Dynamic Blood Oxygen Level-dependent MR Imaging of Muscle: Comparison of Postocclusive Reactive Hyperemia in Young Smokers and Nonsmokers

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Purpose: The role of early stage functional assessment of muscle blood flow response (MFR) by dynamic muscle blood oxygen level-dependent (BOLD) magnetic resonance (MR) imaging is unknown. We investigated the effect of smoking on vascular function according to MFR derived from dynamic muscle BOLD MR imaging during postocclusive reactive hyperemia in young smokers and nonsmokers.

Methods: Sixteen healthy male volunteers (8 smokers, 8 nonsmokers; mean age, 30.4 ± 4.6 years) underwent BOLD MR imaging of the left calf muscle. During reactive hyperemia provoked by a cuff-compression technique, we measured muscle BOLD (mB) using a 3-tesla single-shot multi-echo gradient-echo echo-planar imaging sequence. The 2 key mB variables in the reactive hyperemic phase that we studied were times to half hyperemic peak (T1/2peak) and peak (TTP), each measured from cuff deflation. We used the Welch test to assess differences in these between smokers and nonsmokers.

Results: T1/2peak and TTP were significantly longer in smokers (P < 0.05) in reactive hyperemia. T1/2peak was 13.8 ± 5.4 s in smokers and 7.6 ± 1.5 s in nonsmokers, and TTP was 67.5 ± 18.8 s in smokers and 45.4 ± 7.1 s in nonsmokers.

Conclusion: Dynamic BOLD MR imaging of calf muscle during postocclusive reactive hyperemia demonstrated statistically significant differences in T1/2peak and TTP between young smokers and nonsmokers, indicating the presence of early stage smoking-related deterioration in MFR.

Keywords: blood oxygen level-dependent, magnetic resonance imaging, microcirculation, muscle blood flow response, smoking

Introduction

Smoking, a major risk factor for atherosclerosis,1,2 is strongly associated with coronary, cerebral, and peripheral vascular disease.3 One theory for the mechanism underlying damage of the vascular system by smoking is that smoking damages the vascular endothelium, which leads to endothelial dysfunction,4 increased platelet function, inflammatory processes, hypercoagulability, and eventual atherosclerosis.5 These mechanisms are complex, multifactorial,6 and not fully understood. However, impaired vascular function attributable to effects of early stage smoking has been reported in young smokers.7–9

Blood oxygen level-dependent (BOLD) magnetic resonance (MR) imaging, an established functional imaging technique,10,11 is widely used in neuroradiology to assess the function of organs such as the heart12 and skeletal muscle.13–19 According to Ogawa’s group,10 the signal intensity (SI) of BOLD primarily reflects the concentrations of deoxyhemoglobin and oxyhemoglobin in the local microcir-
culation. Inhomogeneity of the local magnetic field caused by paramagnetic deoxyhemoglobin in the microvasculature results in decreased apparent transverse relaxation time (T₂*). In contrast, oxyhemoglobin, a diamagnetic molecule, causes less signal dephasing. Ischemia decreases oxygen saturation in the capillary bed and increases the concentration of deoxyhemoglobin, resulting in reduced BOLD SI, whereas hyperemia increases the concentration of oxyhemoglobin, resulting in increased BOLD SI. Thus, changes in the response of BOLD SI to ischemia-reperfusion are useful imaging biomarkers of microcirculatory function and tissue oxygen metabolism. Many other factors besides concentrations of oxyhemoglobin and deoxyhemoglobin, including perfusion and capillary bed volume, affect BOLD SI. Changes in dynamic skeletal muscle BOLD SI during ischemia-reperfusion has been investigated in healthy volunteers and disease states.

Noninvasive techniques are needed for assessing early stage vascular dysfunction, and BOLD MR is potentially such a technique. Dynamic BOLD MR imaging of calf muscle is reportedly useful for assessing microcirculatory function and muscle oxygen metabolism during postocclusive reactive hyperemia. However, its usefulness for assessing early stage vascular dysfunction is unclear.

We postulated that dynamic BOLD MR analysis of reactive hyperemia could be useful for evaluating early stage reduction in blood flow response in calf muscle (MFR) and selected young smokers as suitable candidates for such study. In this study, we prospectively investigated the effect of smoking on MFR using BOLD MR imaging of calf muscle during postocclusive reactive hyperemia in young smokers and nonsmokers and evaluated the differences in BOLD SI between them to determine the utility of this noninvasive technique for detecting early stage reduction in blood flow response in calf muscle.

