

Clinical implications of skeletal muscle blood-oxygenation-level-dependent (BOLD) MRI

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Abstract Blood-oxygenation-level-dependent (BOLD) contrast in magnetic resonance (MR) imaging of skeletal muscle mainly depends on changes of oxygen saturation in the microcirculation. In recent years, an increasing number of studies have evaluated the clinical relevance of skeletal muscle BOLD MR imaging in vascular diseases, such as peripheral arterial occlusive disease, diabetes mellitus, and

chronic compartment syndrome. BOLD imaging combines the advantages of MR imaging, i.e., high spatial resolution, no exposure to ionizing radiation, with functional information of local microvascular perfusion. Due to intrinsic contrast provoked via changes in hemoglobin oxygen saturation, it is a safe and easy applicable procedure on standard whole-body MR devices. Therefore, BOLD MR imaging of skeletal muscle is a potential new diagnostic tool in the clinical evaluation of vascular, inflammatory, and muscular pathologies. Our review focuses on the current evidence concerning the use of BOLD MR imaging of skeletal muscle under pathological conditions and highlights ways for future clinical and scientific applications.

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Introduction

Blood-oxygenation-level-dependent (BOLD) magnetic resonance imaging (MRI) was introduced in functional neuroimaging studies in 1990 [1]. Since that time, BOLD MRI has become the mainstay for analyzing activated neuronal tissues. Neuronal activation induces changes of the ratio between oxy- and deoxyhemoglobin concentration in the local microcirculation [2]. Oxygen saturation influences $T2^*$ signal intensity (SI) in gradient echo MRI sequences. According to experimental setup and MR sequence selection, other confounding variables, such as inflow, blood volume, and extravascular contributions, are reduced to a large extent. The correlation between $T2/T2^*$ SI and blood oxygen concentration made BOLD MRI the ideal diagnostic tool for functional neuroanatomical studies

and clinical applications, such as neurosurgical planning and monitoring of treatment outcomes.

In the 1990s, BOLD MRI was also evaluated as a diagnostic tool in other human tissues. Myocardial infarction has been shown to create a significantly reduced BOLD response in damaged muscle tissue [3]. The same study also demonstrated that BOLD SI changes are reversible after coronary intervention via percutaneous transluminal angioplasty. Rest and stress-induced BOLD MRI also differentiated successfully between ischemic, nonischemic, and normal myocardial segments in a population of patients with coronary artery disease [4]. Besides myocardial pathologies, BOLD MRI is valuable for investigating physiological blood oxygenation level changes in the human kidney and uterine myometrium [5, 6]. As human tumors are often characterized by increased oxygen consumption leading to hypoxic conditions and neoangiogenesis, BOLD MRI could also be a valuable tool for functional tumor imaging. However, in breast and prostate cancer, BOLD measurements showed heterogeneous results with respect to extent of hypoxia prior to chemotherapy, most likely due to differing tumor neovascularization and stroma tissue [7, 8].

Concerning MRI of the musculoskeletal system, BOLD MRI has been extensively studied in recent years because of the easy accessibility of human skeletal muscle to different kinds of imaging paradigms (i.e., exercise- and cuff compression paradigms that allow generation of ischemic and hyperemic conditions) and the great variation of perfusion strength between resting and activation state. As BOLD MRI relies on hemoglobin oxygen saturation as endogenous contrast, one has to provoke changes in the local ratio of oxy- and deoxyhemoglobin prior to MRI. The musculoskeletal system permits several different imaging paradigms to shift the tissue oxygenation level: Oxygen delivery to skeletal muscle can be increased via increased oxygen inhalation or vasodilator drugs and decreased via temporal vascular occlusion or vasoconstrictors. Oxygen consumption can be increased via muscular exercise. By using these experimental setups, a large number of studies has focused on the origin of skeletal muscle BOLD signal and its physiological dependencies. This information is helpful for interpreting results of clinical muscle BOLD MRI studies. As skeletal muscle is the end organ of the peripheral vascular system, BOLD MRI provides important information of vascular pathologies, such as peripheral arterial occlusive disease, diabetes mellitus, and rheumatic diseases.

In this review, we focus on the clinical implications of skeletal muscle BOLD MRI. We highlight current evidence concerning the diagnostic application of functional MRI of the skeletal muscle and discuss recent and future developments. After a short overview of the theory behind

skeletal muscle BOLD effect, the technical principles of the procedure are reviewed in detail with a focus on the current interpretation of skeletal muscle BOLD signal.

