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REVIEW

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Contrast-enhanced ultrasound for quantification of tissue perfusion in humans

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Abstract

Contrast-enhanced ultrasound is an imaging technique that can be used to quantify microvascular blood volume and blood flow of vital organs in humans. It relies on the use of microbubble contrast agents and ultrasound-based imaging of microbubbles. Over the past decades, both ultrasound contrast agents and experimental techniques to image them have rapidly improved, as did experience among investigators and clinicians. However, these improvements have not yet resulted in uniform guidelines for CEUS when it comes to quantification of tissue perfusion in humans, preventing its uniform and widespread use in research settings. The objective of this review is to provide a methodological overview of CEUS and its development, the influences of hardware and software settings, type and dosage of ultrasound contrast agent, and method of analysis on CEUS-derived perfusion data. Furthermore, we will discuss organ-specific imaging challenges, advantages, and limitations of CEUS.

KEYWORDS

contrast-enhanced ultrasound, perfusion imaging

1 | INTRODUCTION

In vivo quantification of microvascular perfusion provides valuable information regarding organ function, since tissue perfusion partly regulates the exchange of oxygen, nutrients, and waste products between tissue and blood. To gain insight into microvascular hemodynamics of vital organs, perfusion measurements in superficial tissues such as skin, bulbar conjunctiva, and sublingual mucosa are often used in research settings as these imaging techniques are easy and noninvasive. However, measurements of superficial tissues cannot be directly translated to other tissues in all conditions.[1]

Contrast-enhanced ultrasound allows for quantification of perfusion of various deep organs, including skeletal muscle, heart, adipose tissue, the kidneys, liver, and brain. It involves the use of microbubble contrast agents and specific hardware and software for imaging of these microbubbles. The use of blood-borne contrast agents in CEUS is crucial, since blood cells are poor reflectors of ultrasound waves.[2] Using the CEUS technique, tissue hemodynamics can be expressed by different parameters, including MBV, that is, the proportion of tissue volume existing of blood, MFV, that is, the speed of red blood cells, and MBF, that is, the volume of blood passing a section of tissue per unit of time.[3] MBV, in contrast to MBF, is considered more important to the extraction rate of oxygen and

Abbreviations: AI, acoustic intensity; AU, arbitrary units; AUC, area under the curve; CEUS, contrast-enhanced ultrasound; CV, Coefficients of variation; MBF, microvascular blood flow; MBV, microvascular blood volume; MFV, microvascular flow velocity; MI, mechanical index; MTT, mean transit time; PET, positon emission tomography; PI, Peak intensity; ROI, region of interest; ROI, regions of interest; TIC, time-intensity curves.

nutrients to target tissues, since it reflects the blood volume in direct contact with the vascular endothelium.[4]

The earliest report on the application of CEUS dates back from 1968.[5] Since then, investigators have rapidly gained experience and progress has been made in the development of ultrasound machines and contrast agents. Over the years, universal guidelines regarding the application of CEUS in clinical settings have been developed.[6-8] However, there are no guiding principles regarding the quantification of tissue perfusion in research settings, allowing investigators to choose several variables that influence CEUS data, including the selection of hardware, echo contrast agent and dosage, application mode (for example pulse-interval versus real-time imaging and constant infusion versus bolus injection), and data analysis. The objective of this review is to provide an overview of the use of CEUS for quantification of tissue perfusion in general, as well as organ-specific applications, advantages, and limitations. Aside from perfusion imaging, CEUS has been applied in clinical practice for visualizing specific anatomical structures,[9] for molecular imaging[10] and used as therapeutic agent to allow site-specific drug delivery.[2] However, these applications of CEUS have been extensively reviewed elsewhere and are beyond the scope of this review.

1.1 | Ultrasound machine, transducers, and settings

Several companies, such as Philips®, Siemens®, and Samsung®, produce ultrasound machines with a contrast imaging mode. In these ultrasound systems, the investigator controls contrast-specific system settings, such as gain, dynamic range, mechanical index, or frequency and amplitude. These settings, described in detail in the next paragraph, determine how raw acoustic signals are translated into an image intensity. It is important to choose the appropriate setting for each study protocol and to maintain identical settings during a study, because these settings affect image intensity and thereby quantification of tissue perfusion. In addition, each ultrasound system includes different types of transducers. These transducers differ in size and shape of the ultrasound beam, and more importantly the ultrasound frequency range. Each imaging frequency is a compromise between imaging depth and spatial resolution. A high ultrasound frequency enables imaging with high spatial resolution, which is required to define small and superficial anatomical structures.[11] As a result, high-frequency transducers are restricted to superficial measurements. Low ultrasound frequencies provide lower spatial resolution but a higher AI in deeper tissues, enhanced by the fact that most microbubbles resonate most strongly at low ultrasound frequencies. Therefore, the optimal transmitted frequency should be determined before initiating a study protocol.

