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An Investigation of the Detection Capability of Pulsed Wave Duplex Doppler of Low Grade Stenosis using Ultrasound Contrast Agent Microbubbles – an in-Vitro Study

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Title: An investigation of the detection capability of pulsed wave duplex Doppler of low grade stenosis using ultrasound contrast agent microbubbles – an in-vitro study

Running Title: Detection of low grade stenosis with UCA Doppler

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Abstract

Objective: The objective of the study was to investigate whether clinically used ultrasonic contrast agents improved the accuracy of spectral Doppler ultrasound in the detection of low grade (< 50%) renal artery stenosis. Low grade stenoses in the renal artery are notoriously difficult to reliably detect using Doppler ultrasound due to difficulties such as overlying fat and bowel gas.

Methods: A range of anatomically-realistic renal artery phantoms with varying low degrees of stenosis (0, 30 and 50%) were constructed and peak velocity data was measured from within the pre-stenotic and mid-stenotic regions in each phantom, for both unenhanced and contrast-enhanced spectral Doppler data acquisitions. The effect of a 20mm overlying fat layer on the ultrasound beam distortion and phase aberration, and hence on the measured peak velocity data, was also investigated.

Results: The overlying fat layer produced a statistically significant underestimation ($p<0.01$) in both the peak velocity and peak velocity ratio [Stenotic Region(V_{max})/Pre-stenotic Region(V_{max})] for the 0% and 30% stenosis models, but not the 50% model. A statistically significant increase ($p<0.01$) in the peak velocity was found in the contrast-enhanced Doppler spectra; however, no significant difference was found between the unenhanced and contrast enhanced peak velocity ratio data, which suggests that the ratio metric has better diagnostic accuracy. The peak velocity ratios determined for each of the contrast-enhanced phantoms correctly predicted if the phantom had a stenosis and furthermore correctly classified the degree of stenosis.

Conclusion: Contrast-enhanced Doppler ultrasound could significantly assist in the early detection of renal artery disease.

Highlights

- Anatomically realistic flow phantoms
- Contrast-enhanced Doppler spectra
- Fat Mimicking layers

KEY WORDS: Doppler Ultrasound; Ultrasound Contrast Agents; Anatomical Renal Artery Phantoms; Peak Velocity; Peak Velocity Ratio.

1 Introduction

2 Renal artery stenosis (RAS) is the most common cause of potentially curable secondary
3 hypertension, with the most frequent cause being due to atherosclerosis (63 %) in patients
4 older than 50 years and fibromuscular dysplasia (32 %) in younger patients. Secondary
5 hypertension is potentially curable, and so its early detection is critical as this offers the
6 possibility of noninvasive antihypertensive drug treatments rather than more invasive
7 procedures such as re-vascularization through percutaneous transluminal renal angioplasty or
8 surgery [1-5]. If left untreated, this progressive disease has many associated morbidities
9 including renal insufficiency, myocardial infarction, congestive heart failure, stroke and death.
10 Consequently, there is significant interest in the detection of atherosclerotic disease in the
11 renal artery and whether more accurate stenosis assessment techniques would improve patient
12 outcome [6]. Ultrasound is considered to be an ideal first-line imaging technique for RAS due to
13 the fact that it is non-ionizing, non-invasive, of low cost [7-9] and in this regard, it is a promising
14 technique for RAS detection, specifically duplex ultrasound (DUS), which combines the direct
15 visualization of the renal arteries via B-mode imaging with Doppler measurement of the
16 velocity of blood flow in the main renal artery and within the kidney [10, 11]. In comparison
17 with the “gold standard” technique of X-ray Intra-Arterial Digital Subtraction Angiography (IA-
18 DSA), which has a sensitivity of 94-100% and specificity of 65-97% for RAS detection, DUS has a
19 sensitivity of 67–98% and a specificity of 54–99% [6-8, 11-14]. DUS has several limitations,
20 including its dependence on the skill of the operator, its ability to visualize accessory renal
21 arteries, its determination of high velocities at depth without aliasing, and a lack of Doppler
22 measurement sensitivity when imaging obese patients and those with overlying bowel gas [9,
23 15]. However, with an experienced operator, Duplex ultrasound has been found to be reliable
24 [16].

