ting et al., 1999). The next line (0.1 μg/g creatinine) indicates the cut point established by the

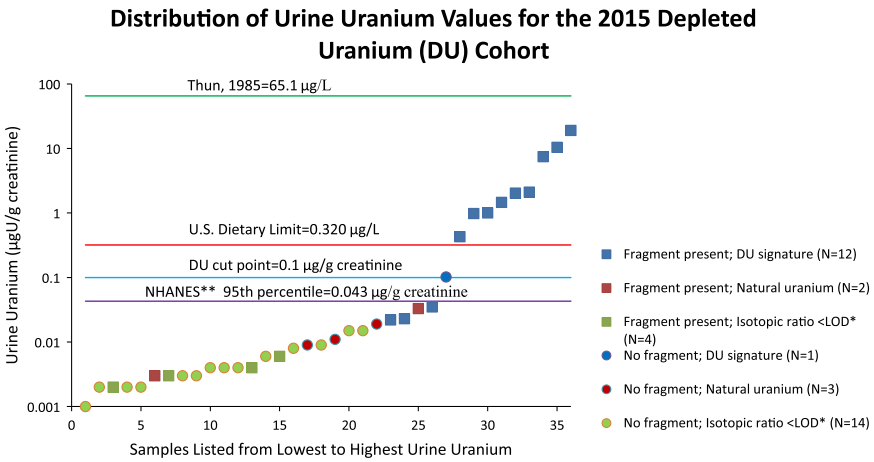

Fig. 1. Displays the urine uranium (uU) distribution from low to high values of the 2015 cohort of participants with comparison populations. The symbols used represent: total uU, isotopic signature and fragment status of individual. The top line (65.1 µg/L) represents the mean total uU found in a sub-cohort of uranium fabrication workers in 1980 (Thun et al., 1985). The next line (0.320 µg/L) is an upper limit for the dietary contribution of uranium in urine for a general population from drinking water (ICRP, 2002; McDiarmid et al., 2000). The next line (0.1 µg/L) indicates the cut point established by the DU Follow-up Program to define low vs. high uU exposure groups (McDiarmid et al., 2000). The next line (0.043 µg/g creatinine) represents the 95th percentile for uU concentration for adults from the 2001–2002 National Health and Nutrition Examination Survey (NHANES) (USDHHS CDC, 2012). * Limit of detection.

DU Follow-up Program to define low vs. high urine uranium exposure groups (McDiarmid et al., 2000). The bottom line (0.043 µg/g creatinine) represents the 95th percentile for urine uranium concentration for adults from the 2001–2002 US. National Health and Nutrition Examination Survey (NHANES) (USDHHS CDC, 2012). The NHANES survey assesses the health and nutritional status of adults and children in the United States using interviews, physical examinations and laboratory assessment.

For the DU cohort results reported on here, the concentrations of uU ranged from a low of 0.001 µg U/gram creatinine, to a high of 19.044 µg U/gram creatinine. Eighteen of the 36 participants, (50%) of the group, had retained metal fragments as determined previously by plain film skeletal X-ray (Hooper et al., 1999; Squibb and McDiarmid, 2006). Those participants possessing metal fragments and a DU isotopic signature are seen at the high end of the distribution, suggesting again that the fragment is a depot of on-going U metal mobilization into the systemic circulation. One participant with an isotopic signature had previously had a fragment removed. We note also that many of the values below the cut point of 0. 1 µg U per gram creatinine in cohort members with only an inhalation exposure are at or below the NHANES U 95th percentile value for the population, and that many of these lower values approach the laboratory limit of quantification to assess the isotopic signature for the sample (~10 ng/L). Participants with fragments, but without a DU isotopic signature, are thought to possess non-DU metal fragments from other sources, such as from non-DU tank armor and/or from secondary impaction of other material in the trajectory of the projectile and carried into a wound. These observations of fragment status (yes/no) generally tracking with elevated uU have been consistent throughout the almost 25 years of follow-up.