The BOLD effect

Functional MRI is based on the principle that transverse relaxation rate of water protons inside or in the vicinity of small blood vessels is influenced by the oxygenation status of hemoglobin [1]. Oxyhemoglobin concentration in the microvascular environment of a certain tissue is mainly influenced by blood flow, intravascular volume, and capillary oxygen extraction. A sole increase of tissue perfusion with oxygenated hemoglobin at constant levels of oxygen extraction results in a higher oxy- and lower deoxyhemoglobin concentration in the local microcirculation and small venous vessels [9–11]. Deoxyhemoglobin is paramagnetic and thus causes local magnetic field distortions in its vicinity. At conditions of increased tissue perfusion, the lower local deoxyhemoglobin content leads to a decrease of the apparent transverse relaxation rate ($1/T_2$) of water protons and an increase of T_2 and T_2^* signal through a reduced intravoxel spin dephasing (i.e., positive BOLD contrast) [1, 9]. Due to a phase dispersion of water proton signals, GRE MRI sequences emphasize this effect [9, 12].

Different physiological parameters have been postulated to influence skeletal muscle BOLD signal, such as blood flow, intravascular blood volume, cellular pH and fluid shifts, vessel diameter, and orientation. However, it is generally accepted that the bulk of BOLD signal alterations in GRE sequences of skeletal muscles are due to the oxygenation level of hemoglobin [10, 13–21]. Regarding macro- and microvascular tissue blood flow, good correlations with the muscular BOLD signal have been found in various studies using different paradigms to provoke BOLD responses and different techniques for flow measurements such as plethysmography, arterial spin labelling, and laser Doppler flowmetry [14, 17, 22–24]. The relative extent of other factors contributing to BOLD contrast depends on the imaging protocol. Alterations of blood volume, for example, can lead to changes in T_2^* signal [23]. According to a recent study using short isometric muscle contractions to provoke BOLD SI changes in the calf muscles, these changes depend on the oxygenation status of hemoglobin [24]. Thus, if blood oxygen saturation is at high levels, near 100%, an increase of blood volume in the leg will cause an increase of T_2/T_2^* , whereas a low oxygen saturation of about 50% results in a T_2^* decrease. Furthermore, two studies found a prolongation of the BOLD signal increase after increased muscular blood flow had normalized [25, 26]. This could be explained by increased oxygen saturation of venous blood and increased

tissue oxygen tension. Exercise-induced osmotic changes of the intracellular milieu (i.e., water shift from the circulation to the intracellular space) could also alternate the T2 signal from muscle tissue [27]. However, metabolic changes during ischemia, such as increasing lactate or phosphate concentration or pH-decrease, have been reported to occur later than usually measured BOLD signal changes [28]. Additionally, vessel orientation has been proposed to influence muscle BOLD signal, as most capillaries and small vessels are oriented along the axis of the muscle fibers and thus have a determined orientation inside the static magnetic field [29]. However, a study using a leg rotation protocol found no influence on BOLD signal in an arterial occlusion paradigm [15]. Primarily due to secondary alterations of the variables and mechanisms discussed above, physiological parameters of the imaged person such as age, training state, and intake of vasoactive drugs can have impact on BOLD signal [26, 30–32].

Scientific evidence supports the fact that the BOLD signal of skeletal muscle is influenced by different factors which are—to a certain extent—paradigm dependent. However, using optimized GRE MRI sequences and experimental conditions, muscle BOLD signal reflects changes of hemoglobin oxygenation in muscle microvessels that primarily depend on perfusion and oxygen consumption. As muscle tissue is the end organ of the limb vasculature, BOLD MRI is a promising noninvasive imaging method for functional evaluation of diseases affecting peripheral macro- and microvasculature. The following section describes methodological principles that are usually applied in muscle BOLD studies. Several of these principals are necessary to measure true BOLD effects and reduce the influence of confounding parameters.

Principles of skeletal muscle BOLD MRI

Patient selection

BOLD MRI of skeletal muscle can be a valuable diagnostic tool under many different clinical conditions that impair peripheral vasculature and thus result in a reduced oxygen delivery to skeletal muscle [14, 33–35]. Patients undergoing muscle BOLD imaging therefore usually bear cardiovascular risk factors, such as hypertension, smoking, hypercholesterolemia, diabetes mellitus, and obesity. In diagnostic studies comprising a patient and control group, both groups must be controlled for these confounding variables. Moreover, patient age and training state influence skeletal muscle BOLD signal [24, 25, 30, 31, 36]. To date, there is no literature concerning muscle BOLD signal under inflammatory vascular or skeletal muscle diseases,

but—as an influence on tissue oxygenation and perfusion cannot ruled out—one should also control for these disorders. Further exclusion criteria for muscle BOLD studies are typical contraindications of MRI examination, e.g., claustrophobia and ferromagnetic implants.