25
26 Ultrasound microbubble contrast agents are routinely used in contrast enhanced harmonic
27 imaging for enhancing the visibility of the arterial system, and have been found to enhance the
28 Doppler signal thereby, improving the diagnostic capability for RAS grading where the signal can
29 be affected by overlying fat layers [17]. Specifically, the use of ultrasound microbubble contrast

agents were found to increase the number of examinations which were deemed to be diagnostic in multicenter studies [18, 19]. This improvement in the diagnostic efficacy was found to aid the operator's ability to visualize the anatomy of the renal arteries, thereby decreasing the number of inadequate Doppler studies [18]. However, several studies which investigated the effect of ultrasound contrast agents on the measured spectral Doppler velocities, both *in vitro* and *in vivo*, reported conflicting results regarding an artificial increase in the peak systolic velocity due to the contrast agent itself [20-22].

Furthermore, it has been found in simple flow phantom *in-vitro* experiments that the presence of an overlying fat layer can result in underestimation of the peak velocity measurement, leading to a consequent incorrectly classified severity of stenosis. Given the physiology of the body, particularly in elderly patient populations, overlying subcutaneous fat layers are a common problem in renal artery imaging, and their impact can be confounded if the PSV parameter alone is used to classify the severity of a stenosis, thereby negatively impacting the patient's management and treatment [23]. The aim of this study was to perform a systematic investigation of the effect of an overlying fat layer on Doppler measurements of Peak Velocity and Peak Velocity Ratio using two commercially-available contrast agents, using anatomically realistic renal artery flow phantoms with varying degrees of accurately-known stenosis.

Methodology

Phantom Design and Manufacture

The vessel geometry used to fabricate the range of wall-less anatomically realistic renal artery flow phantoms was developed using inverse casting of a physical model produced by a rapid prototyping machine, based on a clinical 3-D X-ray computed tomography dataset of a healthy renal artery where the renal artery lumen diameter was measured to be 6.7 mm [24]. The vessels were manufactured by means of a silicone inverse mould which had a non-stenotic diameter equal to the lumen diameter of the renal artery (7.7 mm), using a methodology described previously [24]. The difference in the in dimensions of the final renal artery lumen

was as a result of the rapid prototyping process that resulted in the model diameter increasing by approximately 1 mm. An agar-based tissue mimicking material (TMM) was used in the flow phantoms [25]. Its composition and manufacture have previously been described by [26] and has been acoustically characterised and found to have good acoustic reproducibility between batch production [27-31]. The wall-less flow phantoms were attached to a flow circuit at the inlet and the outlet using 7.9 mm inner diameter Nalgene PVC plastic tubing (Thermo Fisher Scientific, NY, USA). The flow circuit consisted of blood mimicking fluid (BMF) pumped through the anatomical flow phantom by a high flow rate suction-shoe pump (07002-23, Cole-Parmer, Walden, UK) coupled to a direct-current servo motor (McLennan Servo Suppliers Ltd., Surrey, UK); a schematic of the circuit is shown in Figure 1. The BMF, which incorporated orgasol particles to replicate the red blood cells, has been previously characterised to have acoustic and physical properties similar to that of human blood [32]. An injection port, attached to a syringe via a 3-way tap, was inserted and glued into the plastic tubing, 22 cm from the inlet of the phantom and downstream from the pump for the injection of the contrast agents. Finally, a 20 mm scanning well of either speed-of-sound-corrected water or olive oil layer (i.e. a fat-mimicking layer, representing the fat layer in an average patient) [23, 33, 34] was coupled to the surface of each of the flow phantoms. The transducer was positioned longitudinally over the vessel and held in place using a clamp attached to a micrometer manipulator on a translation stage. The transducer was held at an angle, $\theta = 30^\circ$, to the surface of the scanning well and the total distance from the face of the transducer to the center of the unstenosed vessel was 82 mm (representing the depth of the renal artery in the average patient) [34], as can be seen in Figure 1.

The motor was driven by a servo amplifier (Aerotech Ltd, Berkshire, UK), multifunctional I/O board (PCI-6036E National Instruments, Austin, TX, USA) and a computer controller program developed in LABVIEW (National Instruments, Austin, TX, USA) was used to output a steady-state mean velocity of 26 cm s^{-1} which was used for all measurements. The steady-state mean velocities output by the pump system was calibrated using timed weight collection. It was important to ensure that the flow was laminar; therefore, the tube was straightened to an inlet length of 26 cm, as determined by: $L = 0.04 d \text{Re}$, where L =inlet length, d = diameter and $\text{Re} =$

Reynolds number, for a maximum velocity of 40 cm s^{-1} and BMF with a viscosity of 4 mPa s^{-1} . The temperature of the flow phantom and BMF was maintained at $22 \pm 1 \text{ }^{\circ}\text{C}$ throughout.