Table 2 displays the urinary concentrations of 13 other metals which were also assessed, as they have been shown to be present in fragments analyzed from other veterans of the Iraq/Afghanistan conflict (Squibb et al., 2012; Centeno et al., 2014). It is hypothesized that a participant with a retained DU fragment would be more likely to have other urinary metal elevations resulting from other metals mobilizing from either the same alloyed-DU fragment or from other fragments embedded at the time of injury. Only aluminum concentrations were seen to be statistically different between the two uU exposure groups, with measures higher in the high U group. One may posit that aluminum may also be present in the retained fragments and is thus available for mobilization in the urine, as is U. However, other dietary and environmental sources of aluminum must also be considered as an explanation for this elevation. Importantly, however, the mean concentration of aluminum was found to be within normal ranges. We note also that the arsenic concentrations, while not statistically different, are just outside the normal range for the low uU group. Arsenic speciation revealed that all results were from organic (dietary) sources, so this observation is not a threat to health.

7. Clinical findings

A clinical battery of hematology, chemistry, bone metabolism, neuroendocrine and thyroid parameters have been performed in this DU-exposed cohort since inception of the surveillance protocol with the results revealing no consistent U-related differences observed between the low and high U groups over time. For the present assessment, no statistically significant differences between U exposure groups were observed for hematologic endpoints and all values were within normal limits. A complete chemistry panel reflecting electrolytes, lipids and liver function were likewise not different between U exposure groups and mean group results were also within normal limits with the exception of LDL cholesterol, mildly outside the normal range, and higher in the low U group (data not shown).

Four neurocognitive indices of accuracy, speed, throughput and cognitive efficiency were computed. Mann-Whitney *U* tests were conducted to evaluate neurocognitive performance between the low versus high U groups. No significant differences were observed between the two U exposure groups as displayed in Table 3.

Measures of bone metabolism outcomes have also been of interest as bone is a long-term storage depot of U. Only the blood estradiol level was found to be different in the battery of bone metabolism measures, Table 4, with the results in the high U group (35.9 pg/ml) being greater than in the low group (27.9 pg/ml), but still below the upper limit of normal (39 pg/ml). The smoking status of the participants was not observed to affect this outcome. This statistical difference in estradiol was not observed previously, and the many outcomes measured imply that a significant difference will be observed on occasion, due to chance alone. We will watch for this effect during future health appraisals to assess its persistence, which would imply a potentially important clinical observation.

7.1. Biomarkers of renal effects

Surveillance for kidney effects has been a focus of this health assessment since its inception as the kidney is the known 'critical'

Table 2
Urinary metals concentrations.

Laboratory test (reference range[a])	Low uU group[b] Means+S.E.[d] (µg/g creatinine)(N=26)			High uU group[c] Means+SE (µg/g creatinine) (N=10)			Mann-Whitney *p*
Aluminum (< 30 µg/g cre[e])	3.79	±	(0.53)	8.20	±	(0.98)	0.00
Arsenic (< 60 µg/g cre)	55.04	±	(22.01)	14.00	±	(5.50)	0.26
Cadmium(< 1.0 µg/g cre)	0.24	±	(0.02)	0.20	±	(0.02)	0.61
Chromium (< 2.0 µg/g cre[e])	0.26	±	(0.06)	0.39	±	(0.09)	0.18
Cobalt (< 1.0 µg/g cre)	0.21	±	(0.03)	0.21	±	(0.04)	0.99
Copper (< 40 µg/g cre[f])	5.21	±	(0.80)	4.63	±	(0.49)	0.64
Iron (< 250 µg/g cre[f])	6.73	±	(1.31)	5.77	±	(0.92)	0.96
Lead (< 2.0 µg/g cre)	0.39	±	(0.08)	0.26	±	(0.04)	0.77
Manganese (< 2.0 µg/g cre[f])	0.24	±	(0.15)	0.13	±	(0.05)	0.41
Molybdenum (< 122 µg/g cre)	34.72	±	(6.07)	32.04	±	(4.85)	0.57
Nickel (< 8 µg/g cre[f])	0.23	±	(0.05)	0.29	±	(0.18)	0.26
Tungsten (< 0.4 µg/g cre)	0.04	±	(0.01)	0.06	±	(0.02)	0.27
Zinc (< 1100 µg/g cre[f])	591.02	±	(60.44)	686.12	±	(111.04)	0.39