Experimental setup

BOLD MRI of human skeletal muscle is usually performed in a whole-body MRI device that, in principle, allows imaging of every muscle within the patient's body; however, according to typical clinical questions, routinely, arm or leg muscles are examined [11, 17, 26, 37]. The extremity is placed within a vascular array coil, and foam braces can be used to minimize motion artifacts in exercise paradigms [22]. Prior to imaging, a resting period of 5–15 min of the examined individual is required due to the influence of position changes on the venous filling state and functional vascular adaptations that have been shown to influence BOLD SI [23]. To evoke changes in the local hemoglobin oxygen saturation that correspond to voxel contrastation in T2/T2* MRI sequences, typically, one of the following paradigms is applied: (1) arterial occlusion; (2) muscle exercise; (3) oxygen inhalation.

1. Arterial occlusion has been widely used in a variety of muscle BOLD studies [10, 11, 13, 14, 23, 30, 38]. It requires an easily applicable experimental setup with a standard air cuff wrapped around the proximal part of the examined extremity (Fig. 1a). If the pressure manometer contains ferromagnetic parts, a safe distance from the magnet should be achieved by using an extended tube [30]. Ischemia is achieved by fast inflation of the cuff to an occlusion pressure at least 50 mmHg above the individual brachial systolic blood pressure [14, 30, 33, 36, 38–40]. Extensive arterial calcification will make higher cuff pressures necessary, especially in patients with diabetes. Fast inflation is necessary to prevent immoderate filling of the venous system. This can be achieved via automatic cuff inflation systems or fast manual inflation. However, manual inflation lasts over several cardiac cycles and thus could lead to increased venous filling. Applied cuff pressure and duration of ischemic phase is always a compromise between patient compliance, degree of discomfort and pain, possible complications, and assumed optimal muscle BOLD effect (also refer to “[Paradigm selection in clinical settings](#)” below). MRI measurements can be started during resting state, ischemic phase, or after cuff deflation, i.e., during reactive hyperemia phase. Besides sustaining suprasystolic cuff compression in the examined leg, this paradigm is mostly independent from patient

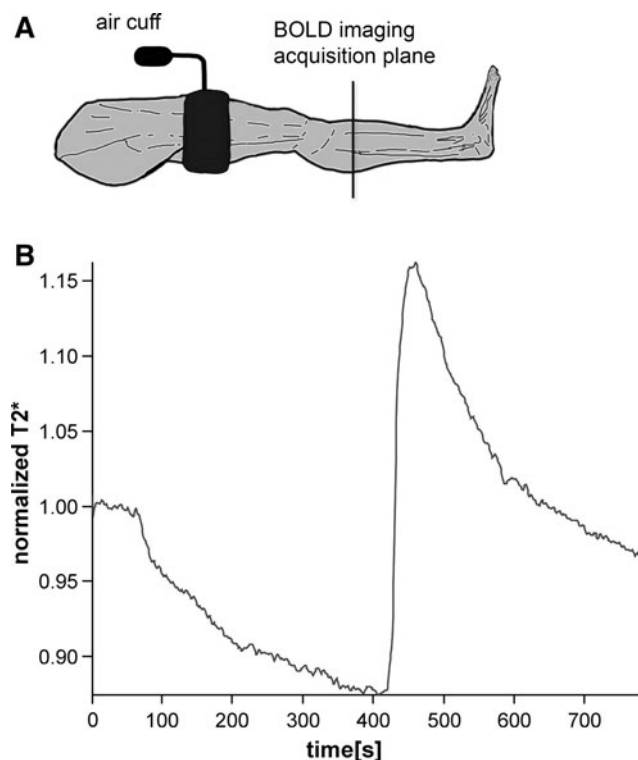


Fig. 1 **a** Experimental setup of the widely used cuff compression paradigm in skeletal muscle blood-oxygenation-level-dependent (BOLD) magnetic resonance imaging (MRI). Inflation of a standard air cuff at midhigh level allows BOLD examination of the calf microvasculature during ischemia and reactive hyperemia. In T_2^* maps calculated from echo planar imaging (EPI) data, regions of interest (ROIs) are usually positioned at the maximal calf muscle diameter. Areas with larger vessels are excluded. As T_2^* images offer a low spatial resolution, T1-weighted images of the corresponding calf region are concomitantly acquired for anatomical reference. **b** Extracted T_2^* (BOLD) time course of a healthy 46-year old woman volunteer with a 60-s resting period, 6 min of ischemia, and 6 min of reactive hyperemia

compliance. However, in patients with critical leg ischemia or ulcerations, BOLD MRI examination via cuff compression should be considered with caution due to massive pain or even worsening of the clinical condition.