Three different stenosis phantoms were used: 0% (normal, un-stenosed), 30% stenosis and 50% stenosis (diameter reduction determined the stenosis, as shown in Figure 2. The stenosis were placed 10 mm from the inlet end of the phantom as it has been found that 60% of renal artery stenosis have been shown to occur at this point [35]. The centre of the vessel was located at a depth of 62 mm below the surface of the TMM phantom, representing the depth of the renal artery in the average patient [34].

Contrast Agents

Two contrast agents were used in this study: SonoVueTM (Bracco, Italy) and LUMINITY[®] (Lantheus Medical Imaging, USA). Both agents were reconstituted immediately prior to experiments and were discarded after a period of 4 hours to ensure consistency of the microbubbles used in the experiments. The agents were diluted in saline to a concentration of 1:1000, and 5 ml of the diluted contrast solution was injected manually over 2 s via the injection port described above. For each of the experiments, a comparison was made between the non-enhanced and contrast-enhanced Doppler spectra obtained from the BMF flowing through the phantom. For the contrast experiments, the BMF was not recirculated.

Measurements

An ATL HDI 3000 (Philips Medical Systems, Bothwell, WA, USA) ultrasound system with a broadband curvilinear transducer C4-2 (nominal frequency 2.5 MHz) was used to collect the peak velocity data in each of the anatomically-realistic renal artery flow phantoms. Peak velocity measurements (V_{max}) were acquired at both the pre-stenotic and mid-stenotic regions within each of the phantoms, and from these measurements the peak velocity ratio [Stenotic Region(V_{max}) / Pre-stenotic Region(V_{max})] was determined. The transducer was positioned longitudinally over the region of interest and held in place using a clamp attached to a micrometer manipulator on a translation stage which translated the transducer between the

two positions. The focal zone was positioned in the centre of the vessel and the time gain compensation control was adjusted to produce a uniform image of the TMM of the phantom. Spectral Doppler was turned on and Doppler velocity measurements were recorded with a gate length of 7.5 mm, the same diameter as the vessel lumen and the depth of the gate length was 82 mm (Figure 2). The following settings were used throughout the study. The pulse repetition frequency was adjusted so that the measured maximum flow velocity (without contrast) was no greater than 75% of the maximum spectral display to minimise the effect of Doppler aliasing. The wall filter was set to the lowest setting. The transducer was manually positioned to give a Doppler angle of 60° , as typically used clinically. The mechanical index was set to 0.1 and the Doppler gain was adjusted to 66% to obtain a strong Doppler waveform which was free from extraneous noise as determined prior to contrast agent administration. Doppler recording was continued without change in position of the range gate or ultrasound machine settings (such as gain, wall filter and transmit power) throughout the contrast effect. The goal was to make uninterrupted Doppler recordings throughout the contrast effect [22].

A cine-loop of the Doppler waveform was obtained. This was repeated three times for each of the renal artery flow phantoms (0%, 30%, 50%), at both pre-stenotic and mid-stenotic positions, for each contrast agent and for both the water and oil baths (36 Doppler spectra data sets). One observer, who was not blinded to the study, manually undertook the measurement of the peak velocities and peak velocity ratios from each of the spectra obtained (n=4 measurements at each site) for peak-enhancement and post-enhanced of the contrast agent. Statistical analyses was carried out using a dedicated software (SPSS, IBM, Armonk, NY, USA). Independent sample t-tests were used to compare (1) the Doppler measurement with no contrast agent with the water and oil layers and (2) the Doppler measurement with no contrast agent and the two types of contrast agents with the water and oil layers.

Results

The effect of the overlying oil layer on the accuracy of peak velocity estimations was determined for each of the anatomically renal artery flow phantoms and the results are

presented in Table 1. A statistically significant underestimation in the peak velocity was measured for the 0% and 30% stenosis models ($p < 0.01$), while no difference was found for the 50% stenosis model. The effect of the overlying oil layer on the peak velocity ratio was determined and found to be underestimated when there was an overlying oil layer in all three of the anatomically realistic models; these results for the un-enhanced data are also presented in Table 1. It can be seen from Figure 3 (a) – (b) that the overall brightness of the B-mode image and the Doppler spectrum were decreased due to the increased attenuation of the overlying oil layer.