[a] All reference values are rounded from calculated and available data (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2012, Lauwerys and Hoet, 2001, Burtis and Ashwood, 2001, University of Iowa, Department of Pathology, 2013, Cleveland Clinic Laboratories, 2015).
[b] Low urine uranium (uU) group < 0.10 µg/g creatinine (n=24).
[c] High uU group≥0.10 µg/g creatinine (n=10).
[d] S.E.=Standard error.
[e] Source reference value converted from µg/L to µg/g creatinine (cre) assuming urine output of 1.2 L/24 h and 1.2 g cre/24 h (American Conference of Governmental Industrial Hygienists (ACGIH, 1991)).
[f] Source reference value converted from µg/24 h to µg/g creatinine assuming urine output of 1.2 g cre/24 h (American Conference of Governmental Industrial Hygienists (ACGIH, 1991).

organ, that is, the organ first perturbed, for soluble U toxicity (Parkhurst et al., 2005). Table 5 displays the common, clinical renal function measures obtained, which are within normal limits both for endpoints measured in blood and urine matrices. There are no statistical differences between mean results when comparing the low versus high uU groupings for any measure in Table 5, implying the absence of a U-driven effect.

7.2. Urinary low molecular weight proteins: biomarkers of renal proximal tubule effects

It is specific segments of the kidney's proximal tubule that are the target of soluble U and therefore, we also obtained spot urine samples to measure low molecular weight, filtered proteins [microalbumin, $\beta_2$microglobulin, $\acute{\alpha}_1$microglobulin and retinol binding protein (RBP)], which are normally reabsorbed in the proximal tubules, to assess renal tubular function. We also obtained measures of renal tubular cytotoxicity (IAP and NAG) which may be found in the urine as a consequence of the sloughing of cells into the tubular lumen.

Similar to results obtained during recent past surveillance visits, there were no statistically significant differences (at the $p < 0.05$ value) between the mean values for proteins in the low vs high uU groups (Table 6). This observation remained unchanged when the cohort members diagnosed with diabetes were removed from the analysis.

This lack of differences between U groupings extends to the three acute kidney injury markers we also assessed (IL-18, KIM-1 and NGAL). Borrowed from the pharmaceutical industry, these markers are used as more sensitive indicators for detecting acute kidney injury (AKI) in drug induced renal toxicity (Coca et al., 2008). These biomarkers have also recently been used to detect adverse effects of chronic exposures to renal environmental toxicants such as metals and have allowed early detection of injury (Prozialeck et al., 2009; Zhou et al., 2008; Zhang et al., 2014). Although not reaching statistical significance, results presented in Table 6 for KIM-1 and NGAL demonstrate a 40–100% higher mean value in the high uU group compared to the low uU group potentially suggesting signs of a U-effect. The lack of statistical significance may be due to the high variability associated with the data, to the broad range of normal values and the standard error observed. Of interest, however, this trend of higher mean results in these kidney injury markers have been observed previously in the high uU group (McDiarmid et al., 2015) and may suggest detection of an early U-related renal effect.

7.3. Pulmonary effects

Pulmonary function measurements obtained by spirometry provide a non-invasive test to monitor for physiologic changes to the lungs, a known target of insoluble uranium particulate exposure. Spirometry results for the 2015 cohort are shown in Table 7 and demonstrate values that fall within the normal clinical range for the group overall.

As well, for all parameters displayed (FVC% predicted, FEV1%

Table 3
Neurocognitive measures.

Automated Neuropsychological Assessment Metrics (ANAM)	Low uU group[a] Means+S.E.[c] (N=20)			High uU group[b] Means+S.E. (N=9)[d]			Mann-Whitney *p*
Accuracy index	0.16	±	(0.11)	0.19	±	(0.14)	0.53
Speed index	0.14	±	(0.12)	0.15	±	(0.19)	0.83
Throughput index	0.16	±	(0.20)	0.24	±	(0.29)	0.63
Index of cognitive efficiency	771.11	±	(124.13)	727.80	±	(187.00)	0.42

[a] Low urine uranium (uU) group < 0.10 µg/g creatinine.
[b] High uU≥0.10 µg/g creatinine.
[c] S.E.=Standard error.
[d] The N does not add up to total number of participants for 2015 due to the fact that some were unable to complete neurocognitive testing.

Table 4
Markers of bone metabolism.