1.2 | Ultrasound settings

1. The *amplitude (P), or the acoustic pressure amplitude*, is related to the strength ("loudness") of the applied ultrasound. It is defined as the difference between the peak pressure value and the mean pressure value of the waveform and is expressed in Pascal (Pa) or decibel (dB). The amplitude of the ultrasound beams decreases when it travels through tissue. This is called attenuation.
2. The *frequency (f)* is defined as the number of sound waves per second. It is expressed in Hertz (Hz). The ultrasound spectrum is defined at frequencies higher than the upper audible limit of human hearing, approximately above 20 kHz. The choice of transmit frequency selection depends on the target tissue. Higher frequencies improve spatial resolution at the expense of penetration depth.
3. The MI is a measure of ultrasound power, which is defined as the amount of energy transfer. It can be calculated by dividing the amplitude by the square root of the frequency. Choosing the appropriate MI is important for adequate perfusion quantification, because this parameter affects microbubble behavior, which is further explained in the paragraph "pulse-interval vs. real-time imaging."
4. The *gain* refers to the amplification of the received signal. It can be used to compensate for attenuation and controls how bright the image appears. When the gain is increased, the received signal is amplified and the image becomes brighter. An excessive increase in gain will increase the signal but will also add "noise" to the image. Vice versa, when the gain is reduced, signal and noise become weaker. For perfusion quantification using CEUS, the gain is usually set to the highest value at which the image is still almost dark in the absence of microbubbles.[11] It is key that the optimal gain with the highest signal-to-noise ratio is determined prior to initiation of a study.
5. The optimal *image depth* depends on the localization of tissue of interest. Deeper tissues require a lower transmitted frequency, which increases the maximal imagine depth but reduces image resolution. Higher frequencies result in better image resolution, but limit the image depth. The image resolution will increase when the total depth of the image plane is reduced. Therefore, it is key to set the image depth so that only the tissue of interest is visualized.
6. The *focus* is the area within the beam where lateral spatial resolution is at its optimum. The focus of an image can be positioned at the high of the target tissue or deeper to achieve a more uniform acoustic field, which improves sensitivity to the agents and reduces the risk of bubble disruption.[12]
7. The *frame rate* refers to the amount of pictures captured per second and is also expressed in Hz. A higher frame rate allows better assessment of microvascular flow velocity. However, increased frame rates can augment bubble destruction specifically at high MI.[13]
8. There are many different algorithms and filters which may be applied to the raw image before it is displayed. These settings are called postprocessing settings and include but are not limited to dynamic range, edge, delta, and color map settings. The *dynamic range* refers to the range of ultrasound intensities that can be displayed by the ultrasound machine. A wide dynamic range results in increased shades of gray, whereas a

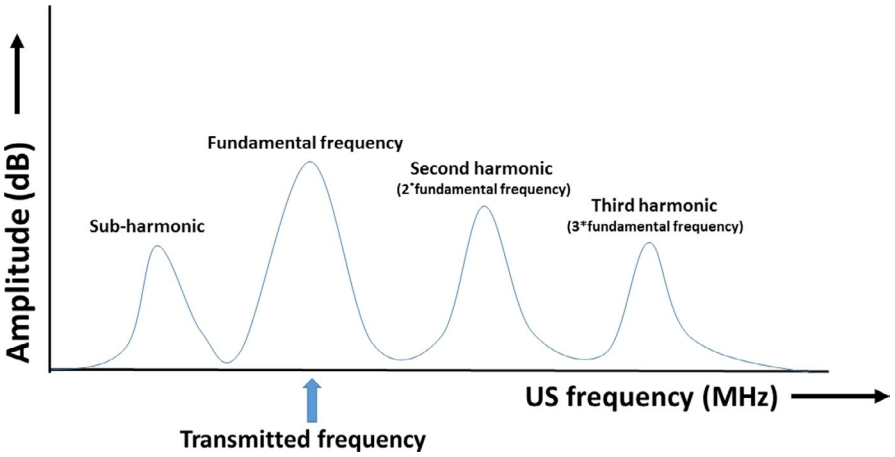

FIGURE 1 Harmonic imaging. Schematic representation of a frequency spectrum received from microbubbles, including both the fundamental frequency and harmonic frequencies (subharmonic, second harmonic, and third harmonic frequency)

low dynamic range results in a more black and white image.[12] A small dynamic range is preferred in cases of very low signal. For perfusion quantification studies, a wide dynamic range should be used to avoid signal saturation. When acquiring a series of cases whose appearances are to be compared, it is adviced to keep the dynamic range and other postprocessing settings constant.

