BOLD effect on calf muscle groups in elderly females with different bone mineral density

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***Abstract*—This study examined the BOLD effect on calf muscles in elderly subjects with different bone mineral density. The purpose was to investigate the oxygenation characteristics in different calf muscle groups for the elderly females and compare the muscle oxygenation among groups with different bone mineral density. Temporary vascular occlusion was induced with air-cuff compression of the thigh and BOLD-MRI data curve was fitted to derive quantitative parameters. Three muscle groups, gastrocnemius muscle (lateral head), soleus muscle, and tibialis anterior muscle, were investigated individually. Quantitative CT measurement was conducted on each subject, based on which subjects were classified into normal, osteopenia, and osteoporosis groups. The BOLD signal in soleus muscle showed the lowest minimum ischemic value during ischemia and the steepest slope during hyperemia. As soleus muscle is mainly composed by slow-twitch oxidative muscle fibers, current results may be due to a higher vascular bed density and better endothelial function in such muscle. By t-test, the half-life of the BOLD signal decay during ischemia in both gastrocnemius and soleus muscles was significantly prolonged in osteoporosis group, indicating a degenerated muscular oxygen metabolic capacity in osteoporotic patients.**

I. Introduction

Lower-extremity peripheral artery disease affects roughly 1 in 20 adults aged above 55 years [1]. Current methods used to measure skeletal muscle blood flow are either limited by spatial resolution (e.g. PET) or are invasive (e.g. DCE-MRI). Development of non-invasive techniques for evaluating vascular function would be highly desirable. Functional MRI relies on the blood oxygenation level-dependent (BOLD) effect that is sensitive to changes in local tissue deoxyhemoglobin concentration [2]. This technique is widely used in human brain activation mapping and also in skeletal muscle perfusion studies [3-7]. Skeletal muscle oxygenation level varies with local metabolic process and oxygen supply by the feeding arteries or capillaries. Muscle BOLD effect in the lower limb may be observed by applying a T2*-weighted sequence while an air-cuff is inflated above the knee to induce ischemia. On releasing air-pressure, post-ischemic reactive hyperemia may be assessed by measuring the rate of T2* signal recovery [6-10].

The BOLD effect in the skeleton muscle can reflect hemoglobin oxygenation inside the muscle's capillary bed, which is mainly influenced by blood inflow, fluid shifts, metabolic factors, vascular architecture, and magnetic field angulation [1, 11]. Previous studies indicated that osteoporotic bone had a reduced perfusion in bone marrow while remained a normal perfusion function in the surrounding muscles [12,13]. Perfusion process reflects the blood inflow in the tissue, which only partially indicates the local oxygenation situation. If the muscle oxygenation keeps at the normal level in osteoporotic patients is unknown yet. BOLD examination on muscle would reveal the muscle function change in osteoporosis in terms of the muscle oxygenation function.

Therefore, this study was proposed to examine the BOLD effect on calf muscles in elderly subjects with different bone mineral density (BMD) to investigate the oxygenation characteristics in different calf muscle groups and the difference in osteoporotic patients. Temporary vascular occlusion was induced with air-cuff compression of the thigh and BOLD-MRI data curve was fitted to derive quantitative parameters. Three muscle groups, gastrocnemius muscle (lateral head), soleus muscle, and tibialis anterior muscle, were investigated individually. Subjects were classified into three groups (normal, osteopenia, and osteoporosis) based on the bone mineral density (BMD) measured by the quantitative CT (QCT).

II. Methodology

A. Subjects

Subjects were recruited in a common community by public advertisement. In order to avoid gender influence, only female subjects were included in current study. Subjects were excluded if they had (a) clinical or imaging evidence of renal osteodystrophy or other metabolic bone disease other than osteoporosis or a known malignancy, (b) a history of lumbar spinal surgery or irradiation, or (c) MR imaging evidence of large intravertebral disk herniation, hemangioma, or moderate to severe vertebral fracture of L3. Finally, 87subjects (age 65.4

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±4.3yrs) in total were involved in this study. The study was approved by the Ethics committee, Chinese University of Hong Kong with all participating subjects submitting written consent.

B. Data acquisition

Quantitative CT (QCT) was conducted on subjects'L3 of lumbar spine by Lightspeed VCT 64. Standard phantom was put under the lumbar during the imaging. Bone mineral density was then derived according to the CT values from the bone and the phantom.

Each subject was also required to undergo BOLD imaging of the lower limb using a 3T whole-body scanner (Achieva TX, Philips Healthcare). An eight-channel SENSE knee coil was used for signal reception and a T2*-weighted sequence (TR/TE 372/40 ms; slice thickness 5 mm; NEX 1; FOV 330 mm; dynamic measurements 2400; scan time 900s) was employed. An air-cuff was placed just above the left knee with an imaging plane selected 20-25 cm away from the distal side of the air-cuff. Air-cuff pressure applied was 50mmHg above systolic pressure. The cuff was inflated at 1 min and maintained for 5 minutes. Followed cuff deflation, the scanning continued for a further 9 minutes. Regions of interest (ROI) were drawn on the gastrocnemius muscle (lateral head), soleus muscle, and tibialis anterior muscle on the T1 images (Fig.1), which was co-registered onto the BOLD image series. The BOLD signals in the corresponding ROIs were measured from T2* images.