Laboratory test (normal range)	Low uU group[a] Means+S.E.[c] (N=26)			High uU group[b] Means+S.E. (N=10)			Mann-Whitney *p*
Serum/Blood							
Estradiol (0–39 pg/ml)	27.86	±	(1.66)	35.90	±	(2.63)	0.01
Parathyroid hormone (intact) (10–65 pg/ml)	48.04	±	(2.92)	46.40	±	(6.88)	0.57
Bone specific alkaline phosphatase (7.6–14.9 µg/L)	12.38	±	(0.69)	10.91	±	(1.16)	0.45
Vitamin D 1,25 (18–72 pg/ml)	49.42	±	(3.33)	53.50	±	(6.41)	0.57
Vitamin D 25–0H (30–100 ng/ml)	34.31	±	(2.27)	37.59	±	(5.70)	0.82
Calcium (8.4–10.2 mg/dL)	9.08	±	(0.06)	9.26	±	(0.11)	0.10
Phosphate (2.7–4.5 mg/dL)	3.57	±	(0.12)	3.65	±	(0.19)	0.82
Urine							
Sodium (40–220 mEq/24 h)	173.31	±	(14.92)	172.90	±	(23.85)	0.88
Calcium (100–300 mg/24 h)	153.79	±	(19.88)	177.49	±	(32.26)	0.44
PO_4 (0.4–1.3 g/24 h)	0.90	±	(0.05)	0.85	±	(0.08)	0.59
N-Telopeptide (9–60 nMol BCE/mMol creatinine)	20.38	±	(1.35)	17.20	±	(2.11)	0.34

[a] Low urine uranium (uU) group < 0.10 µg/g creatinine.
[b] High U≥0.10 µg/g creatinine.
[c] S.E.=Standard error.

Table 5
Renal parameters.

Laboratory test (normal range)	Low uU group[a] Means+S.E.[c] (N=26)			High uU group[b] Means+S.E. (N=10)			Mann-Whitney *p*
Urine							
Creatinine g/24 h (0.6–2.5)	1.94	±	(0.09)	1.87	±	(0.14)	0.76
Glucose g/24 h (0–0.5)	1.24	±	(0.96)	18.47	±	(18.38)	0.52
Ca mg/24 h (100–300)	153.79	±	(19.88)	177.49	±	(32.26)	0.44
PO_4 g/24 h (0.4–1.3)	0.9	±	(0.05)	0.85	±	(0.08)	0.59
Magnesium mEq/24 h (1.4–14.0)	7.84	±	(0.84)	7.9	±	(1.27)	0.32
Total protein conc mg/dL (0–12)	13.02	±	(4.79)	4.05	±	(1.12)	0.16
Uric acid g/24 h (0.25–0.75)	0.67	±	(0.05)	0.65	±	(0.07)	0.14
Potassium mEq/24 h (25–125)	65.38	±	(4.27)	65.5	±	(4.45)	0.74
Serum/Blood							
Glucose mg/dL (70–105)	106.5	±	(6.53)	111.6	±	(14.52)	0.54
C-Reactive Protein (0–10 mg/L)	10.07	±	(4.30)	6.32	±	(2.25)	0.85
Creatinine mg/dL (0.9–1.3)	1	±	(0.04)	0.97	±	(0.05)	0.69
Calcium mg/dL (8.4–10.2)	9.08	±	(0.06)	9.26	±	(0.11)	0.50
Phosphate mg/dL (2.7–4.5)	3.57	±	(0.12)	3.65	±	(0.19)	0.09
Uric acid mg/dL (3.4–7)	6.59	±	(0.27)	5.79	±	(0.39)	0.82
Sodium mEq/L (133–145)	138.54	±	(0.28)	137.8	±	(0.73)	0.37
Magnesium mg/dL (1.8–2.5)	2.04	±	(0.03)	2.12	±	(0.06)	0.56
Calculated Glomerular Filtration Rate ml/min/SA[d] (90–120 ml/min/SA 1.73 m^2)[e]	88.27	±	(3.59)	94.7	±	(8.67)	0.61
Beta 2 Microglobulin µg/L (0–2510)	1937.37	±	(113.43)	1902	±	(109.25)	0.61

[a] Low urine uranium (uU) group < 0.10 µg/g creatinine.
[b] High uU ≥0.10 µg/g creatinine.
[c] S.E.=Standard error.
[d] Surface area.
[e] National kidney foundation.

predicted, and FEV1/FVC ratio) mean values are within normal clinical limits for both the low and high uU groups, and there is no statistical difference in outcomes between U exposure categories. This is consistent with observations made during the 2011 and 2013 surveillance visits (McDiarmid et al., 2013, 2015). Ever smokers tended to have lower FEV1/FVC ratios which could represent a smoking effect. A greater proportion of smokers were found in the low uU group. When adjusted for smoking, there still remained no differences in any spirometry measure in low compared to high uU groups.