2 | CONTRAST AGENTS; COMPOSITION, GENERATIONS, AND BRANDS

Contrast-enhanced contrast agents consist of small gas-filled bubbles encapsulated in biodegradable shells, such as phospholipids or albumin. The typical diameter of a microbubble is a few microns, allowing them to pass through the (lung) capillaries. In addition, the microbubbles are confined to the vascular compartment and circulate in a similar manner as red blood cells. Over the years, researchers have improved the stability of the microbubbles by stabilizing the shell and using gases with lower solubility such as sulfur hexafloride (SF_6) and octafluoropropane (C_3F_8). This prevents quick dissolution of the microbubble and allows these microbubbles to persist in the circulation for several minutes. Overviews of the composition of the different ultrasound contrast agents used in imaging research have been provided previously.[14-16] In short, the first generation contrast agents consisted of air-filled microbubbles with a lipid shell while the second generation microbubbles contain gases with a low solubility in water. Currently, newer (third and fourth generation) microbubbles are used for molecular imaging and site-specific drug delivery. The microbubble gas eventually dissolves in plasma and is cleared via the lungs,[3] whereas the shell components of the microbubbles are cleared by liver and kidneys. At present, there are three major manufacturers of ultrasound contrast agents: Bracco Imaging, GE Healthcare, and Lantheus Medical Imaging. In the United States, two contrast agents (Optison, GE Healthcare; Definity, Lantheus Medical) have been approved for left ventricular opacification. Only recently in 2016, the first agent (Sonovue or Lumason in the US) was approved for noncardiac imaging. A fourth agent, Sonazoid (GE Healthcare) is used in Japan for liver imaging but has not been approved by the FDA.[17]

3 | NONLINEAR ULTRASOUND IMAGING

Microbubbles reflect ultrasound by expanding and compressing in response to positive and negative ultrasonic pressure, referred to as nonlinear behavior of the microbubbles. This nonlinear oscillation of microbubbles results in resonating ultrasound waves that reflect at harmonic frequencies ("harmonics") and is key in discriminating between microbubble- and tissue-reflected ultrasound waves. A harmonic wave has a frequency which is typically a multiple of the fundamental frequency (ie, the frequency of the transmitted wave), or a fraction of that frequency (subharmonic imaging) (Figure 1). The harmonics can be distinguished and separated from the fundamental frequency.[18] There are different contrast-specific imaging techniques implemented in ultrasound machines which are all based on nonlinear echogenic behavior of the microbubbles: harmonic frequency filtering or harmonic imaging techniques, pulse inversion techniques, power modulation techniques, and cadence contrast pulse sequencing.

3.1 | Harmonic frequency filtering

Since harmonic signals emanate from microbubbles rather than surrounding tissue (Figure 2A), the ultrasound machine equipped with a specific "contrast-mode" can detect microbubbles by filtering out harmonic ultrasound frequencies from the signal.[19] In the first nonlinear imaging techniques, a "simple" bandwidth filter was used to eliminate waves at the fundamental frequency from the total reflected echo signal.[20] With this technique, the fundamental frequency is filtered out and frequencies that have more likely emerged from harmonic echoes are used to generate the image. The downside of this method is that narrowing the bandwidth of received ultrasound signals reduces the axial resolution. Selection of the cutoff frequency is therefore a compromise between harmonic frequency signal loss and contamination by the fundamental