In healthy individuals, a fast T_2^* signal decay is observed at the beginning of ischemia, followed by a slower decrease (Fig. 1b) [13, 14, 38]. During reactive hyperemia, a fast surge in BOLD signal is observed with peak values after approximately 30–60 s and a subsequent decrease to a steady state value around baseline [30, 33]. The extent of BOLD signal changes depends on experimental setup, physiological variables of the examined patient, and magnetic field strength [11, 13, 17]. To analyze muscle BOLD data in a quantitative manner, several time-course parameters can be calculated, including:

- $T_{2^*_{\min}}$ or MIV: minimal T_2^* value in percent during ischemia;
 - $T_{2^*_{\max}}$ or HPV: maximal T_2^* value during reactive hyperemia;
 - Time to peak (TTP): time from cuff deflation to hyperemia T_2^* peaking;
 - Declining slope (DS): average decline of T_2^* curves during ischemia or reactive hyperemia.
2. As skeletal muscle work results in local increases of blood flow due to local vasodilation triggered by the accumulation of metabolites, voluntary contractions of the imaged individual can be used to provoke a skeletal muscle BOLD response. Several studies showed that brief contractions of about 1–3 s are sufficient to produce a measurable BOLD response [17, 18, 25]. Motion artifacts are one major drawback in these studies, so that sophisticated fixation devices or foam braces need to be installed prior to imaging. Significant BOLD responses appear 8–15 s after individual muscle contractions [17, 18]. As contraction intensity is related to BOLD SI changes, this paradigm depends largely on patient compliance [22].
 3. By sequential inhalation of ambient air and 100% oxygen from a mask with a reservoir, BOLD response of skeletal muscle can be provoked by repeatedly increasing and decreasing blood oxygen content (O_2 -enhanced MRI) [10]. However, this kind of paradigm has some drawbacks that constrain its widespread use in scientific or clinical applications. As oxygen saturation of hemoglobin in healthy young individuals is near 100%, oxygen ventilation primarily leads to an increase of dissolved O_2 in the blood, whereas the ratio between oxy- and deoxyhemoglobin does not change extensively. O_2 is paramagnetic and leads to changes of T1, T2, and T_2^* . Studies using this paradigm therefore must be analyzed with care regarding true BOLD effects that depend on hemoglobin oxygenation. Furthermore, the paradigm has not been extensively evaluated with regard to how large the influence on true BOLD signal or perfusion is.

Muscle BOLD MRI needs high-speed image acquisition methods, typically based on echoplanar imaging (EPI) [41]. Spiral imaging techniques are more prone to image artifacts due to a higher sensitivity to off-resonance effects compared with EPI [42]. Water proton susceptibility differences depending on oxygen hemoglobin saturation are emphasized on gradient-echo MRI sequences [1, 12]. BOLD signal alterations of conventional single-shot EPI are sensitive to changes in T_2^* and T2—reflecting oxygenation—and initial BOLD signal intensity (S_0) [30, 33]. S_0 is influenced by several confounding parameters, such as blood inflow, changes in T1, and baseline drifts [16, 43].

Multiecho GRE EPI sequences with fat suppression can be used to separate oxygenation-related changes ($T2^*$) from these other effects [16, 33]. EPIs are superposed to a corresponding T1-weighted spin-echo (SE) image of the same acquisition plane to allow exact placement of the region of interest (ROI) in a designated muscle (Fig. 1a).

Image analysis

Regarding image analysis, $T2^*$ sequences (multiecho acquisition) comprise the highest sensitivity for studying the true BOLD effect in skeletal muscle. In case of multiecho EPI, $T2^*$ maps are generated by using a pixel-by-pixel least-square fit of a monoexponential decay to the signal intensities of the different echo images [14, 30]. $T2^*$ values, normalized to a baseline value set to 100% (i.e., at resting state) can then be extracted for every examined ROI. Larger vessels should be excluded to minimize inflow artifacts. A BOLD time course can be calculated subsequently (Fig. 1b).

Peripheral arterial occlusive disease

Assessment and diagnosis

Peripheral arterial occlusive disease (PAOD) is one of the leading manifestations of atherosclerosis. Existence of this disease has a significant impact on the survival of patients with generalized atherosclerosis [44, 45]. Due to stenoses and occlusions in the macrovasculature, malperfusion of the distal tissues (skeletal muscle and skin) occurs [46]. Specifically, in the skeletal muscle, the process of atherosclerosis seems to lead to changes in microvessel architecture and composition of muscle fiber types [47–49]. These factors interact and may significantly reduce oxygen delivery to and alter oxygen consumption in the skeletal muscle microcirculation [10].