Materials and Methods

Our institutional review board (IRB) approved the study protocol, and each subject provided written informed consent prior to entering the study. All subjects’ records/information were deidentified prior to analysis.

Subjects were 16 healthy male volunteers (8 smokers, 8 life-long nonsmokers) aged 24 to 36 years (mean, 30.4 ± 4.6 years). None had manifestations of either peripheral arterial occlusive disease (PAOD; as defined by the guidelines of the Inter-Society Consensus for the Management of Peripheral Arterial Disease [TASC II]) or venous insufficiency (as described by the classification of clinical manifestations, etiologic factors, anatomical distribution, and pathophysiologic dysfunction [CEAP] for chronic venous disease). Candidates were excluded if they had a recognized Brinkman index (number of cigarettes smoked per day multiplied by number of years of smoking) ≥ 200, were taking any medication, demonstrated cardiovascular risk factors, such as hypertension and diabetes mellitus, and had any contraindication to MR imaging.

Study protocol

First, to evaluate reproducibility, we subjected 4 normal nonsmoking volunteers to muscle BOLD MR imaging twice on the left leg with an interval of at least one day between first and second assessments. Nine additional volunteers were then enrolled. We also assessed the left-sided ankle-brachial index (ABI) and brachial artery flow-mediated dilation (FMD) using an ultrasound system equipped with semiautomatic tracking and a real-time monitoring system. The subjects were instructed to avoid strenuous exercise at least 12 hours prior to the MR imaging and FMD test. Further, the subjects were asked to respond to the short form version of the International Physical Activity Questionnaire (IPAQ) to calculate weekly total physical activity of each subject.

Cuff-compression paradigm for reactive hyperemia

We provoked reactive hyperemia using a cuff-compression technique previously described. We applied a conventional sphygmonanometer air cuff for the thigh (86 × 21 cm; Welch Allyn Japan, Tokyo, Japan) to the middle of the left thigh (Fig. 1a) (40-s rest phase), compressed the cuff by applying pressure of 50 mm Hg above the individual’s brachial systolic blood pressure to induce ischemia (300-s ischemic phase), and then rapidly reduced compression by opening the air valve, provoking reactive hyperemia (360-s hyperemic phase) (Fig. 1b). One author performed all cuff inflation (15 s) and deflation (2 s). Subjects rated the discomfort of occlusion from 0 (no discomfort) to 10 (maximal discomfort).

MR image acquisition

All MR imaging was performed with a 3-tesla system (Achieva, Philips Medical Systems, Best, The Netherlands) using an 8-channel knee coil. The BOLD MR imaging protocol was a single-shot multi-echo gradient-echo echo-planar imaging se-
quence with fat suppression with parameters: repetition time (TR), 1000 ms; echo time (TE), 6 echoes at 15.5, 45.2, 75.0, 104.7, 135.5, and 164.2 ms; slice thickness, 4 mm; field of vision (FOV), 240 to 260 mm; and imaging matrix, 224 × 224. The slice position was set at the proximal quarter of the lower leg (Fig. 1a). We performed dynamic measurement with temporal resolution of one measurement/s for consecutive series for 700 measurements of whole phases. We obtained a standard gradient-echo T1-weighted image at the same slice position to assess anatomical features.

**Image analysis**

We analyzed the images of the second TE of 45.2 ms (TE45) (Fig. 1c) and T2* maps. We generated T2* maps from 6-echo echo-planar datasets by a pixel-by-pixel least-square fit of a monoexponential decay function using the reconstruction system on the MR console unit. We calculated the decay function by: \( S(\text{I}_0, T_2^*) = I_0 \exp\left(-\frac{\text{TE}}{T_2^*}\right) \), where \( I_0 \) refers to the initial signal intensity, modulated by proton density, T1, and perfusion,14 and TE is the echo time.

One author, a board-certified diagnostic radiologist blinded to the subjects’ identities and smoking status, performed further image and data analyses. First, using our prototype software written by R (R Foundation for Statistical Computing, Vienna, Austria) and RNiftyReg package (https://github.com/jonclayden/RNiftyReg), we performed non-rigid registration using a free-form deformation algorithm28 to compensate for moving effect. Then, we set regions of interest (ROI) in the TE45 images and T2* maps and obtained dynamic SI changes in the ROI. Using the region growing method, we set the ROIs for all calf muscles to exclude large vessels (Fig. 1d). We validated the accuracy of ROIs
visually with $T_1$-weighted images and made corrections manually.