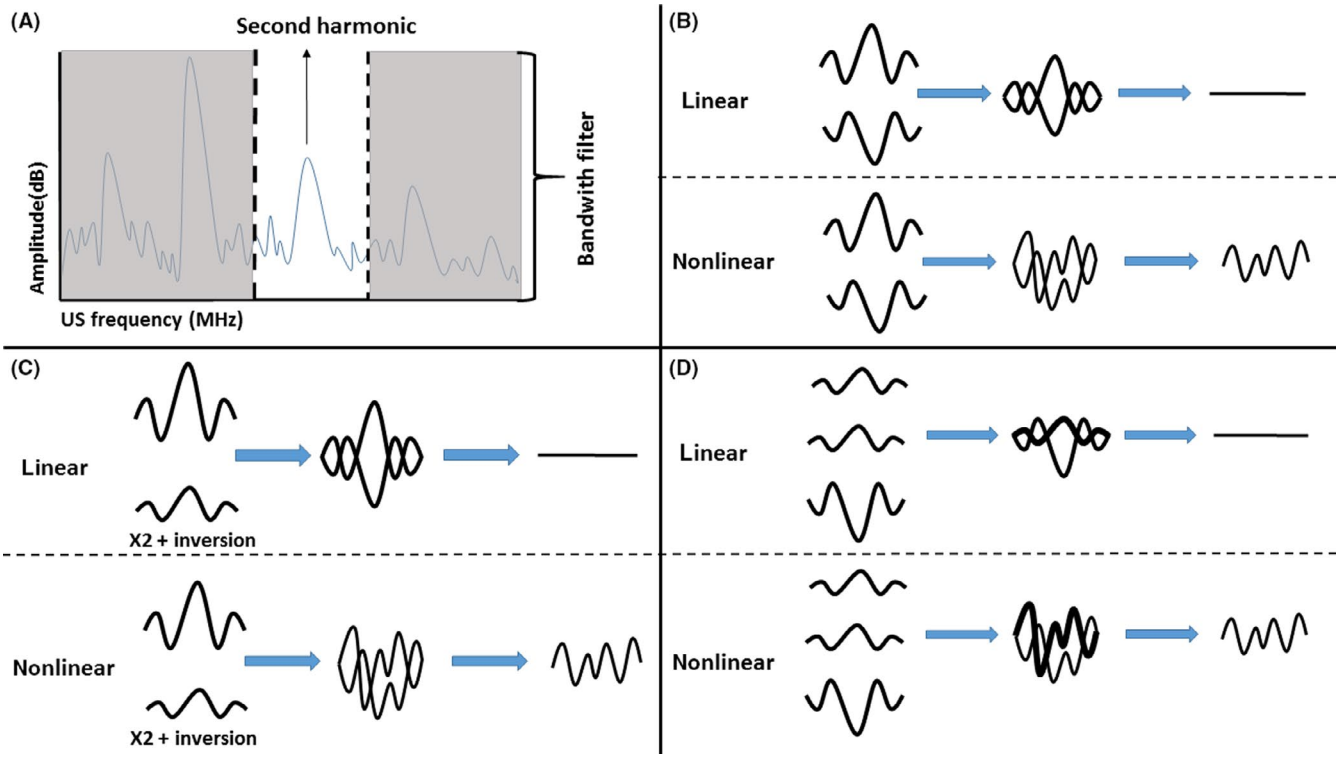

FIGURE 2 Nonlinear imaging techniques. A, Harmonic frequency filtering uses a bandwidth filter to eliminate the fundamental frequency from the reflected echo signal. With this technique, frequencies that have more likely emerged from harmonic echoes are used to generate the image. B, Pulse inversion is used to eliminate the linear response and preserve the nonlinear content from the signal by sequentially emitting two pulses with a 180° phase difference. Whether the echo response is linear or nonlinear depends upon the acoustic properties of the scatterers. The linear scattering components of the echo signal cancel each other out, while nonlinear scatterers show amplification of the amplitude. C, Power modulation techniques use two consecutive pulses of identical shape but a twofold difference in amplitude. With linear scatterers, this results in identical reflections other than the expected two fold difference in amplitude. The reflection from the second wave with smaller amplitude is subsequently doubled and subtracted from first reflection, resulting in canceling out of the signal in linear reflections. The same two pulses, when reflected by the nonlinear tissues, would differ from each other not only in amplitude but also in their shape, resulting in residual signal in nonlinear scatterers. D, Cadence contrast pulse sequencing uses a set of three pulses consisting of a pulse pair of 0° pulses and one amplitude modulated and phase inverted 180° pulse. The amplitude modulation is twice the 0° pulse. When all the three pulse responses are summed together the linear responses will cancel each other out while the nonlinear contribution remains

frequency signal. To increase signal-to-noise ratio while preserving axial resolution, multi-pulse sequencing strategies, such as pulse inversion, power modulation, and cadence contrast pulse sequencing have been developed.

3.2 | Pulse inversion

The principle behind pulse inversion is sequential emission of two ultrasound waves/pulses with a 180° phase difference to eliminate reflected waves with the fundamental frequency and preserve the ultrasound waves at nonlinear frequencies in the reflected ultrasound signal (Figure 2B). Whether the reflected ultrasound signal is linear or nonlinear depends upon the acoustic properties of the reflectors (ie, microbubbles or tissue). The linear components of the reflected ultrasound signal negate each other by interference, while the amplitude of waves from nonlinear reflectors is amplified. This technique is also termed phase cancelation or temporal cancelation.

3.3 | Power modulation

Power modulation imaging uses alternating pulses of identical shape but a twofold difference in amplitude (Figure 2C). With linear reflectors, this results in identical reflected waves with a twofold difference in amplitude. Every second reflected wave, with a smaller amplitude, is subsequently doubled and subtracted from the preceding reflected wave, resulting in disappearance of the reflected signal at linear frequencies. When subsequent reflected pulses have nonlinear frequencies, they not only differ from each other in amplitude but also in frequency, resulting in residual signal from nonlinear reflectors.

3.4 | Cadence contrast pulse sequencing

Cadence contrast pulse sequencing is another multi-pulse sequencing technique (Figure 2D), which uses the nonlinear property of the microbubbles to distinguish between the vascular compartment and the surrounding tissue by modulation of amplitude and phase of the

ultrasound pulses. This technique uses a combination of power modulation and pulse inversion, in sequences of three ultrasound pulses consisting of two equal pulses and a third with twice the amplitude of the first two waves and a 180° inverted phase. When reflections of all three pulses are summed together, the reflected waves at linear frequencies are quenched, while the nonlinear component of the reflected wave is preserved.