MR angiography (MRA) detects and localizes stenoses and occlusions on a macrovascular level but does not allow a statement about the function of the microvasculature in the distal tissues [50, 51]. The ankle brachial index (ABI) is an easily applicable but observer-dependent diagnostic tool to evaluate the functional status of patients with PAOD [52, 53]. Performing this method is limited when the patient has incompressible calcified arteries. This is due to a much larger cuff pressure necessary to compress the vessel under this condition [54]. PAOD in a patient with arterial calcification thus will not be detected by ABI measurements. Moreover, there is a lack of noninvasive diagnostic tools to detect and quantify PAOD disease improvement in terms of adaptation of the vascular system and effectiveness of vessel stimulation therapies [40, 55]. As previously mentioned, BOLD MRI allows conclusions about alterations in

tissue oxygenation of the skeletal muscle, with a high spatial resolution [19, 43, 56–60]. Thus, it is a potential diagnostic tool for evaluating microcirculation function in the skeletal muscle of patients with PAOD.

There are three studies investigating muscle BOLD MRI in PAOD: Ledermann et al. [33] compared skeletal muscle BOLD signal of 17 patients with symptomatic PAOD to a control group consisting of 11 healthy volunteers of similar age. They performed a $T2^*$ -weighted single-shot multiecho EPI sequence on a whole-body magnetic resonance scanner at 1.5 T and used an arterial occlusion paradigm with cuff compression to provoke postischemic reactive hyperemia. The patient group demonstrated a lower $T2^*_{\max}$ and a more than twofold prolongation of the TTP value. Both differences were highly significant. Impaired microcirculation in the human skeletal muscle of the patient group is accompanied by a change in the $T2^*$ time course. The delayed TTP might be explained by the lack of oxygenated blood in the microvasculature, most possibly due to diminished microperfusion after vascular stenoses. It is difficult to understand the underlying mechanisms of the significant $T2^*_{\max}$ -decrease. A variety of effects could underly this mechanism. For instance, the longer contact time of the blood with the capillaries might lead to a more efficient deoxygenation of hemoglobin inside the capillaries. Another factor might be the circulation of a lower blood volume in patients with PAOD owing to damaged capillaries in the skeletal muscle [61, 62]. Further studies correlating BOLD measurements with the assessment of perfusion (e.g., via laser Doppler flowmetry or arterial spin labeling) and oxygenation (e.g. via near-infrared spectroscopy) are warranted to elucidate the exact pathophysiological mechanisms underlying these BOLD time-course changes in PAOD patients. An example of a $T2^*$ time course during reactive hyperemia from a patient suffering from PAOD is shown in Fig. 2a.

Another study investigated muscle BOLD response during the ischemic phase in 15 patients with PAOD versus ten healthy volunteers also using a cuff compression paradigm [38]. Differences in mean $T2^*_{\min}$ values and $T2^*$ time courses were observed between both groups: In the patient group, a rapid decrease of the $T2^*$ time course was followed by a slower decrease and finally ending in a constant $T2^*_{\min}$ value that did not change over time. The control group revealed a rapid decrease of the $T2^*$ value during the entire ischemic period, resulting in a significantly lower $T2^*_{\min}$ compared with the patient group. The observed differences in the $T2^*$ time course are most likely explained by a reduced oxygen consumption in patients with PAOD, because $T2^*$ decrease was not only diminished but also retarded when compared with healthy individuals. This effect may be caused by morphological (i.e., reduced capillary density) and/or metabolic alterations in the