8. Discussion

The epidemiology of U fabrication workers and animal evidence from the DU implantation experiments, have informed the content and conduct of the Department of Veteran's Affairs longitudinal surveillance program initiated in the early 1990s. The dual toxicities of U derived from both its chemical, heavy metal properties as well as from its radioactivity have influenced the protocol as have likely exposure routes. Both inhalation and ingestion exposure routes could be anticipated for respirable aerosols encountered in confined spaces, such as inside a tank hatch, likely permitting acute exposure at the time of DU penetrator impact. In addition, chronic absorption of metal ions from retained fragments of shrapnel which were either embedded in tissue or which contaminated superficial wounds posed an added health risk to those affected and argued for on-going surveillance.

Two primary target organs of the DU oxide exposures sustained during these friendly fire events were thought to be the kidney and the pulmonary system, for the soluble and the insoluble DU particles respectively (The Royal Society, 2001, 2002; Parkhurst et al., 2005). For this reason, we have continued to focus on these organ systems. While kidney U concentration in animal experiments have been shown to reach peak levels at six months and plateau thereafter (Pellmar et al.,

Table 6
Biomarkers of renal proximal tubular effects.

Laboratory test[d] (normal range)	Low uU group[a] Means±S.E.[c] (N=26)			High uU group[b] Means±S.E. (N=10)			Mann-Whitney *p*
IAP U/g creatinine (< 2 U/g)	0.44	±	0.11	0.41	±	0.12	0.79
NAG U/g creatinine h (< 5U/g)	1.00	±	0.18	0.85	±	0.12	0.90
Micro Albumin mg/g creatinine (< 17 mg/g)	46.29	±	33.38	10.84	±	3.02	0.39
beta2 microglobulin mg/g creatinine (< 0.20 mg/g)	0.07	±	0.01	0.08	±	0.01	0.37
alpha1 microglobulin mg/g creatinine (< 8 mg/g)	2.79	±	0.49	2.48	±	0.65	1.00
RBP mcg/g creatinine (< 300 mcg/g)	35.33	±	3.71	47.83	±	17.62	0.90
IL-18 ng/g creatinine (< 20 ng/g)	10.11	±	1.47	12.94	±	3.14	0.54
KIM-1 ng/g creatinine (< 560 ng/g)	273.75	±	40.11	366.70	±	91.25	0.43
NGAL ng/g creatinine(< 730 ng/g)	492.08	±	41.75	965.79	±	339.15	0.61

[a] Low urine uranium (uU) group < 0.10 μg/g creatinine.
[b] High uU ≥0.10 μg/g creatinine.
[c] S.E.=Standard error.
[d] Lab test abbreviations defined:IAP (intestinal alkaline phosphatase);NAG ((N-acetyl-β-D Glucosaminidase);RBP (retinol-binding protein);IL-18 (Interleukin–18);KIM-1 (kidney injury molecule-1);NGAL (neutrophil gelatinase-associated lipocalin).

1999), uranium concentrations in bone and other organ systems showed accumulation of U over a long period of time. Therefore, in light of the potential for build-up of U concentrations exceeding tissue-specific threshold concentrations over time, we obtain a full clinical laboratory battery at every biennial visit. The protocol thus continues to examine the impact of uU exposure on other organ systems, such as hematological, immune, CNS, bone metabolism, and neuroendocrine systems.

