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● *Original Contribution*

EFFECT OF CARBON DIOXIDE ON THE TWINKLING ARTIFACT IN ULTRASOUND IMAGING OF KIDNEY STONES: A PILOT STUDY

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Abstract—Bone demineralization, dehydration and stasis put astronauts at increased risk of forming kidney stones in space. The color-Doppler ultrasound "twinkling artifact," which highlights kidney stones with color, can make stones readily detectable with ultrasound; however, our previous results suggest twinkling is caused by microbubbles on the stone surface which could be affected by the elevated levels of carbon dioxide found on space vehicles. Four pigs were implanted with kidney stones and imaged with ultrasound while the anesthetic carrier gas oscillated between oxygen and air containing 0.8% carbon dioxide. On exposure of the pigs to 0.8% carbon dioxide, twinkling was significantly reduced after 9–25 min and recovered when the carrier gas returned to oxygen. These trends repeated when pigs were again exposed to 0.8% carbon dioxide followed by oxygen. The reduction of twinkling caused by exposure to elevated carbon dioxide may make kidney stone detection with twinkling difficult in current space vehicles. (E-mail: jcsimon@uw.edu) © 2016 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Ultrasound, Kidney stones, Doppler, Space, Bubbles, Twinkling artifact, Carbon dioxide.**

INTRODUCTION

Astronauts are at an increased risk of forming kidney stones because of the bone demineralization, dehydration, stasis and alkalinization of urine that occur in spaceflight (Jones et al. 2008; Sibonga et al. 2008). Although generally innocuous in the kidney, a stone often causes debilitating pain as it passes and, even worse, may become obstructive, which could lead to sepsis, urinary tract infection, renal failure and even death (Glowacki et al. 1992; Pearle et al. 2005; Scales et al. 2012). Current ground-based technologies to detect kidney stones, such as plain-film x-ray and computed tomography, are unsuitable for flight because of the size of the equipment and/or exposure to ionizing radiation. B-Mode ultrasound can also be used to detect kidney stones; however, the sensitivity of the technique is highly dependent on the skills of the operator and has been reported to be as low as 30% (Fowler et al. 2002; Ulusan et al. 2007). The color Doppler ultrasound "twinkling artifact" (TA), which highlights hard objects such as kidney stones with rapidly changing color, has been found to improve the sensitivity of ultrasound for stone detection (Dillman et al. 2011; Lee et al. 2001; Mitterberger et al. 2009; Rahmouni et al. 1996; Winkel et al. 2012); however, the inconsistent appearance of the TA has limited its use in the clinic. Lu et al. (2013) proposed that stable crevice microbubbles on the kidney stone surface cause twinkling, and bubbles are expected to be very sensitive to aspects of space travel, including changes in ambient pressure and the composition of vehicle air. Here we report the influence of one aspect of space travel, namely, exposure to elevated levels of carbon dioxide, on twinkling in an *in vivo* porcine pilot study.

Twinkling is an artifact that was first observed in 1996 by Rahmouni et al. (1996). Several explanations have been proposed to explain twinkling, including the interaction of the acoustic field with the stone; an imperfection in the ultrasound machine; and stable crevice microbubbles on the kidney stone surface (Chelfouh et al. 1998; Kamaya and Rubin 2003; Lu et al. 2013). The

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primary evidence for stable crevice bubbles on the stone surface is that twinkling is suppressed by hydraulic overpressure and returns when the overpressure is released (Lu et al. 2013). Overpressure is known to shrink bubbles and drive them into solution. Bubbles in crevices are stabilized against dissolution from overpressure because the meniscus turns inward and surface tension acts to stabilize, not dissolve, the bubbles (Apfel and Holland 1991). That twinkling was first suppressed by overpressure and then observed to return when the overpressure was released supports the hypothesis that crevice microbubbles exist on the kidney stone surface; none of the other proposed mechanisms are affected by static overpressure. Furthermore, when a kidney stone was submerged in ethanol, a wetting agent with a surface tension much lower than water, twinkling was suppressed as the ethanol displaced the bubbles from the crevices (Lu et al. 2013). Although these results constitute strong evidence for twinkling being caused by crevice microbubbles on the kidney stone, the hypothesis is still under debate as the bubbles have not been directly observed (Tanabe et al. 2014). Nevertheless, specific conditions that occur in spaceflight are likely to perturb these bubbles and, therefore, influence the appearance of twinkling on kidney stones.