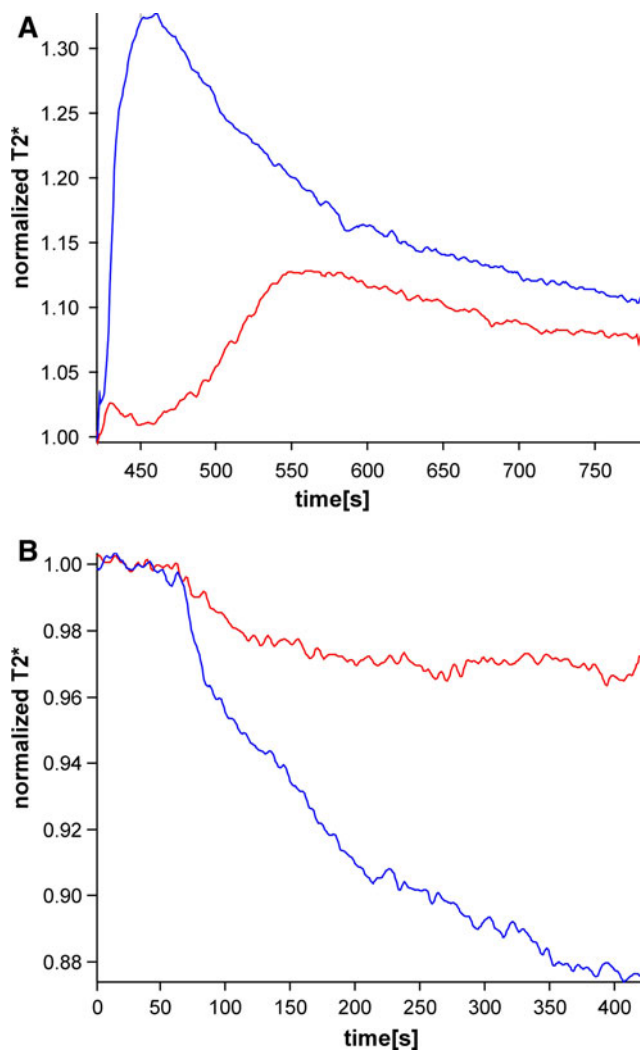


Fig. 2 **a** T_2^* time courses during reactive hyperemia of a 79-year-old woman suffering from peripheral arterial occlusive disease (PAOD) with signs of intermittent claudication and restrictions of pain-free walking distance >200 m (Fontaine Stage IIa, red line) and a 46-year old healthy woman volunteer (blue line). **b** T_2^* time courses during the ischemic period of a 74-year-old woman suffering from PAOD with severe claudication and a pain-free walking distance of about 80 m (Fontaine Stage IIb, red line) and the same volunteer as in **a** (blue line)

skeletal muscle of patients with PAOD, leading to reduced oxygen consumption in the distal muscle tissue [47, 63]. In Fig. 2b, an example of a T_2^* time course during ischemia from a patient suffering from PAOD is demonstrated.

A study from Versluis et al. [64] deals with reproducibility of different MRI imaging methods, including muscle BOLD MRI for assessing patients with PAOD. Postischemic reactive hyperemia with a cuff compression paradigm was provoked in healthy volunteers and PAOD patients and a multishot single-echo EPI sequence with fat suppression for acquiring the BOLD response was performed. All muscles were assessed together with one large ROI over

the entire cross-section of the calf. A poor interscan reproducibility was determined in this study for the maximal T_2^* change [patients: $T_{2^*_{\max}} = 13.2 \pm 6.4\%$, coefficient of variation (CV) = 26.7%, repeatability coefficient (RC) = 9.7%; volunteers: $T_{2^*_{\max}} = 13.3 \pm 5.9\%$, CV = 21.5%, RC = 7.9%]. However, for the TTP value, fair reproducibility was demonstrated (patients: TTP = 116 ± 26.8 s, CV = 10.6%, RC = 33.8 s; volunteers: TTP = 60.7 ± 15.9 s, CV = 15.9%, RC = 26.7 s). According to the authors, tissue oxygenation might change over time. This can be explained by many intrinsic and extrinsic factors that influence the day-to-day oxygenation status of the calf muscle, including but not limited to hormones, insulin secretion, temperature, day time, and eating habits [65]. The authors concluded that acquiring reliable functional data with muscle BOLD MRI in patients with peripheral arterial disease using a 1.5-Tesla MRI scanner is not advisable in clinical settings, with the exception of the TTP value. A further cause of the poor interscan reproducibility could be the missing exclusion of larger vessels from the ROI that included the entire soft tissue of the analyzed calf diameter. This may have caused inflow artefacts in the acquired T_2^* data. Additionally, the used single-echo EPI sequences makes the BOLD measurement even more prone to alterations of inflow, T1-, and baseline changes. Considering these limitations and the fact that several other published studies evaluating BOLD muscle MRI in PAOD patients showed significant time course alterations in relatively small patient collectives, reproducibility of skeletal muscle BOLD MRI could be higher than evaluated by Versluis et al. [64]. However, due to lack of supporting evidence, it is necessary to investigate the reproducibility of muscle BOLD signal in larger studies. This must inevitable include taking care of maximal standardization during the imaging procedure and the use of multiecho sequences to minimize confounding parameters.

In summary, noninvasive diagnosis and follow-up MRI investigations in patients with PAOD are feasible with muscle BOLD MRI. By evaluating microcirculation function in the calf, muscle BOLD MRI has the potential to serve a noninvasive low-risk (no radiation dose, no contrast agent) diagnostic tool for monitoring conservative and invasive therapeutic approaches. Moreover, skeletal muscle BOLD MRI assesses end-organ function and serves as a quantitative method for functional imaging in PAOD. In contrast to other MRI imaging techniques developed with the same purpose, such as first-pass gadolinium-enhanced MRI, muscle BOLD MRI does not require any contrast agent, making repeated measurements possible [66]. With MRI as a comprehensive diagnostic tool in PAOD, a macrovascular morphological visualization could be combined with microvascular functional measurements using muscle BOLD MRI [21, 67, 68]. Such a comprehensive

diagnostic tool might be of high value in clinical settings. Further investigations in larger patient cohorts are warranted to confirm these encouraging results and to determine the value of muscle BOLD MRI in clinical settings, including sensitivity and specificity.

