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● *Original Contribution*

RELIABILITY OF CONTRAST-ENHANCED ULTRASOUND FOR THE ASSESSMENT OF MUSCLE PERFUSION IN HEALTH AND PERIPHERAL ARTERIAL DISEASE

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Abstract—We investigated the reliability of contrast-enhanced ultrasound (CEUS) in assessing calf muscle microvascular perfusion in health and disease. Response to a post-occlusive reactive hyperaemia test was repeated on two occasions >48 h apart in healthy young (28 ± 7 y) and elderly controls (70 ± 5 y), and in peripheral arterial disease patients (PAD, 69 ± 7 y; n = 10, 9 and 8 respectively). Overall, within-individual reliability was poor (coefficient of variation [CV] range: 15–87%); the most reliable parameter was time to peak (TTP, 15–48% CV). Nevertheless, TTP was twice as long in elderly controls and PAD compared to young (19.3 ± 10.4 and 22.0 ± 8.6 vs. 8.9 ± 6.2 s respectively; $p < 0.01$), and area under the curve for contrast intensity post-occlusion (a reflection of blood volume) was ~50% lower in elderly controls ($p < 0.01$ versus PAD and young). Thus, CEUS assessment of muscle perfusion during reactive hyperaemia demonstrated poor reliability, yet still distinguished differences between PAD patients, elderly and young controls. (E-mail: andre.vanrij@otago.ac.nz) © 2015 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Muscle perfusion, Peripheral arterial disease, Contrast-enhanced ultrasound.**

INTRODUCTION

Contrast-enhanced ultrasound (CEUS) combines traditional ultrasound imaging with intravenous contrast added into the systemic circulation. The contrast is a homogeneous, opaque suspension of lipid-shelled gas microbubbles, which are 1–5 μm in diameter. Importantly, the microbubbles are smaller than red blood cells and therefore can pass throughout the circulation, including microcirculation, but they cannot pass through vessel walls. This containment in intravascular spaces and the increased echogenicity are advantages of their use in perfusion studies; that is, the contrast in a tissue is an indicator of the perfusion and, therefore, the blood volume in that tissue. CEUS is increasingly being used in clinical practice for echocardiography (Wei et al. 1998) and in the assessment of liver and renal perfusion (Kalantarinia et al. 2009) and tumor vascularity (Dietrich et al. 2012). More recently, a role in the evaluation of angiogenesis (Leong-Poi et al. 2003) and contrast-mediated gene delivery has also been proposed (Smith et al. 2011). However, other ultrasonography practices may also benefit from this developing technique.

One such practice in which CEUS may have potential value is the assessment of skeletal muscle perfusion. Current methods for assessing limb blood flow tend to provide an assessment, directly or indirectly, of flow in conduit vessels of the limb rather than in the specific tissues. The direct examination of microvascular perfusion within a specific muscle or group of muscles is limited at present (Roustit and Cracowski 2012). Given the unique advantage of the CEUS technique to specifically assess perfusion within a tissue or organ, it may provide a robust tool to quantify and improve understanding of muscle perfusion in healthy and diseased populations. CEUS has been used in several investigations of muscle perfusion in recent years (Amarteifio et al. 2011,

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2013b; Duerschmied et al. 2009; Krix et al. 2009, 2011), but its reliability is not known. Specifically, none of these CEUS perfusion studies have made an effort to assess the reproducibility of the technique - an important step in the transition from feasibility studies to regular use, whether in a research or in a clinical role.

Patients with peripheral arterial disease (PAD) suffer from the common, debilitating symptom of intermittent claudication (*i.e.,* walking-induced pain), likely caused by reduced muscle perfusion. The specific changes in muscle perfusion with disease progression and the effects of different therapies are not well understood. This makes PAD patients an important group in which to test the utility of CEUS in assessing muscle perfusion.

Compromised blood flow, as occurs in PAD, is best revealed when there is an increased demand for blood supply, such as during exercise. However, exercise testing has limitations, particularly in diseased populations (*e.g.,* claudication, cardiorespiratory impairment, mobility issues unrelated to the vascular system and lack of motivation). Post-occlusive reactive hyperaemia (PORH) induces a standardized ischemic episode resulting in reperfusion, which is reproducible and more practical than exercise (Krix et al. 2011). The PORH test has been used in studies with laser Doppler (Roustit and Cracowski 2012) and during CEUS examinations of lower limb muscle perfusion in healthy volunteers (Krix et al. 2011) and patients with PAD (Amarteifio et al. 2011, 2012) and diabetes (Amarteifio et al. 2013b). The test has been validated against exercise (Krix et al. 2011), which is important to maintain the external validity of the dynamic test used. Therefore, we used the PORH provocation test to determine the reliability of CEUS in measuring muscle perfusion on two repeat occasions. The secondary aim was to explore whether there were differences in perfusion between elderly individuals with or without PAD, which has been suggested by others but has not previously been established in studies using CEUS. To our knowledge, no scientific study has yet directly compared CEUS assessment in PAD patients with that in similarly aged healthy controls, or used it to determine age-related changes in a healthy population.

METHODS

Study population

We studied three groups: (i) healthy young controls (young, n = 10); (ii) healthy elderly controls, of the same age range as the patients (elderly, n = 9); (iii) patients with PAD (PAD, n = 10; 2 patients were excluded before analysis, see Results). Demographic characteristics are listed in Table 1. The study was approved by the Lower South Regional Ethics Committee, and conformed to the standards set by the Declaration of Helsinki. Participants were informed of experimental procedures and possible risks involved, screened for comorbidities relating to the administration of contrast (*e.g.,* known or suspected right-to-left cardiac shunt, hypersensitivity to perflutren) and invited to take part. For the PAD patients, this occurred after clinical diagnostic tests in the laboratory, if they met inclusion criteria (exercise-induced claudication and an exercise-induced drop in ankle–brachial index [ABI]). The ABI is a widely used screening test for PAD; it is the ratio of the systolic blood pressure at an ankle artery to that at the brachial artery. Both the resting and post-exercise ABI contribute to diagnosis of PAD (Stein et al. 2006). Written informed consent was obtained before participation.

Table 1. Characteristics of participants (mean ± standard deviation)

	Young	Elderly	PAD
n	10 (6 males)	9 (8 males)	8 (7 males)
Age (y)	28 ± 7	70 ± 5	69 ± 7
Height (cm)	176 ± 9	174 ± 8	171 ± 10
Mass (kg)	77 ± 11	82 ± 16	75 ± 11
Body mass index (kg m^{-2})	24.8 ± 3.3	27.8 ± 2.5	25.7 ± 2.7

PAD = peripheral arterial disease.

Participant characteristics

Young controls were not taking any medication, all were non-smokers and all were recreationally active, typically engaging in moderate-intensity aerobic activities (*e.g.,* jogging) and/or resistance training (~3 d/wk). Elderly controls were recreationally active, and the absence of PAD was verified using the resting ABI (*i.e.,* index >0.9). One was a current smoker, six were ex-smokers and the remaining two were non-smokers. PAD patients all described claudication; the distance to onset ranged between 50 and 200 m, and symptoms had been noted for at least 1 y in the leg under investigation. The mean resting ABI for PAD patients was 0.79 (range: 1.02–0.50), and all experienced a decrease after exercise (of 0.32 ± 0.15). Three patients were current smokers, six were ex-smokers and one was a non-smoker. None of the participants were diabetic.

Experimental design

The study involved two identical experimental tests, performed >48 h apart at the same time of day. All participants were instructed to limit their physical activity for the morning before the test. After 10 min of supine rest, a venous cannula was inserted into a forearm vein for infusion of the contrast agent.