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● *Original Contribution*

HIGH-FREQUENCY ULTRASOUND FOR *IN VIVO* MEASUREMENT OF COLON WALL THICKNESS IN MICE

Mostafa A. Abdelrahman,* Gemma Marston,* Mark A. Hull,* Alexander F. Markham,* Pamela F. Jones,* J. Anthony Evans,† and P. Louise Coletta*

*Leeds Institute of Molecular Medicine, University of Leeds, St. James's University Hospital, Leeds United Kingdom; and †Leeds Institute of Genetics, Health and Therapeutics, Leeds, United Kingdom

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Abstract—Mouse models are becoming increasingly important in the study of molecular mechanisms of colorectal disease and in the development of novel therapeutics. To enhance this phase of preclinical research, cost-effective, easy to use noninvasive imaging is required to detect and monitor changes in the colon wall associated with disease pathology. This study investigated the feasibility of using 40-MHz (high frequency) B-mode ultrasound (HF-US) to image the normal mouse colon and measure its thickness *in vivo* by establishing a robust imaging protocol and conducting a blinded comparison of colon wall thickness (CWT) measurement between and within operators. The *in vivo* and *ex vivo* appearance of mouse colon under HF-US revealed distinct patterns. Colon wall thickness was reproducibly and accurately measured using HF-US compared with histology measurement. The technique was more sensitive in detecting changes in CWT in distal than proximal colon as it showed the highest level of inter- and intraoperator reproducibility. Using the protocol described, it is possible to detect changes in thickness of 0.09 mm and 0.25 mm in distal and proximal colon, respectively. In conclusion, HF-US provides an easy to use and noninvasive method to perform anatomical investigations of mouse colon and to monitor changes in CWT. (E-mail: P.L.Coletta@leeds.ac.uk) © **2012 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **High frequency ultrasound, Colon wall thickness, Mouse models, Mouse colon, *In vivo* imaging, Preclinical ultrasound.**

INTRODUCTION

The mouse is often the model of choice in the study of human diseases because of their genetic and physiologic similarities, as well as the ease with which the mouse genome can be manipulated and analysed. Several transgenic and experimental mouse models have been developed to investigate diseases that affect the colon and rectum (Heyer et al. 1999; Moser et al. 1990; Wirtz et al. 2007) and changes in colon wall morphology and thickness that are related to disease progression and severity. As such, measuring colon wall thickness (CWT) may provide a useful biomarker for monitoring drug efficacy and response to treatment, as well as evaluating chemoprevention in preclinical and clinical trials. A noninvasive, easy to use, accurate and reproducible method of measuring CWT *in vivo* in mouse models would improve preclinical studies by providing more accurate data from longitudinal studies while also reducing the number of animals used.

Magnetic resonance imaging (MRI) has been shown to be sensitive to changes in CWT (Young et al. 2009) and provides a tool to monitor changes (Melgar et al. 2007). However, MRI is not portable and is not routinely available in animal facilities due to the high cost of specialist infrastructure and operation. Micro-computed tomography (Micro-CT) has also been used to reliably measure CWT (Fredin et al. 2008) but, in addition to its high cost and specialist infrastructure, it exposes animals to high radiation dose in longitudinal studies. Although transabdominal US has been used routinely in imaging the human gastrointestinal tract (Jeffrey et al. 1987; Pradel et al.1987; Hata et al. 1992) and for measuring CWT in children (Haber et al. 2000), it has not been used as yet to investigate CWT or morphology in mouse colon.

High frequency ultrasound (HF-US) (40-MHz) gives spatial resolution of 90 × 30 μm (Turnbull et al. 1995) allowing high definition imaging of mouse organs

Address correspondence to: P. Louise Coletta, Leeds Institute of Molecular Medicine, University of Leeds, St. James's University Hospital, Leeds LS9 7TF, UK. E-mail: P.L.Coletta@leeds.ac.uk