The effect of both commercially-available contrast agents on the measured peak velocity in the absence and presence of an overlying oil layer was determined for each of the anatomically realistic renal artery flow phantoms and the results are presented in Figure 4 (a) – (c). No significant difference was measured in the peak velocity measurements with the overlying oil layer for both LUMINITY® and SonoVue™, compared to the underestimation produced by the oil layer in the absence of a contrast agent, for each of the renal artery flow phantom models. This demonstrates the improvement in the diagnostic accuracy in the challenging imaging situation presented by the overlying oil layers, with the administration of the contrast agents. The measured peak velocities increased between 9 – 20% in each of the renal artery flow phantom models following the administration of both LUMINITY® and SonoVue™ compared to the unenhanced control peak velocity measurements for the BMF.

The data for the peak velocity ratios are presented in Figure 5. In general, it can be seen that the spectra with no oil layer had a higher peak velocity ratio compared with the spectra which were attenuated by the overlying oil layer, for conditions of no contrast agent and with both LUMINITY® and SonoVue™, at 50% stenosis and normal renal artery flow phantom models, respectively. There was no significant difference between the peak velocity ratios for the unenhanced and enhanced measured ratios, which suggests that the ratio has better diagnostic accuracy. All the peak velocity ratios determined for each of the renal artery flow phantoms correctly predicted if the phantom had a stenosis as well as correctly classifying the degree,

except for the unenhanced 50% stenosis in the presence of an overlying oil layer. For this spectrum the peak velocity ratio was underestimated.

Discussion

Renal artery imaging is regarded as technically challenging due to the overlying subcutaneous fat of larger patients and/or in the presence of bowel gas and the corresponding reduction in the Doppler signal [6]. Previous studies have found that the presence of overlying fat layers can cause an underestimation in the measured peak velocity which could result in the patient being incorrectly managed or undertreated [23]. This current study corroborated this underestimation in the peak velocity, by up to 13% when a 20 mm overlying oil layer was present. This underestimation is most likely due to: (i) the lower speed of sound within the oil layer, which causes a decrease in the peak velocity determined by the ultrasound system, and (ii) the additional attenuation, by the oil layer, of the higher Doppler shift frequencies, which further reduces the peak velocity determined by the ultrasound system. Ultrasound contrast agents are known to enhance the amplitude of Doppler signals by increasing the amount of ultrasound beam energy being backscattered, and thus they can provide enhanced visualization of weak Doppler signals that are beyond the detection capabilities of the system due to excessive depth or attenuation [22, 36-38]. In particular, it may increase the detectability of weak low velocity signals at the edge of the vessel as well as higher velocities whose higher Doppler shift frequency would have been more preferentially attenuated due to the overlying fat layers. Thus, in addition to investigating the effects of overlying fat layers, the current study further investigated the impact of ultrasound microbubble contrast agents on the measured peak velocity by providing greater enhancement of the blood mimicking fluid and the corresponding Doppler signal. In studies where attenuation has been a feature affecting the Doppler signal, it has been found that there was no change in the measured peak velocity following the administration of ultrasound contrast agents [20, 22]; specifically, Guberlet et al, found no enhancement in the measured peak systolic velocity in an *in vitro* study of human common carotid arteries [39]. In contrast a number of investigators have argued that there

was in fact an increase in peak velocity following the administration of contrast agents and it was proposed that the observed higher velocities represented real signals that were beyond the detection limits of the Doppler processing unit prior to the administration of the contrast agent [21]. Forsberg et al. argued that this increase was not artificial, but rather is real signal deriving from the relatively small number of red blood cells moving at the highest velocity in the vessel, which prior to the injection of the contrast agent went undetected [40].

These findings were specific to the contrast agent Levovist (Bayer Shering), and the characterization of this potential effect for other contrast agents was not addressed. In the current study, it was demonstrated definitively that the measured peak velocity increased significantly following the administration of two commercially-available contrast agents, SonoVue™ (Bracco) and LUMINITY® (Lantheus Medical Imaging) compared to the unenhanced Doppler spectra.