The principal advantage of these multi-pulse sequencing strategies is that image resolution is preserved, which provides better tissue contrast. However, the imaged tissue must remain identical during the sequence of opposite pulses to allow the pulse summation to result in quenching echoes at linear frequencies. Consequently, motion artefacts can markedly reduce the quality of the contrast image.

4 | PULSE-INTERVAL VS REAL-TIME IMAGING

Contrast-enhanced ultrasound can be performed in two ways: by intermittent imaging, using variable pulse intervals between strong ultrasound pulses that destroy the contrast agent and by real-time imaging with weaker ultrasound, relying on the use of pulses with a high and low MI, respectively. An MI of <0.3 is considered low and will result in oscillation of microbubbles, whereas a mechanical index of >0.7 destroys the microbubbles.[21] The older pulse-interval technique relies on discontinuous echo recordings by high MI pulses, that destroy microbubbles in the area of interest. The imploding microbubbles reflect waves that contain harmonics of the transmitted frequency. In the past years, this technique has largely been replaced by real-time imaging. Real-time uses a low MI to continuously measure the microbubble signal. With low-MI imaging, bubble oscillation rather than bubble destruction generates harmonic signals and is the main source of the image.[22] This imaging mode has several advantages over the older pulse-interval protocol. It is less time-consuming, since inflow-curves can be generated in one turn. Second, it is easy to detect and confirm a steady-state microbubble concentration. Finally, this techniques permits real-time imaging, which allows for detection of rapid changes in perfusion. This is advantageous when measuring quick increases in microvascular blood volume. A disadvantage of real-time imaging compared with the old pulse-interval technique is that it requires higher bubble concentration and is therefore more expensive. Finally and perhaps most importantly, imploding microbubbles can influence endothelial cell function, undesirable when using this imaging technique for evaluating vascular function.[23]

5 | CONTINUOUS INFUSION VS BOLUS INJECTION OF ULTRASOUND CONTRAST AGENTS

Contrast-enhanced ultrasound can be performed by using a continuous microbubble infusion or by injection of a single bolus. Continuous microbubble infusion results in steady-state microbubble concentration. To maintain a constant infusion of microbubbles, power injectors are available that continuously mix the microbubbles during infusion. The signal-to-noise ratio of contrast signal is obviously influenced by the microbubble infusion rate. There are limits to the amount of contrast agent that can be infused at once and in total in each individual. An infusion rate that is too low will result in low signal-to-noise ratios. A higher infusion speed will increase the signal-to-noise ratio, but it will shorten the measurement period or increase the cost of the measurements when more microbubbles are needed. In addition, high contrast agent concentrations in well-perfused tissues may saturate the ultrasound signal, leading to underestimation of the real tissue blood volume. Therefore, it is desirable to determine the optimal contrast infusion speed for each tissue, contrast agent and ultrasound system when developing new study protocols.[11]

Bolus administration produces a rapid rise in contrast agent and signal followed by a slower contrast washout and is the most commonly used form of contrast agent administration for nondestructive imaging. A bolus injection should be immediately followed by 5-10 mL saline bolus to flush the line at the same speed at which the contrast agent was infused. A 3-way tap is used with the saline flush injected through the side port.[17] High pressure should be avoided, because it may lead to destruction of microbubbles.

6 | CEUS IMAGE ANALYSIS

6.1 | Software

Analysis of CEUS images can be performed in several ways. First, many software packages, either commercially available (VueBox®, Qlab) or in-house manufactured scripts (programmed in MATLAB) can be used. These software packages allow for semi-quantification of MBV and MFV and subsequent calculation of MBF, using both continuous infusion and bolus injection.[24] However, different analyses are needed to obtain these data (Figure 3).

The continuous perfusion protocol uses the so-called flash-replenishment technique to measure MBV and MFV by curve fitting the experimental data using the formula $AI = MBV\ (1-e^{-MFV(t-0.5)})$, where AI is the acoustic intensity at t, where t represents the time (s) after microbubble destruction.[25] After a steady state of plasma microbubble concentration has been reached, the flash-replenishment technique uses a high MI pulse—or flash—to destroy all microbubbles in the image plane. After returning to low MI, the refilling of blood vessels—or replenishment—within the image plane is recorded. The AI at the plateau phase corresponds with a measure relative to MBV, and the initial wash-in slope reflects MFV (Figure 3). For the old pulse-interval protocol, a similar inflow curve was generated by repeatedly extending the interval between subsequent microbubble destructions.