Treatment evaluation

Muscle BOLD MRI has the potential to serve as an objective tool for planning and assessing the success of different therapeutic approaches to vascular diseases, such as conservative treatment with drugs or invasive procedures such as stenting or bypass surgery. This is of particular interest due to the capability of muscle BOLD MRI to reveal end-organ function rather than global function of macrocirculation [33]. The study by Hügli et al. [40] investigated the response of muscle BOLD MRI in ten patients with PAOD 1 day before and 6 weeks after percutaneous transluminal angioplasty of the superficial femoral artery. A cuff compression paradigm was used to provoke reactive hyperemia. Treatment of stenoses should lead to improved macrovascular and consecutively also microvascular function. Although not reaching significance level, the investigators found homogenous improvements of the key BOLD parameters TTP (decreased), $T2_{\max}^*$ (increased), and end value (decreased) from the first to the second measurement. The fact that these changes in key BOLD parameters did not reach significance level was explained by the low number of patients, the inhomogeneous patient group, and the relative short follow-up period. Blood inflow over the normal anatomical course of the arteries is faster than over the collateral vessels. That could be the reason TTP decreased in this study. $T2_{\max}^*$ could have increased due to a diminished oxygen extraction at higher microcirculatory flow, according to the Fick principle. This increase of blood inflow could also lead to a faster washout of blood from the calf muscle, thus leading to decreased $T2^*$ values at the end of hyperemia. The increased maximal $T2^*$ change might be based on increased blood oxygen supply. This optimized blood oxygen supply has already been demonstrated with transcutaneous oxygen pressure measurements in PAOD patients [69, 70]. New therapeutic concepts in PAOD aim to induce collateral formation and to improve function of existing arteries by angiogenic growth factors or stem cell administration [71–74]. It might be interesting to see whether muscle BOLD MRI can demonstrate the success of these approaches by showing improved microcirculatory function.

Compartment syndrome

Chronic compartment syndrome has an unclear underlying pathophysiology. The main problem seems to be an

exercise-induced increase of tissue pressure in one of the four compartments of the calf, resulting in a compression of muscles and vessels [34]. Patients show symptoms during and after exercise (i.e., walking, cycling) such as burning, pain, and cramping, which normally resolve under resting conditions [75]. Most often, the anterior compartment of the calf is affected. The elevated compartment pressure (>15 mmHg) causes decreased blood inflow, leading to ischemic pain. To assess chronic pressure effects in muscle compartments, BOLD signal is an attractive method to noninvasively detect microvasculature changes.

In a prospective study, Andreisek et al. [39] investigated muscle BOLD signal alterations in chronic compartment syndrome patients. They expected a lower $T2^*$ signal in patients with chronic compartment syndrome because of impaired perfusion and therefore delayed reoxygenation of the working calf muscle. Nine patients and ten controls underwent muscle BOLD MRI before and after exercise, and $T2^*$ signal in the lower extremity was registered. An immediate response after exercise concomitantly with an increase in $T2^*$ in both groups could be demonstrated. Although the patients reported higher levels of symptoms during exercise than did controls, a significant difference in terms of the $T2^*$ signal between groups could not be found. Moreover, the level of pain or muscle weakness did not show a correlation with the $T2^*$ signal. The $T2^*$ signal 15 min after muscle exercise was still elevated, which is most likely not due to a real BOLD effect but rather to much larger and longer-lasting $T2/T2^*$ effects caused by osmotic changes in muscle after repetitive exercise [76–78].

Another study used frequency-dependent signal intensity mapping after Fourier transformation to show alterations in BOLD response of a patient with chronic compartment syndrome [34]. This method relied on the disappearance of physiological cardiac and respiratory frequency peaks in the ROIs of the affected compartment. As spectral SI changes of BOLD signals after Fourier transformation are absent when excluding visible vessels from the defined ROIs, this method depends on blood inflow rather than oxygenation [79]. Hence, a sufficient imaging paradigm for the diagnosis of chronic compartment syndrome in the clinical setting is still lacking.

Diabetes mellitus

Diabetes mellitus is associated with impaired macro- and microcirculation [80–82]. Especially in this patient group, the early diagnosis of disturbances in the vascular system is crucial to prevent secondary disease complications. Macrovasculature can be visualized with Doppler ultrasound, MR angiography, or plethysmography [83, 84]. Detecting