With the exception of the on-going mobilization of U from fragment depots in soft tissue, manifesting as elevated uU concentrations, the comprehensive surveillance assessment reported here demonstrates no clinically significant uranium-related effects, now twenty-five years since initial exposure. Fig. 1 may help explain this observation as it includes uU results for occupationally- exposed U fabrication workers' (Thun et. al., 1985) (upper line of figure) for comparison with the DU cohort. As shown, only the highest DU cohort members' uU results approach those of the mean uU values recorded in the U fabrication workers in 1975. When the Thun cohort of 36 U mill workers was compared to cement worker referents, the authors showed renal tubular abnormalities including elevated urine B2-microglobulin as a function of exposure duration and mild aminoaciduria in five of 23 amino acids assessed. Although uU results for the entire group were not available, the authors reported historical mean uU values for a sub-group of the population at 65 μg U/L in 1975 as depicted here in Fig. 1. After significant hygiene improvements, the mean uU reported in 1980 was 9 μg U/L. Importantly, the uU biologic action limit was evaluated as acceptable at 15 mcg/L as recently as 2014 (U.S. Nuclear Regulatory Commission (U.S. NRC), 2014).

Also, consistent with the lack of renal findings in the DU cohort are the reports on populations exposed to U via drinking water. In a Canadian cohort whose drinking water source contained uranium at concentrations exceeding 1 ppb and exceeding 100 μg/L for 50% of the group, a positive correlation between proximal tubular markers including urinary glucose, alkaline phosphatase and B2-microglobulin was reported with U intake (Zamora et al., 1998). Urine U concentrations in this cohort ranged from 0.1 to 1.7 μg/L (Zamora et al., 2002).

Kurttio et al. (2006), likewise reported only subtle proximal tubule function effects in a Finnish cohort also exposed via high background U in drinking water. Here, an association between cumulative uranium intake and urinary glucose excretion was reported in a cohort for whom 68% of their current uU samples exceeded 0.03 μg/L, the 95th percentile reference level for uU in a U.S. population sampled between 1988 and 1994 (Ting et. al., 1999). This value is close to the 95th percentile reference level of 0.043 μg/g creatinine reported for a more recently sampled U.S. population (see Fig. 1).

Another North American cohort environmentally exposed to U via drinking water had proximal tubule function assessed using RBP levels in urine with only four of 156 participants having a value above the normal range when the geometric mean for their uU values was 0.100 μg/g creatinine and their arithmetic mean, 0.300 μg/g creatinine (Wyatt et al., 2008). Returning to Fig. 1 in this paper, these uU values reported by Wyatt would lie between the 'DU cut point' used to stratify the DU population into a low or high uranium category, and a reported uU dietary upper limit for other U.S. cohorts exposed via diet or water source, reported by (ICRP, 2002). Such comparisons provide context and orient the DU cohort uU distribution among other industrial and environmentally U-exposed populations, which sustained little to no kidney injury from exposure at these concentrations, similar to our findings here.

Kidney injury thresholds, based on both animal and human

Table 7
Pulmonary function test (PFT) outcomes.

PFT parameter	Reference values	Overall (n=36)	Urine U burden sub-group		Ever smoker	
			Low uU group [a] (n=26)	High uU group [b] (n=10)	No (n=20)	Yes (n=16)
Spirometry[c]	Normal	Mean (S.D.[d])	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
FVC % predicted	≥80	88.56 (13.79)	88.19 (14.0)	89.5 (14.0)	87.05 (14.49)	90.44 (13.09)
FEV1% predicted	≥80	93.81 (14.83)	93.54 (15.5)	94.5 (13.7)	93.5 (14.05)	94.19 (16.2)
FEV1/FVC	≥70	77.69 (6.17)	0.78 (0.07)	0.77 (0.04)	79.4 (4.67)	75.56 (7.24)

[a] Low urine uranium (uU) group < 0.10 μg/g creatinine.
[b] High uU ≥0.10 μg/g creatinine.
[c] Spirometry measuress defined.FVC (forced vital capacity).FEV1 (forced expiratory volume in 1 s).
[d] S.D.- Standard deviation.

evidence, have been set historically to limit harm to Uranium workers, with the traditional threshold of 3 μg U/g kidney set by the ICRP in 1960 (ICRP, 1960). Informing the biokinetic modelling in humans, Pellmar et al. (1999) had previously shown that the biokinetics of DU migrating from implanted pellets in animal experiments had similar biokinetics to forms of U which had been injected or absorbed. This work provided the support for use of this model in assessing mobilization from U-contaminated wounds (Leggett and Pellmar, 2003).

Using the ICRP 69 biokinetic model for forms of U which had been injected or absorbed, Squibb et al. (2005) showed that only the highest U-burdened members of the DU cohort are now approaching that threshold, after 20 years of exposure.