After a bolus injection of contrast agent, so-called TIC are observed, that is, an S-shaped curve reflecting the wash-in of the contrast agent is followed by a nearly exponential washout period.[11]

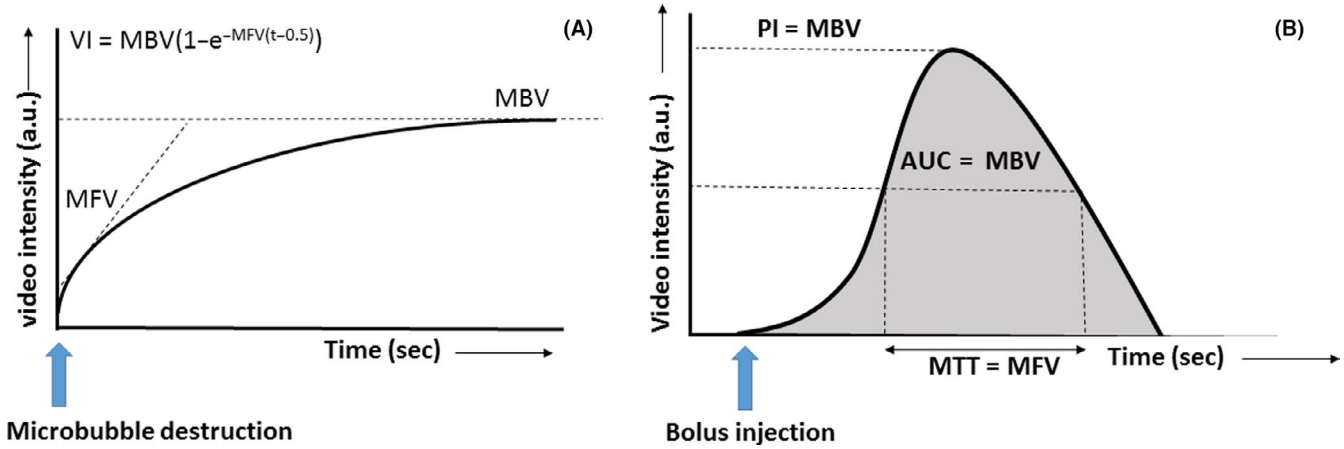

FIGURE 3 Flash-replenishment and time-intensity curve. A, Flash-replenishment curve during continuous perfusion. The curve starts after microbubble destruction. Time in second (s) is displayed on the horizontal axis and the AI in au on the vertical axis. After microbubble destruction, microbubble tissue replenishment can be described as an exponential curve with the corresponding formula: $VI = MBV(1-e^{-MFV(t-0.5)})$. Maximal AI after complete filling of the microvascular bed is a parameter of MBV, and the slope of the initial increase is a parameter of MFV. B, TIC obtained after bolus injection of ultrasound contrast agent. Time in second (s) is displayed on the horizontal axis and the AI in au on the vertical axis. PI and AUC are parameters of MBV, and MTT is a parameter of MVF

The relative MBV is the peak microbubble signal (peak AI) or can be derived by calculating the area under the curve. The MFV is determined by the time between 50% of peak intensities values in the wash-in and washout periods[25] (Figure 3).

6.2 | Regions of interest

Quantitative assessment of perfusion is done using user-defined ROI. Several ROIs can be drawn in one image to determine perfusion in different tissues or different areas of one specific organ. When comparing perfusion characteristics between measurements at different time points within one individual, it is important to use identical ROIs, since depth of the ROI and heterogeneity within a tissue can result in differences in AI. Motion artefacts should therefore be avoided during and between measurements as much as possible, or corrected for by adapting the ROI during analyses of different measurements.

6.3 | Background subtraction

Background subtraction is essential in determining tissue perfusion. Contrast-specific subtraction consists of subtraction of the native background signal, that is, the signal before microbubble infusion, from the maximal tissue intensity so that a microbubble-only image is created.[26] The background signal from surrounding tissues is determined by the selected gain and other ultrasound settings as well as tissue properties and may therefore differ per individual and per measurement. Often, the average AI of the first frames, for example 0.5 seconds after microbubble destruction, is subtracted from the AI of subsequent images. This eliminates not only background signal from surrounding tissues, but also the signal derived from rapidly filling larger arterioles, which contribute much less to nutrient and oxygen exchange.[27,28]

6.4 | Microbubble concentration

Administration of a specific amount of microbubbles can lead to different microbubble concentrations between individuals and measurements, due to differences in volume distribution or microbubble clearance rate. Therefore, correcting for the microbubble concentration in blood by dividing the tissue perfusion signal by a signal obtained from a large artery or the left heart chamber may reduce variation between individuals and measurements.[29]

6.5 | Linearization of ultrasound signal

The signal that is captured by the ultrasound transducer (the raw radiofrequency signal) is processed into the signal displayed on the monitor (video output signal) by log compressing the raw acoustic data. To adequately quantify tissue perfusion, this signal therefore needs to be linearized on a per-pixel basis to avoid wrong determination of perfusion parameters due to averaging.[30] There are commercially available software packages, which allow validated quantification of linearized video signals obtained with different machines and transducers. It is also possible to use home-made scripts for Matlab or other software for this purpose. In the latter case, it is important to know the formula originally used to log-transform the raw data in order to accurately reverse this process.