We normalized the individual TE45 SIs according to the mean SI during the first 40 s (rest phase) as muscle BOLD SIs (mB) and calculated mB time course curves (Fig. 1d). To describe the phases completely, we defined the following 6 variables. For the rest phase, 1) we obtained $T_2^*$ rest (average 40-s rest phase) from the $T_2^*$ map. 2) Ischemia was defined from the mB curves and characterized by time to the half ischemic minimum from cuff inflation at 40 s ($T_1/2_{min}$). Reactive hyperemia was also defined from the mB curves and characterized by the following 4 variables—the 3) mB of the hyperemic peak ($mB_{peak}$); 4) time to half the hyperemic peak from cuff deflation at 340 s ($T_1/2_{peak}$); 5) time to peak from cuff deflation at 340 s (TTP); and 6) time to half of the post-hyperemia normalization from TTP ($T_1/2_{nor}$) (Fig. 1b).

**Statistical analysis**

We calculated the coefficient of variation (CV in %) and intraclass correlation coefficient (ICC) of the mB variables ($T_1/2_{min}$, $mB_{peak}$, $T_1/2_{peak}$, TTP, and $T_1/2_{nor}$) using the duplicate measurements of the 4 initial normal nonsmoking volunteers to evaluate test-retest reproducibility; used the Welch test to assess differences in volunteer characteristics and mB and $T_2^*$ results between nonsmokers and smokers; and used the Spearman’s correlation coefficient to analyze correlations between the key variables of mB, ABI, and FMD. We analyzed statistics using JMP9.0 (SAS Institute Japan, Tokyo, Japan) and R (R Foundation for Statistical Computing, Vienna, Austria) software, with significance level set at $P = 0.05$.

**Results**

MR examinations were successfully performed and relevant variables assessed in all subjects. Figure 2 shows the representative color map of mB images for each phase. Cuff compression was tolerated well. Discomfort scores ranged from 2 to 6 (median 4); there were no adverse effects.

Figure 3 illustrates representative mB curves of a nonsmoker and smoker. Continuous decay and convergence to minimum value occurred during ischemia. After cuff deflation, mB increased rapidly, representing reactive hyperemia. This increase was

![Fig. 2. Representative color-mapped mB (see following definition) images of a nonsmoker. In (a) rest phase, (b) ischemic phase, and (c) hyperemic phase. Color bar shows the signal intensity ratio normalized by average of rest phase. mB, muscle blood oxygen level-dependent (BOLD) magnetic resonance (MR) imaging signal intensity ratio normalized by average of rest phase.](image-url)
followed by an initially steep and subsequently slower decay. Finally, mB reached a steady state value near the rest value.

Table 1 shows the dynamic mB variables and CV and ICC of the 4 nonsmokers.

Table 2 presents relevant characteristics of smokers and nonsmokers, which did not differ significantly between the 2 groups. However, TTP and $T_{1/2peak}$ were significantly longer in smokers ($P < 0.05$, statistical power $> 0.80$) (Table 3). Mean values for TTP were $67.5 \pm 18.8$ s for smokers and $45.4 \pm 7.1$ s for nonsmokers, and mean values of $T_{1/2peak}$ were $13.8 \pm 5.4$ s for smokers and $7.6 \pm 1.5$ s for nonsmokers.

We observed moderate negative correlations between TTP and ABI ($\rho = 0.64$, $P < 0.01$) (Fig. 4). There were no significant correlations between the other variables.

**Discussion**

We investigated the differences in mB time courses between young smokers and nonsmokers during postocclusive reactive hyperemia and found significant differences in the TTP and $T_{1/2peak}$. Apart from smoking, there were no obvious causes for these differences. Further, though physical activity would also be considered to affect MFR, reported physical activity did not differ significantly between young smokers and nonsmokers in our study. We also found no significant differences in ABI and FMD, indicating that mB changes may be more sensitive to vascular dysfunction than ABI or FMD.

Others have identified and discussed the main causes of mB changes in each phase (Table 4)$^{14,15,18–20}$; however, the underlying factors are insufficiently understood. In the rest phase, $T_2^{*}$rest...
represents the steady-state level of muscle oxygen metabolism. In the ischemic phase, because the cuff occlusion technique eliminates input and output flow, which affect BOLD SI, the mB time course shows only functional changes in muscle oxygen consumption and extraction. T_{1/2min} is the functional variable in this phase. Because rest- and ischemia-related variables did not differ significantly in our study, we speculate that in young smokers, there is either a compensatory mechanism for metabolic responses or vascular damage occurs prior to metabolic damage or both.