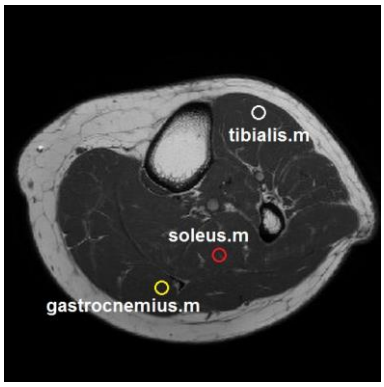

Fig 1.T1 image of the calf slice showing the location of ROIs drawn in gastrocnemius muscle (yellow circle), soleus muscle (red circle), and tibialis muscle (white circle).

C. Signal processing and data analysis

The BOLD-MRI signal was first normalized by the resting stage signal intensity, which is the starting period of the imaging. A curvefitting modelwas employed to analyze BOLD-MRIsignals using PRISM software (ver. 5). After signal normalization, four parameters were selected to characterize the T2* time course as shown in Fig 2:

1. MIV: the minimum ischemic value, which is the minimal BOLD signal during the ischemic phase relative to baseline;
2. HPV: hyperemia peak value, which refers to the maximum height of T2* signal during hyperemia relative to baseline.
3. Half-life: the time interval forthe BOLD signal decaysto half MIV during ischemia as measured from the beginning of the ischemia;
4. Slope: the ratio between the HPV+baseline and the time interval for hyperemia, which is a measure to indicate the rate of T2* signal surge at the moment of cuff release;

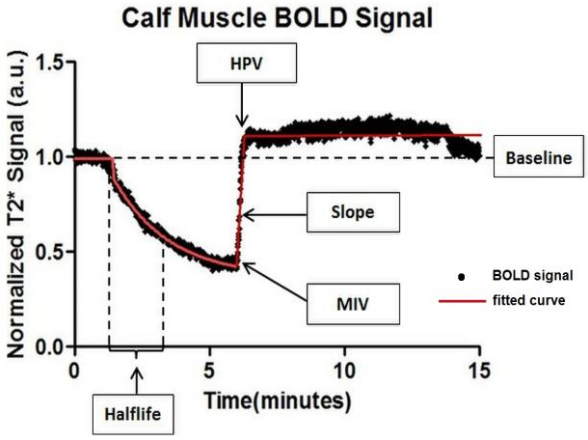

Fig 2. An example of curve fitting model for normalized T2* signal

Subjects were classified into three groups based on the BMD values derived from QCT measurement. According to the American College of Radiology (ACR) [17]: a BMD ≤ 80 mg/cm^{3}indicates osteoporosis; a BMD ≤ 120 mg/cm^3 and > 80 mg/cm3 indicates osteopenia; and a BMD above 120 mg/cm^3 is considered normal.

ANOVA and t-test were performed for the key parameters to distinct difference among muscle groupsand BMD groups. All statistical analyses were performed using SPSS 16 and p=0.05 was set as the statistically significant level.

III. Results

The ANOVA of the parameter values derived from the three muscle groups for the 87 subjects are shown in Table 1. Significant differences in Slope and MIV were observed among the muscle groups ($p<0.05$). Soleus muscle has the highest Slope but the smallest MIV value, compared to the other two muscle groups. Therefore, the soleus muscle should have a steeper enhancement phase during the hyperemia phase. The comparison is further elucidated by Fig.3.

Table1: Muscle groups comparison by ANOVA

Muscle Groups	Slope (a.u./min)	Halflife (min)	HPV (%)	MIV (%)
Gastroc	1.5±0.6	3.0±2.5	3.9±8.6	62.3±14.3
Soleus	1.8±0.7	4.6±4.8	5.8±8.6	55.9±13.0
Tibialis	*1.3±0.4*	*2.3±2.0*	*5.5±7.4*	*68.2±11.0*
P-value	<0.0001	0.065	0.243	<0.0001

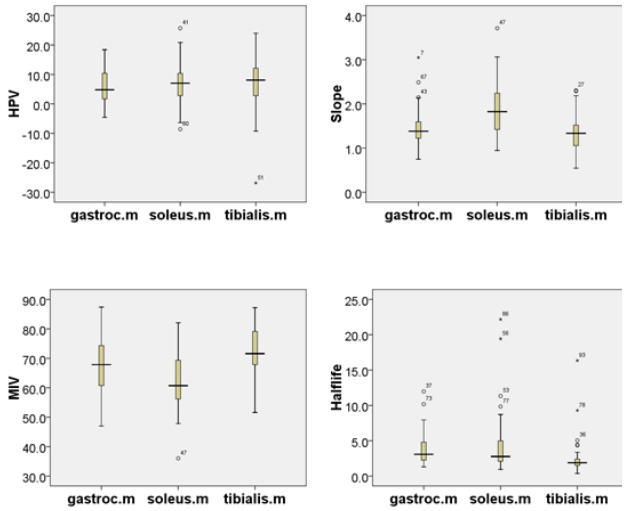

Fig 3.Comparison of the four parameters (Slope, Half-life, MIV, HPV) among 3 muscle groups.