The impact of the overlying fat layer on the PSV measurements was reduced with the administration of both types of contrast agents. In particular it was found that the PSVs in the absence and presence of the fat mimicking layer were comparable which is most likely due to the limited Doppler sensitivity and detection without the contrast agents of the weaker Doppler signals of the maximum velocity. There was no significant difference between the PSV measured in the 50 % stenosis in the absence and presence of the fat mimicking layer this could be due to the increased magnitude of red blood cells travelling at this maximum velocity in the local stenosed region whereas in the normal and 30 % stenosed renal artery phantom in presence of fat the signal amplitude of the PSV was lower due to firstly, the attenuation of the fat mimicking layer and secondly, the lower signal magnitude of the increased PSV of the non-haemodynamically significant stenosis. With both of these factors combine the increased PSV may have been beyond the Doppler detection of system. This requires further investigation as it was expected that the PSV would be underestimated in a similar manner as it was in the normal and 30 % stenosis renal artery flow phantom in the presence of the fat mimicking layer.

All the peak velocity ratios determined for each of the renal artery flow phantoms correctly predicted the degree of stenosis present in the anatomically realistic flow phantom for the

unenanced and enhanced Doppler spectra. For this latter spectrum, the peak velocity ratio was underestimated, which in a clinical situation has the potential to negatively affect the patient management. However, with the application of contrast agents, this degree of stenosis was correctly predicted, with increased peak velocity and peak velocity ratio measured. The use of the RAR instead of the absolute PSV value is preferable where the existence of hypertension is suspected as this can cause increased PSV velocities in all the vessels in hypertensive patients [19] It has been found in multiple studies that an RAR of > 3 successfully identified hemodynamically significant lesions with a sensitivity and specificity of 91 - 92% and 77 - 91%, respectively [19]. However, Doppler examinations were prone to technical failure in due to severe obesity, the use of older US devices, excessive bowel gas or poor flow in the main RA due to severe renal impairment. Therefore, the overlying fat layer may have been the reason as to why the RAR measured in the renal artery with a 50 % was < 2 . Furthermore, the two stenoses evaluated in this study would be regarded as early disease as well as not being considered as haemodynamically significant ($> 60\%$). With the use of ultrasound contrast agents it can be seen that the presence of that both of these lower grade stenoses were differentiated using the RAR parameter from the normal renal artery.

Finally, the use of the two commercially-available contrast agents improved the delineation of the vessel, thereby improving the diagnostic accuracy of the Doppler examination, particularly in the presence of a fat mimic layer which produced a weaker spectrum with lower signal-to-noise ratio and affected the measured unenhanced peak velocity.

Conclusion

A range of anatomically-realistic renal artery flow phantoms with varying degrees of stenosis were used to investigate the effect of a bolus injection of two commercially-available contrast agents, SonoVueTM (Bracco) and LUMINITY[®] (Lantheus Medical Imaging). It was found that the peak velocity measured by Doppler ultrasound increased following the bolus injection of both contrast agents, with no significant difference observed between the contrast agents. All the peak velocity ratios determined for each of the renal artery flow phantoms correctly predicted the degree of stenosis present in the anatomically realistic flow phantom for the unenhanced and enhanced Doppler spectra, except for the unenhanced 50% stenosis in the presence of a fat-mimicking overlying oil layer. For this latter spectrum, the peak velocity ratio was underestimated, which could potentially negatively affect the patient management. However, with the administration of either contrast agent, this degree of stenosis was correctly predicted, indicating that the use of contrast agents improved the diagnostic accuracy of the Doppler examination. Furthermore, the two stenoses evaluated in this study would be regarded as early disease as well as not being considered as haemodynamically significant (> 60 %). With the use of ultrasound contrast agents it can be seen that the presence of that both of these lower grade stenoses were differentiated using the RAR parameter from the normal renal artery. This work would suggest that the use of contrast agents may well become an essential tool for the clinician in the diagnosis of low grade stenosis in an increasing overweight and obese population. In conclusion, the use of ultrasound contrast agents could provide information at an earlier stage of disease progression which can be incorporated into a stratified screening program for higher risk patient groups (such as diabetic patient groups). This could be used to positively alter patient outcomes, specifically provide earlier drug treatment interventions and possibly delay the loss of kidney function and the need for kidney transplant or haemodynamic dialysis.