In the hyperemic phase, previous studies have shown that hyperemia-related variables (T_{1/2peak} and TTP) are useful for assessing microcirculatory function. In our study, these variables were significantly prolonged in smokers. We identified a correlation between TTP and ABI, which was consistent with findings of a previous report. Some subjects with the low-end of normal ABI (<1.1) reportedly have subclinical atherosclerosis, which could explain the correlation we found between TTP and ABI in our young subjects. Because ABI is an index of arterial inflow, we speculate that the reduction in these hyperemia-related variables reflects a reduction in muscle reperfusion. We observed no significant difference between smokers and nonsmokers in mB peak, which represents hyperemic muscle microcirculatory blood volume.

Table 2. Differences in relevant characteristics between nonsmokers and smokers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nonsmokers* (N = 8)</th>
<th>Smokers* (N = 8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.1 ± 4.2</td>
<td>30.6 ± 5.2</td>
<td>0.84</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.8 ± 1.8</td>
<td>21.1 ± 2.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>121.1 ± 7.8</td>
<td>121.0 ± 10.3</td>
<td>0.98</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>81.3 ± 9.4</td>
<td>78.0 ± 8.9</td>
<td>0.49</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>63.5 ± 7.1</td>
<td>64.8 ± 14.6</td>
<td>0.83</td>
</tr>
<tr>
<td>Ankle-brachial index</td>
<td>1.16 ± 0.05</td>
<td>1.13 ± 0.06</td>
<td>0.24</td>
</tr>
<tr>
<td>Flow-mediated dilation (%)</td>
<td>13.8 ± 4.2</td>
<td>12.3 ± 4.0</td>
<td>0.47</td>
</tr>
<tr>
<td>Brinkman index</td>
<td>0</td>
<td>140.3 ± 52.3</td>
<td>—</td>
</tr>
<tr>
<td>Physical activity (METs-min/week)</td>
<td>1968.5 ± 1099.2</td>
<td>1892.4 ± 1011.9</td>
<td>0.89</td>
</tr>
</tbody>
</table>

*Mean value ± standard deviation

Brinkman index, the number of cigarettes smoked per day multiplied by the number of years of smoking; MET, metabolic equivalent of task; physical activity, result from the International Physical Activity Questionnaire Short form.

Table 3. Differences in magnetic resonance (MR)-related variables between nonsmokers and smokers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonsmokers* (N = 8)</th>
<th>Smokers* (N = 8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{2*} peak</td>
<td>26.0 ± 0.6</td>
<td>26.1 ± 1.0</td>
<td>0.71</td>
</tr>
<tr>
<td>mB image</td>
<td>53.6 ± 9.0</td>
<td>53.9 ± 11.1</td>
<td>0.96</td>
</tr>
<tr>
<td>mBpeak</td>
<td>1.08 ± 0.02</td>
<td>1.08 ± 0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>T_{1/2peak} (s)</td>
<td>7.6 ± 1.5</td>
<td>13.8 ± 5.4</td>
<td>0.02</td>
</tr>
<tr>
<td>TTP (s)</td>
<td>45.4 ± 7.1</td>
<td>67.5 ± 18.8</td>
<td>0.01</td>
</tr>
<tr>
<td>T_{1/2nor} (s)</td>
<td>86.3 ± 37.0</td>
<td>100.3 ± 26.7</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*Mean value ± standard deviation

mB, muscle blood oxygen level-dependent (BOLD) MR imaging signal intensity ratio normalized by average of rest phase; mBpeak, mB of hyperemic peak; T_{1/2min}, time to half ischemic minimum from cuff inflation; T_{1/2nor}, time to half of post-hyperemia normalization from time to peak from cuff deflation (TTP); T_{1/2peak}, time to half hyperemic peak from cuff deflation; T_{2*rest}, average T_{2*} value of rest phase.

Fig. 4. Correlation of time to peak from cuff deflation (TTP) and ankle-brachial index (ABI).
and muscle O₂ saturation. We postulate that prolongation of T₁/2peak and TTP in early stage vascular dysfunction leads to decreases in mBpeak after compensatory responses have become impaired. In the post-hyperemia normalized phase, mB changes reflect a combination of functional changes in muscle oxygen metabolism and venous washout. Prolonged normalization may be an additional indication of impaired muscle reperfusion; T₁/2nor was relatively prolonged in smokers, but this did not reach statistical significance.