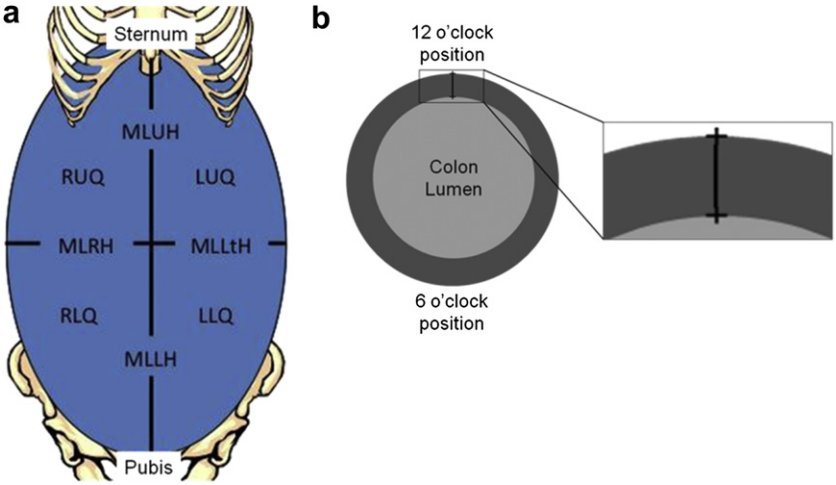

Fig. 1. Reference diagrams for imaging and measuring mouse colon wall thickness. (a) Diagram showing mouse abdomen divided into named quadrants. (b) Positioning of electronic callipers used to measure CWT. Callipers were placed at the 12 o'clock position parallel with the insonation beam direction. Crosshairs were placed on the edges of the colon wall with the crossing arms lying against the wall interface. RUQ: right upper quadrant, LUQ: left upper quadrant, RLQ: right lower quadrant, LLQ: left lower quadrant, MLUH: midline upper half, MLRH: midline right half, MLLtH: midline left half, MLLH: midline lower half.

such as liver, kidney, eyes and heart (Foster et al. 2002; Graham et al. 2005; Jolly et al. 2005; Sun et al. 2008). In addition, HF-US has been used to study tumour growth and development in different human cancer cell xenografts and transgenic models (Cerniglia et al. 2009; Xuan et al. 2007).

To use HF-US in mouse models of colorectal disease, a robust protocol that is operator independent is required to ensure sensitivity and reproducibility. Here we describe a protocol for HF-US imaging of mouse colon and for measurement of normal CWT *in vivo* that is both sensitive and reproducible. It shows that the thickness of normal C57BL/6 mouse colon can be accurately measured in longitudinal studies without any bowel preparation and, therefore, provides a fast and easy to use technique for *in vivo* measurement of CWT.

METHODS

Animals

All animal work was performed under licence and in accordance with the UK Animals (Scientific Procedures) Act 1986 following local ethical review and procedures. Twenty 8-week old female C57BL/6 mice were used. Animals were housed under specific pathogen free conditions with free access to diet and water. Prior to imaging, fur was removed from the abdominal region by shaving followed by the topical application of a hair removal cream. Animals were anaesthetized with 5% (v/v) isoflurane (Merial Animal Health Ltd., Essex, UK) in medical air at a flow rate of 2 L/min. Animals were placed on a heated imaging platform (VisualSonics Inc., Toronto, Canada) in supine position with anaesthesia maintained at 3% (v/v) isoflurane and with vital signs monitored. After the final imaging session, animals were sacrificed and tissues taken for histology.

Ultrasound imaging

Ultrasound imaging was performed independently by two blinded operators using a VisualSonics Vevo770 ultrasound system (VisualSonics Inc., Toronto, Canada). After applying coupling gel (EcoGel 100™; Eco-Med Pharmaceuticals Inc., Mississauga, Canada) to the skin, ultrasound scans were performed in B-mode with the transducer positioned above the animal in a holder. The imaging platform and transducer positions were manipulated as appropriate to image the colon. A 40-MHz mechanical single element transducer (RMV-704; VisualSonics Inc.) with a nominal focus at 6 mm depth was used. At best this should provide lateral and axial resolutions of 90 μm and 30 μm, respectively. However, using a thin nylon wire test object we concluded that the 90 μm value is only achieved within 0.3 mm of the focal depth (data not shown). Therefore, to achieve optimal measurement accuracy we carried out all measurements at as close to a depth of 6.0 mm as was practical. Contrast (8) and brightness (0) were used at default settings, the time gain compensation (TGC) was set at 10 and the field of view (FOV) was 10 $\times$ 10 mm. For spatial and descriptive reference, the abdominal region was divided into quadrants as shown in Figure 1a.

Scan protocol and image acquisition

All animals were scanned by two operators at day 0, day 7 and day 7+ to determine intra- and interoperator