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Figure 1. Schematic of the flow circuit used for the renal artery contrast-enhanced flow phantom measurements

Figure 2: (a) Illustration of the method used to determine the degree of stenosis; (b) Photograph of the 0%, 30% and 50% stenosis models; and (c) ultrasound image and Doppler spectrum for the 50% stenosis renal artery flow phantom.

Figure 3: The top row shows the Doppler Spectrum measured in the 50% stenosis renal artery flow phantom at the point of the stenosis with the 20 mm overlaying water layer (left) and 20 mm overlaying fat mimicking olive oil layer (right).

Figure 4: Peak Velocity measurements at the point of the stenosis in (a) 0% (normal, unstenosed); (b) 30% stenosis and (c) 50% stenosis renal artery flow phantom unenhanced and with peak enhancement following administration of the commercially-available contrast agents LUMINITY® and SonoVue™, with a 20 mm water layer and a 20 mm fat-mimicking olive oil layer.

Figure 5: Peak Velocity Ratio measurements at the point of the stenosis in (a) 0% (normal, unstenosed); (b) 30% stenosis and (c) 50% stenosis renal artery flow phantom unenhanced and with peak contrast enhancement following administration of the commercially-available contrast agents LUMINITY® and SonoVue™ with a 20 mm water layer and a 20 mm fat mimicking olive oil layer. The normal peak velocity ratio is indicated by the red line and the ≤50% stenosis is indicated by the blue line.

Table 1. The peak velocity ($V_{\max}$) and Peak Velocity ratio [$\text{Stenotic Region}_{(V_{\max})}/\text{Pre-stenotic Region}_{(V_{\max})}$] measured at the site of stenosis at a set mean steady velocity of 26 cm s^{-1} in the 0%, 30% and 50% stenosed renal artery flow phantom with the scanning well filled with speed of sound corrected water and the olive oil fat mimic.

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Figure 1. Schematic of the flow circuit used for the renal artery contrast-enhanced flow phantom measurements

Figure 2: (a) Illustration of the method used to determine the degree of stenosis; (b) Photograph of the 0%, 30% and 50% stenosis models; and (c) ultrasound image and Doppler spectrum for the 50% stenosis renal artery flow phantom.

Figure 3: Doppler Spectrum measured in the 50% stenosis renal artery flow phantom at the point of the stenosis with the 20 mm overlaying water layer (left) and 20 mm overlaying fat mimicking olive oil layer (right).

Figure 4: Peak Velocity measurements at the point of the stenosis in (a) 0% (normal, unstenosed); (b) 30% stenosis and (c) 50% stenosis renal artery flow phantom unenhanced (n=12) and with peak enhancement following administration of the commercially-available contrast agents LUMINITY® (n=12) and SonoVue™ (n=12), with a 20 mm water layer and a 20 mm fat-mimicking olive oil layer.

Figure 5: Peak Velocity Ratio measurements at the point of the stenosis in (a) 0% (normal, unstenosed); (b) 30% stenosis and (c) 50% stenosis renal artery flow phantom unenhanced (n=12) and with peak contrast enhancement following administration of the commercially-available contrast agents LUMINITY® (n=12) and SonoVue™ (n=12) with a 20 mm water layer and a 20 mm fat mimicking olive oil layer. The normal peak velocity ratio is indicated by the red line and the $\leq 50\%$ stenosis is indicated by the blue line.

Table 1. The peak velocity (V_{max}) and Peak Velocity ratio [Stenotic Region_(Vmax)/Pre-stenotic Region_(Vmax)] measured at the site of stenosis at a set mean steady velocity of 26 cm s⁻¹ in the 0%, 30% and 50% stenosed renal artery flow phantom with the scanning well filled with speed of sound corrected water and the olive oil fat mimic.

Stenosis	Scanning Well	V_{max} (cm s⁻¹)	Peak Velocity ratio
[% difference]			
Normal	No oil	70 ± 2.5	1.3
	Oil	60 ± 2.5	1.0
		[-13]†	
30%	No oil	98 ± 2.5	1.7
	Oil	92 ± 2.5	1.5
		[-6]†	
50%	No oil	109 ± 2.5	2.2
	Oil	109 ± 2.5	1.8
		[No difference]	

† Statistically significant $p < 0.01$