7 | TISSUE-SPECIFIC CEUS MEASUREMENTS

Contrast-enhanced ultrasound can be and has been used to quantify perfusion in many different tissues and organs including muscle, fat tissue, heart, brain, liver, and kidney. Each of these tissues has specific properties that makes CEUS more or less suitable compared with

other techniques. In the following paragraph, advantages and disadvantages of tissue-specific CEUS measurements will be summarized.

7.1 | Heart

Real-time CEUS imaging was first described by Wei et al for quantification of myocardial perfusion in dogs.[22] Use of CEUS in the heart not only requires knowledge of contrast-specific ultrasound imaging, but also of cardiac-specific ultrasound settings and transducer placement. For example, a four-chamber view of the heart allows for quantification of perfusion of the cardiac interventricular septum, the apex, and the lateral wall, whereas the two-chamber view allows for quantification of perfusion in the anterior wall of the heart. Ultrasound images should be obtained using ECG-triggering to allow acquisitions of subsequent contrast images in the same moment within the cardiac cycle. Advantages of myocardial measurements include the possibility to simultaneously measure other cardiac parameters, such as cardiac output, and correction for the blood microbubble concentration in each measurement by dividing the muscle signal by the signal within the left ventricle. To this end, it is key to choose microbubble infusion rates and ultrasound settings in which the signal is not saturated in the left ventricle cavity, but is still high enough (with adequate signal-to-noise ratio) to measure perfusion in the myocardium.[30] In addition, myocardial perfusion is relatively high, leading to high signal-to-noise ratios. Moreover, CEUS in the heart is the only application of CEUS that has been validated in vitro and in vivo in humans using PET and therefore allows absolute quantification of myocardial perfusion.[29] Drawbacks of myocardial perfusion measurements include variability in quality of intercostal ultrasound windows the continuous movement of the myocardium, and motion artefacts due to breathing. These continuous movements complicate the analysis of the images, and regions of interest need to be drawn one frame at a time.

7.2 | Skeletal muscle

Skeletal muscle images are relatively easy to obtain, since the transducer can be placed over any muscle in the body. Blood perfusion measurements of the arm are likely to have less signal variation compared with measurements of the leg, because there are more anatomical structures to be used as waypoints for reproducible transducer placement. When measuring at rest, motion artefacts are rare. This technique has been used many times to measure insulin-induced microvascular recruitment.[31,32] Furthermore, this technique has been used to measure muscle perfusion during or directly after exercise. However, these exercise protocols require specific equipment to stabilize the transducer.[33]

7.3 | Subcutaneous adipose tissue

Studies of adipose tissue perfusion using CEUS are relatively scarce compared with studies of skeletal muscle perfusion. Sjoberg et al were the first to show that this technique can be used in adipose tissue, but mentioned that the technique was only feasible in female participants who had thicker layers of subcutaneous adipose tissue compared with male participants.[34] In addition, it has been suggested by experts that adipose tissue itself—perhaps due to the large, round adipocytes—may produce a lot of tissue harmonics, which complicates the discrimination between blood and surrounding adipose tissue.

7.4 | Kidney

Contrast-enhanced ultrasound has been applied to determine kidney perfusion. It has been shown that CEUS can detect responses to several stimuli, including meal-induced and drug-induced changes in blood flow. Kidney perfusion measurements can be complicated by probe placement, which sometimes requires that the participants are able to lie on one side. This may not be feasible in critically ill or otherwise difficult to mobilize patients/participants. Furthermore, during the measurements, participants are requested to hold their breath to minimize motion artefacts. An important advantage of the CEUS technique in this organ over other techniques that use other contrast agents (CT/MRI) is that microbubbles are not nephrotoxic.

7.5 | Brain

Contrast-enhanced ultrasound can also be used for quantification of cerebral perfusion.[35] The method can be used to identify perfusion abnormalities in stroke patients.[36] The major challenge with cerebral perfusion measurements lies in the crossing of the skull. The skull largely absorbs the acoustic signals, requiring high acoustic power to reach the cerebral circulation. However, this high power may cause the microbubbles to burst during insonation. Therefore, transcranial CEUS is performed taking advantage of the temporal bone window. However, the temporal bone window often has an irregular thickness and varying in thickness from individual to individual. These properties of the temporal window result in distortions of the ultrasound beam and an intrinsic frequency-dependent attenuation. Moreover, cerebral perfusion measurement can only be performed in patients with a sufficiently large temporal bone window (approximately 85% of all individuals). There are only a few anatomical structures in the brain that can be visualized by ultrasound, which is necessary for proper orientation of the transducer. First, the butterfly-shaped mesencephalic brain stem can be visualized. Second, when tilting the ultrasound probe by 10-20° toward the parietal lobe, the third ventricle plane can be assessed.