changes of the microvasculature still remains a challenge. BOLD signal time course and corresponding values reflect the peripheral microvascular function and thus might be an interesting diagnostic tool that can be applied in patients with diabetes mellitus [24, 31]. In a study from Slade et al. microvascular function was assessed in patients with diabetes mellitus types I and II and in a matched control group using muscle BOLD MRI. An exercise paradigm with single-muscle contractions was performed. Regarding BOLD signal of ankle dorsiflexors, no differences were found between the patients and healthy controls. This was explained by the relatively short duration of persistent diabetes in the patient group. In this study, it could be confirmed that the BOLD response depends significantly on age. Influence of age on the BOLD signal during ischemia and reactive hyperemia was already demonstrated in previous studies using arterial occlusion paradigms [30, 36]. Another BOLD study aimed at detecting blood volume and oxygenation status in diabetes mellitus type 2 patients and obese and lean individuals following isometric dorsiflexion contractions [85]. A dual GRE EPI sequence with two different echo times of 6 and 46 ms was applied to measure MRI signal intensity alterations. The postcontraction signal intensity differences were calculated, predominantly indicating altered blood volume (for TE of 6 ms) or oxygenation status (for TE of 46 ms) [20]. Considering the echo time of 6 ms, signal intensity differences were lower in diabetic and obese individuals compared with the lean group with respect to the extensor digitorum longus muscle. When evaluating the echo time of 46 ms, signal intensity differences were only lower in diabetic patients compared with lean controls. These findings reveal altered blood volume status in diabetic and obese individuals and altered oxygenation status only in diabetic patients. These studies show that small-vessel impairment in patients with diabetes or obesity can be detected with functional MRI in the absence of overt macro- or microvascular complications. This technique might thus be helpful for detecting preclinical disease states.

Paradigm selection in clinical settings

Muscle BOLD signal shows a dynamic response to vascular diseases such as PAOD. To induce measurable changes in the $T2^*$ signal, it is necessary to apply a suitable paradigm. Under resting conditions, patients with vascular diseases could have a nearly normal oxygenation status in the calf muscle, but under cuff compression an impaired microcirculation function is seen because the usual metabolic demand cannot be compensated by a sufficient blood inflow. All muscle BOLD MRI studies in PAOD patients used an arterial occlusion paradigm to provoke either

ischemia or reactive hyperemia. This paradigm has some advantages, particularly when investigating vascular diseases:

1. Blood inflow and outflow can be completely prevented by the suprasystolic pressure.
2. Through fast cuff inflation, the influence of venous filling on the BOLD signal is minimized.
3. Patient compliance is not required.
4. This paradigm can be standardized for use in clinical settings.
5. Motion artifacts are a well-known problem in functional MRI of the brain; in the cuff-compression paradigm, such motion artifacts could be kept to a minimum.

Points 3 and 5 are particularly relevant advantages of this paradigm in the clinical setting when compared with muscle-contraction paradigms. Furthermore, paradigm selection is a matter of measurable BOLD SI changes. Regarding patients with diabetes mellitus, oxygenation-sensitive functional MRI revealed smaller $T2$ signal intensity changes than did sequences sensitive for blood volume [31, 85]. Arterial occlusion paradigms, showing promising results in PAOD evaluation, have not been tested in diabetic or obese individuals. For clinical implementation of skeletal-muscle BOLD MRI, the diagnostic power of different paradigms should be further evaluated through mutual analysis. Prior to doing this in patient collectives, we see the potential to test exercise and arterial occlusion in older volunteers, where both paradigms successfully showed BOLD signal alterations.

As for muscle exercise, arterial occlusion has also some important drawbacks. A subset of patients complains about discomfort. In one study, mild to moderate discomfort was reported [33]. In another study, however, severe discomfort during cuff inflation was observed in three of eight patients with PAOD and in three of ten healthy volunteers [64]. In the third study, all patients tolerated the cuff-compression paradigm well [40]. Patients with necrosis or gangrene should be excluded from this paradigm, because suprasystolic cuff compression implies the risk of disease progression. In patients with bypass surgery, safety concerns exist regarding the theoretical risk of bypass occlusion through the suprasystolic cuff pressure.

Conclusion

The authors of this study conclude that muscle BOLD MRI is a valuable tool to noninvasively assess peripheral microvasculature. In PAOD, BOLD MRI of skeletal muscle has been proven to successfully differentiate between patients and healthy individuals. To what extent BOLD

imaging bears sufficient sensitivity and specificity for routine clinical implementation is yet to be demonstrated. Initial reports on other vascular diseases, such as compartment syndrome and diabetes, have revealed somewhat controversial results. This may reflect the need for consistent and efficient experimental muscle BOLD imaging setups and the use of effective imaging paradigms. However, several pathological conditions affect the peripheral vasculature not yet studied by BOLD imaging, i.e., vasculitides, connective tissue diseases, and other inflammatory conditions. With well-designed studies, it will be possible to show under which of these conditions BOLD MRI of skeletal muscle is helpful in basic research, diagnostics, classification, and treatment evaluation. As BOLD MRI predominantly reflects oxygenation changes in the microvasculature, new insights into the pathogenesis of these diseases may be revealed.

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