On the International Space Station, reported carbon dioxide levels have generally ranged from 2.3 to 5.3 mm Hg (10–20 times the concentration on Earth, equivalent to 0.4%–0.8% CO_2), with significant spatial and temporal variations (Alexander et al. 2012; James et al. 2011; Law et al. 2010). Local pockets of carbon dioxide form because of the reduced air convection in microgravity; according to a computational fluid dynamics model, without sufficient ventilation, these pockets of carbon dioxide can reach 9 mm Hg in as little as 10 min when the astronaut is sleeping (Alexander et al. 2012). The elevated concentrations of carbon dioxide have been reported to correlate with the reported incidence of headaches and have also been thought to contribute to increased intracranial pressure (Alexander et al. 2012; Law et al. 2010, 2014). Because of the link between symptoms and carbon dioxide levels, flight surgeons have lowered their action threshold to 5 mm Hg from the flight rule permissible exposure limit of 7.6 mm Hg (Law et al. 2010). Carbon dioxide is almost 30 times more soluble in blood than oxygen (O'Brien and Parker 1922), and long-term exposure to elevated carbon dioxide levels has been suggested to increase the risk of kidney stone formation because of the increased alkalinity of the urine (Brandis 2015; Tomoda et al. 1995). The variation in gas concentrations in the tissue and urine, as well as the physiologic adaptations from exposure to elevated levels of carbon dioxide, is likely to influence the formation and appearance of all bubbles in the human body, including those that may be present on the surface of kidney stones.

Our goal here is to present experimental evidence from a pilot study performed to determine that exposure to elevated levels of carbon dioxide reduces the magnitude and persistence of the color Doppler ultrasound twinkling artifact in an *in vivo* porcine model. Pigs were exposed to an anesthetic carrier gas that oscillated between oxygen and elevated carbon dioxide at 8000 ppm in air (20 times the concentration of carbon dioxide on Earth, the upper end of the reported ambient carbon dioxide levels found on the International Space Station) while twinkling was monitored qualitatively during the course of the experiment and quantitatively from the saved in-phase quadrature (I/Q) data.

METHODS

All animal procedures were approved by the University of Washington Institutional Animal Care and Use Committee. Five farm-bred pigs were injected intramuscularly with Telazol pre-medication before being intubated and maintained at a surgical plane with isoflurane in oxygen. The animals were instrumented to monitor heart rate, blood oxygenation, temperature (Ultraview SL, Spacelabs Healthcare, Snoqualmie, WA, USA) respiration rate and the partial pressure of exhaled carbon dioxide (CO_2 SMO, Novametrix Medical Systems, Wallingford, CT, USA). The abdomen and torso of the animal were carefully shaved and depilated with Nair to improve ultrasound coupling. Then, *ex vivo* human calcium oxalate monohydrate kidney stones (approximately 4 mm in diameter) that had been stored in water for at least a week and known to display the TA *in vitro* were implanted into the animals *via* retrograde ureteroscopy. Care was taken to place the two stones in separate calyces of the kidney. At the end of the stone implantation, a catheter was left in the bladder for urine collection. Through use of a Doppler-guided ultrasound needle, a catheter was placed in the femoral artery for blood collection, and baseline blood and urine samples were collected. The kidney was imaged by a sonographer with a Verasonics research ultrasound system (Redmond, WA, USA) and Philips/ATL P4-2 (Bothell, WA, USA) transducer to locate the stones. The stone with the best visualization of ultrasound twinkling was chosen for the study, after which the transducer was clamped in place for the duration of the study with only minor adjustments to position. A baseline twinkling was collected for at least 15 min before the anesthetic gas carrier was changed in four of the pigs from oxygen to elevated carbon dioxide at 8000 ppm in compressed air; the fifth pig was maintained on oxygen for more than 1 h to serve as a sham. The four treated pigs continued to breathe the increased carbon