Controversy exists as to the relative importance of the kidney as a long term storage site for U (discussed in Russell and Kathren (2004)). A new report of U tissue content in three males not thought to have occupational exposure to U, revealed measured renal concentrations an order of magnitude lower than the estimate for Reference Man, thus challenging the notion of a long term storage compartment of U in the kidney (Kathren and Tolmachev, 2015). In the veteran cases presented here, the on-going mobilization of U from fixed metal depots in soft tissue and its excretion from the systemic circulation chronically 'presents' U to the kidney. In addition, uranium's deposition in bone (Priest et al., 1982) and potential mobilization from bone due to age and other factors, further adds to continued 'exposure' of the kidney to U. Leggett thus, underscores the need to consider the "continual but diminished inflow of uranium released from the bone and other tissues" when assessing the kidney's retention of uranium.

This line of reasoning suggests that a focus on kidney surveillance remains appropriate for the cohort described here. Also recommending the continued assessment of kidney insult is the debate surrounding the evidence used to set the traditional "safe" threshold value of 3 μg U/g kidney. Questions regarding laboratory methods of decades ago determining urine U on the one hand and measures of renal injury, on the other have led some to suggest that the injury threshold value should be lowered, perhaps by an order of magnitude (Leggett, 1989).

Also a target of soluble U exposure, the CNS has been assessed using markers of neurocognitive function. Likewise here, consistent with results from previous assessment visits, no significant differences in neurocognitive function emerged between the two U exposure groups. The lack of a cross-sectional association between neurocognitive function and uranium burden does not preclude the possibility of a prospective relationship. That is, the impact of accumulated U, especially in light of its ability to penetrate the blood-brain barrier in animals (Pellmar et al., 1999) and given that the nervous system is a principal target of other heavy metals (Clarkson, 1987), may have a latent impact on brain-behavior relationships. It is possible that over time, as the U concentration continues to increase, a critical threshold may be exceeded, leading to a discernible, deleterious impact on neurocognitive function. Therefore, continued surveillance of neurocognitive function is planned and neurocognitive performance over time is currently being assessed longitudinally.

We note also that the present DU surveillance protocol is quite broad, permitting the identification of perturbations in most any organ systems, not only those in expected target organs. Hence, regarding the provocative observation made by Kathren and Tolmachev (2015) suggesting the thyroid as a potential long term storage site for U, we are able to report no apparent differences in thyroid function parameters (data not shown), but will remain vigilant to this possibility, as these endpoints will be retained in our surveillance battery.

As described above, the consequences of insoluble U oxide exposure would manifest in the pulmonary system. Veterans with high U body burdens continue to have no significant differences in lung function compared to those with low U body burdens. Mean FEV1 and FVC % predicted values obtained in 2015 were similar to values obtained in 2011 (93.9% and 90.9% predicted, respectively). While mean percent predicted values for each year's surveillance results appear clinically normal, an assessment of mean rate of decline in FEV1 and FVC over time will provide a better understanding of longitudinal change in lung function in this population. Furthermore, evaluation of the small airways using Impulse Oscillometry may provide additional understanding of distal lung function in DU-exposed Service members. This technique may allow us to recognize whether there are abnormalities that would otherwise be unrecognized using conventional measures of pulmonary function (Oppenheimer et al., 2007).

9. Conclusion

Now, more than 25 years since first exposure to DU, an aging cohort of military veterans continues to show no U-related health effects in known target organs of U toxicity. The results of an extensive health assessment have also shown no other clinically significant health effects as a function of U burden. However, with the accrual of new data challenging historical assumptions regarding target organs and storage depots, a broader net may need to be cast in surveillance efforts for exposed populations, as has been done for this cohort, so as not to miss an unexpected sentinel result. Also, as tissue concentrations of metals from retained fragments continue to accrue with exposure duration, critical tissue-specific U concentration thresholds may be reached, thus recommending continued surveillance for populations such as the one described here (NRC, 2008).

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The work described in this article has been carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans*.

The opinions and/or assertions expressed herein are the private views of the authors, and shall not be construed as official or as reflecting the views of the Department of Veterans Affairs, the U.S. Department of Health and Human Services, the U.S. Food and Drug Administration or the U.S. Federal Government.

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