8 | SAFETY

The overall safety of microbubble contrast agents is generally accepted with a safety profile that is at least as good as conventional contrast agents.[37,38] It is unclear whether there are significant differences in safety among the products that are currently available. Serious adverse events such as bradycardia, hypotension, or

anaphylactic shock have been reported rarely (<1 out of 10 000 patients).[39,40] Nevertheless, available resuscitation facilities and equipment are mandatory when using microbubble contrast agents.[17] The most common adverse events reported are mild, transient, and similar to those seen with other types of contrast media, that is, headache, warm sensation, and flushing. Less frequent events include nausea and vomiting, dizziness, chills and fever, altered taste, dyspnoea, and chest pain. However, these symptoms may not be related to the ultrasound contrast agents since they have also been observed in placebo-control groups.[38] Currently, the only FDA contraindications to intravenous microbubble contrast agents is a history of allergic reaction.[17] However, according to the European Medicines Agency, SonoVue® must not be used in patients known to have right-to-left shunts, severe pulmonary hypertension, uncontrolled hypertension, or adult respiratory distress syndrome. Its use is no longer contraindicated in patients with acute coronary syndrome and clinical unstable ischemic cardiac disease as well as during pregnancy and lactation.[41] However, in this patient population, specific recommendations and precautions have been included in the summary of product characteristics and the package leaflet, including a careful risk/benefit assessment and close monitoring of vital signs during administration.

9 | COSTS

Costs of a contrast-enhanced ultrasound measurement depend mainly on the costs of the contrast agent used, next to once-only purchase of a suitable ultrasound machine and, if needed, power injector. Other costs, such as intravenous cannulas and lines, are negligible. The costs of a microbubbles vial range between 70 and 130 euro, depending on the manufacturer. The total costs also depend on the research protocol, that is, amount of vials that are needed per measurements. This depends on the required microbubble concentration and measurement duration or number of measurements.

10 | ADVANTAGES OF CONTRAST ULTRASOUND

Contrast-enhanced ultrasound has several advantages over other techniques for quantifying tissue perfusion. CEUS is fast and can be performed at bed-side, allowing repeated measurements. In addition, CEUS is considered safe, since it does not use ionizing radiation and the microbubble contrast has a safety profile that is at least as good as conventional contrast agents. In particular, CEUS contrast agents are not nephrotoxic. Furthermore, CEUS provides measurements of blood volume and blood flow, whereas other imaging techniques in humans provide only blood flow as outcome variable.

11 | LIMITATIONS OF CONTRAST ULTRASOUND

One of the bigger downsides of CEUS compared with other techniques that measure tissue perfusion is the lack of absolute values. Apart from perfusion measurements in the heart, CEUS expresses MBV and MBF in arbitrary units instead of ml/min. MBF quantification in the heart was shown to show small technical variations and to correlate well with MBF measured by PET,[29] measurements in different individuals in skeletal muscle and adipose tissue may reach 100-fold differences within the same study. Therefore, comparisons can only be made when first assessing differences within individuals. The percentage change in MBV between two research settings (for example with and without insulin) in the same individual is usually calculated to identify the effects on perfusion of a specific intervention. A related current limitation of the CEUS technique is the poor reproducibility.[42] Large variations in blood volume measurements have been detected, which may result from changes in transducer orientation or physiological day-to-day variation. Transducer orientation is particularly important because many tissues display large heterogeneity in tissue perfusion. Small shifts in the location of the transducer—as with motion artefacts—can therefore result in incorrect quantification of tissue perfusion. Furthermore, there are many variables, such as chronic and acute physical activity, fasting status, smoking, drinking, and the use of recreational drugs that may influence CEUS measurements resulting in large day-to-day variations. In addition, ultrasound techniques are operator-dependent, which also results in intra-researcher variation. CVs described in literature vary largely and depend mainly on research settings and research techniques with lower CVs in animal studies, during higher microbubble concentrations and when better perfused tissues are examined.[42]

12 | CONCLUSION

In conclusion, CEUS is a relatively new imaging technique that enables (semi)quantification of MBV, MFV and MBF in deep organs, such as liver, kidney and skeletal muscle, and adipose tissue. In addition, the technique is fast and minimally invasive, allowing for quick and repeated bed-side measurements. The development of this technique and the gained experience of researchers over the past decades have led to improvements in signal-to-noise ratio and increased implementation of the technique. However, CEUS also has several limitations including high day-to-day and inter-individual variability as well as limited possibility to express tissue perfusion in absolute units, as validation studies have been performed in the heart only. Therefore, to imply this technique in research settings, knowledge on the limitations of the technique as well as enough practice is required. We therefore recommend sufficient testing prior to data collection in order to achieve the optimal signal-to-noise ratio and to prevent high variation.

CONFLICT OF INTEREST

None.

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