The comparison of the muscle parameters among different BMD groups is summarized by Table 2.

Table2: ANOVA for muscle parameter among BMD groups

Groups		gas.m	soleus.m	tibia.m
Half-life (min)	*0*	*3.3±2.5*	*2.9±1.6*	2.2±0.8
	1	2.3±1.5	3.2±3.7	2.2±1.6
	2	4.3±3.7	4.5±6.1	2.9±3.4
	P-value	--	--	--
MIV (%)	*0*	*61.5±16.8*	*56.7±12.5*	69.1±11.0
	1	62.9±14.0	56.7±12.5	67.4±11.7
	2	60.7±12.4	58.3±11.8	68.5±9.7
	P-value	0.912	0.447	0.902
slope (a.u./ min)	*0*	*1.6±0.6*	*1.8±0.7*	*1.4±0.6*
	1	1.5±0.5	2.0±0.7	1.3±0.4
	2	1.4±0.6	1.7±0.5	1.2±0.3
	P-value	0.601	0.206	0.269
HPV (%)	*0*	*3.6±10.4*	3.5±9.0	*5.7±5.0*
	1	3.6±10.4	6.7±8.8	5.3±8.6
	2	2.1±9.7	5.7±7.8	6.0±6.9
	P-value	*0.531*	*0.388*	*0.939*

Note: 0 represents normal group;1 represents osteopenia group, and 2 represents osteoporosis group

No significant change by ANOVA is observed from Table 2. However, the Halflifemean value of both gastrocnemius and soleus muscles is much higher in osteoporosis group compared to the normal group. Due to the large variance, ANOVA was failed in the analysis. When we further investigated the difference by t-test, the Halflifevalue was significantly larger in osteoporosis group compared to the normal group. This result indicates that osteoporotic patients would have a longer decay halflifeduring the ischemia stage for the gastrocnemius and soleus muscles.

Table3: p-value from the t-testonHalflifeamong BMD groups

Muscle	0 vs 1	1 vs 2	0 vs 2
gastroc.m	0.045	0.36	0.04
soleus.m	0.06	0.13	0.016

Note: 0 represents normal group; 1 represents osteopenia group, and 2 represents osteoporosis group

IV. DISCUSSIONS

Functional MRI is a promising tool for noninvasiveinvestigation of ischemia and reperfusion process ofskeletal muscle. Although no standard procedurefor experiment and data, manystudies have characterized the BOLD signal intensity curve by quantitative parameters [1]. BOLD signal changes reflect muscle oxygenationand in particular the degree of hemoglobin oxygenation. Several physiologic parameters have been postulated to influence the muscle BOLD signal, such as muscle blood volume, perfusion, fluid shifts, metabolic factors, vascular architecture, and magnetic field angulation [6,9]. Due to their mutual interference and paradigm-dependence, dissecting the impact of every single factor on the muscle BOLD effect is difficult. This study was proposed to investigate the muscle oxygenation difference in the elderly females with different BMD by using quantitative parameters, such as MIV, HPV, Slope, and Halflife.

First, the BOLD effect of the three muscle groups was investigated for all the subjects. It was found that soleus muscle had a significant lower MIV during ischemia than the other calf muscles. Parameter MIV reflects oxygen utilization while Slope reflects vascular reactivity [1]. Conversely, the soleus also showed the steepest slope upon reperfusion indicating highest vascular reactivity or density. These changes are in line with expected findings. The soleus muscle mainly helps maintain balance and is mainly consists of slow-twitch oxidative muscle fibers which tend to have a higher capillary density and myoglobin content compared to fast-twitch dominant muscles, such as the gastrocnemius muscle. In this study, using a non-invasive technique, we have been able to demonstrate different oxygen requirements, metabolism and re-perfusion capability in different calf muscle groups. Vascular compromise could potentially affect some muscle groups differently to others.

Secondly, a significantly prolonged Halflifewas observed in gastrocnemius and soleus musclesof osteoporotic subjects compared to those with normal BMD. Parameter Halflifereflects the rate of oxygen metabolism or the efficiency of the oxygen uptake. This difference indicates a reduction of the muscular oxygen metabolic capacity in osteoporotic patients. Taking the comparison results of the muscle groups, it appears that muscle group with more slow-twitch fibers would have a prolonged Halflife during ischemia. The current result may imply that in osteoporotic patients, more muscle fibers transformed to slow-twitched fibers. Actually this implication can be supported by a previous study on muscle fiber of osteoporotic patients by muscle biopsy [14]. In that study, fast-switched muscle fiber was found positively correlated to the BMD. In other words, osteoporotic patients would have more slow-twitched muscle fibers compared to normal subjects. Such muscle degeneration

in osteoporosis should be further investigated, which may reveal other mechanism of osteoporosis.

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