Smoking-related reductions in flow response have been identified by measuring FMD and coronary artery diameter by angiography, and diminished flow responses have been shown in the cerebrovascular systems of smokers by visually evoking cerebral vasomotor responses. Considering these findings, the significant reductions we found in dynamic hyperemia-related variables are likely attributable to smoking-related impairment of MFR. Although previous studies have suggested that smoking affects endothelial-dependent vasodilation, the hyperemia-related variables we measured did not correlate significantly with FMD, which is thought to represent endothelial vascular function. However, this finding is not surprising because BOLD SI cannot be directly compared with reperfusion patterns measured by FMD or strain gauge plethysmography that infuses endothelium-dependent and -independent vasodilators. We speculate that because these hyperemia-related variables reflect overall vascular function, including endothelial-dependent and -independent vascular function, microcirculatory volume, and arterial inflow, the variables are more sensitive than FMD to smoking damage. However, further study is needed to confirm this possibility.

Researchers have investigated the capability of dynamic muscle BOLD MR imaging of postocclusive reactive hyperemia to assess patients with PAOD and age-related changes. Patients with PAOD reportedly have significantly prolonged TTP and lower peak values because of the impaired inflow to their muscle microcirculations. Age-related changes in BOLD variables are still disputed, both these studies reported relatively low peak values in elderly subjects but contradictory findings for TTP. These 2 studies and the present study concur that mB can noninvasively distinguish non-PAOD and PAOD, the young and the elderly, and nonsmokers and smokers. In addition, shortening of TTP in healthy volunteers by infusion of heme arginate indicates that mB is sufficiently sensitive to assess post-ischemia reperfusion in muscle. Our findings also suggest that mB could be used to assess early stage vascular dysfunction as decreases in MFR.

The reproducibility of our hyperemia-related mB variables was acceptable, but our study had only a few subjects. A previous study using a 1.5-tesla system reported relatively poor reproducibility of BOLD variables except TTP. However, a recent study using a 3-tesla system showed improved inter-session CVs for BOLD variables, which was presumably attributable to the increased signal-to-noise ratio and BOLD SI contrast with the higher field strength.

Our study has several limitations. First, there were few subjects. Nevertheless, statistical significance was reached for key mB variables. Second, because of their more prominent time course, we used TE45 images rather than T₂* maps to measure the time course. Their more prominent time course may be attributable to the relatively long ΔTE for multi-echo in our study, but there were limits to shortening the ΔTE while retaining appropriate time and image resolution for our assessments. Previous researchers have used T₂* maps to assess “true” BOLD phenomena, thus eliminating inflow effects. However, because our aim was to as-

### Table 4. The main causes for dynamic muscle blood oxygen level-dependent (BOLD) images in each phase

<table>
<thead>
<tr>
<th>Phase</th>
<th>Presumed main cause in muscle</th>
<th>Variable</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>Steady-state O₂ metabolism</td>
<td>T₂*_rest</td>
<td>—</td>
</tr>
<tr>
<td>Ischemia</td>
<td>O₂ consumption</td>
<td>T₁/2min (s)</td>
<td>(15)</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>O₂ saturation, blood volume</td>
<td>mB_peak</td>
<td>(18–20)</td>
</tr>
<tr>
<td></td>
<td>Inflow, O₂ saturation</td>
<td>T₁/2peak (s), TTP (s)</td>
<td>(14,19)</td>
</tr>
<tr>
<td>Normalization</td>
<td>Outflow, O₂ consumption</td>
<td>T₁/2nor (s)</td>
<td>(18,20)</td>
</tr>
</tbody>
</table>

mB, muscle BOLD magnetic resonance (MR) imaging signal intensity ratio normalized by average of rest phase; mB_peak, mB of hyperemic peak; T₁/2min, time to half ischemic minimum from cuff inflation; T₁/2nor, time to half of post-hyperemia normalization from time to peak from cuff deflation (TTP); T₁/2peak, time to half hyperemic peak from cuff deflation; T₂*_rest, average T₂* value of rest phase.
T2
this, simultaneous measurement of perfusion and ful MR imaging machines are required to achieve factors underlying muscle BOLD.

Moreover, as previously documented, 33 TE45 is reported. 20 Although more sophisticated and powerful MR imaging machines are required to achieve this, simultaneous measurement of perfusion and T2* change would facilitate understanding of the factors underlying muscle BOLD.

Conclusion
Dynamic BOLD MR imaging of calf muscle during postocclusive reactive hyperemia demonstrated statistically significant differences in T1/2peak and TTP between young smokers and nonsmokers, providing evidence of early stage smoking-related deterioration in MFR.

References
8. Dalla Vecchia L, Palombo C, Ciardielli M, et al. Contrasting effects of acute and chronic cigarette smoking on skin microcirculation in young healthy sub-


