Use of BOLD MRI to Study Muscle Microvascular Function and Dysfunction

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Introduction

Skeletal muscle is the body's largest organ and is essential to many aspects of human physiology, including movement, glucose uptake and storage, thermogenesis, and total body water balance. MRI methods allow investigators to assess many aspects of muscle structure and function. A key point of focus for this abstract and presentation will be the muscle microvasculature, the proper function of which is essential to all of the activities noted. The purposes of this abstract and presentation are 1) to describe the changes to muscle microvascular structure and function that occur in obesity and Type 2 Diabetes Mellitus (T2DM) and 2) to describe the use of BOLD MRI-based methods for studying muscle microvascular function in obesity and diabetes.

Changes to the Muscle Microvasculature in Obesity and Diabetes

Diabetes is a severe and costly disease whose prevalence in Western countries is increasing steadily. Most of these cases are T2DM, which involves defects in both insulin secretion and sensitivity. Persons with T2DM have elevated risks for stroke, heart disease, hypertension, adult-onset blindness, non-traumatic amputation, kidney disease, and neuropathy (1). In the United States, the rate of new diabetes diagnoses is expected to double between 2010 and 2050, and the overall prevalence is expected to increase from 14% to 21% (2). Moreover, with obesity and obesogenic lifestyles being central to the development of T2DM, it is noteworthy that in 2009-2010, the prevalence of obesity was 37.5% among adult Americans and 16.9% among adolescent Americans (3).

Skeletal muscle is one of the principal sites of microvascular injury in T2DM. Structural changes to the skeletal muscle microcirculation in diabetes include thickened capillary basement membranes (4), lower capillary density (4,5), smaller terminal arteriole diameter (5), and reduced capillary surface area for transport (6). Functionally, there are deficits to endothelium-dependent vasodilation in skeletal muscle (7-9), due to reduced nitric oxide (NO) synthesis and/or enhanced NO quenching (10-12). NO-dependent vasodilation deficits are improved following Metformin

treatment (13). There may also be impaired endothelium-independent vasodilation (14), possibly due to increased adrenergic tone in the vascular smooth muscle (5,15,16). An increase in sympathetic tone, resulting from neuropathy of the Vagus nerve, may be an early contributor to autonomic dysfunction (17).

The health consequences of these impairments are manifest in at least two ways. First, skeletal muscle is the body's principal site for glucose uptake and storage (18). Insulin increases skeletal muscle blood flow through an NO-dependent mechanism, and so deficits in this pathway and muscle perfusion more generally may lead to insulin resistance (19). Also, muscle metabolic deficits and vascular dysfunction reduce exercise capacity in T2DM (20-23). During exercise, there is normally a global increase in sympathetic outflow. Within the exercising muscles themselves, paracrine signaling phenomena produce a "functional sympatholysis" that produces a local vasodilatory response. The obesity- and T2DM-related microcirculatory deficits cited above impair this process. This limits the ability of an obese, pre-diabetic or T2DM patient to use exercise to control blood glucose independently of insulin or receive the health benefits of regular exercise.

In euglycemic, normotensive obesity, the muscle microcirculation undergoes similar structural and functional impairments to those in T2DM, including reduced capillary density (24-26), increased arterial stiffness (27), and impaired NO-dependent signaling (28). There is enhanced α_1/α_2-adrenergic vasoconstrictor tone in the obese Zucker rat (29) and in obese humans, there is increased sympathetic tone (30,31) that may elevate blood pressure (32). These changes may individually or collectively contribute to the reduced insulin-stimulated muscle microvascular recruitment (33,34), reduced endothelium-dependent vasodilation (35), increased spatial heterogeneity in skeletal muscle blood flow (16), and decreased exercise blood flow (36,37) that have been observed in obesity. Collectively, these findings support the views that structural and functional microvascular and metabolic impairments may be not just be consequences, but also causes, of insulin resistance and T2DM.

Understanding the role of human muscle microvascular dysfunction in obesity and T2DM requires the use of well characterized, image-based tools. *In vivo* imaging and spectroscopy methods can quantify tissue structure, physiology, and pathophysiology in the most translationally relevant contexts possible. In particular, MRI methods that are based on BOLD contrast: 1) have been shown to reflect important physiological quantities such as blood volume

and %HbO$_2$; 2) are non-invasive; 3) can test for muscle-specific responses and detect intramuscular heterogeneity; 4) have high temporal resolution; 5) do not require sophisticated tissue modeling and are widely available on clinical MRI systems; 6) are uninfluenced by subcutaneous fat thickness; and 7) use neither ionizing radiation nor an exogenous contrast agent. This last point is especially important for T2DM patients in the era of nephrogenic systemic fibrosis (38).

BOLD MRI to Study Muscle Microvascular Function

The muscle BOLD effect was identified 15 years ago (39-41). Muscle BOLD effects are largest for T_2^*, but there is a T_2 effect also (41-45). In general, BOLD effects result from deoxyhemoglobin's paramagnetism (46), creating a magnetic susceptibility mismatch between deoxyhemoglobin and the diamagnetic water surrounding it. BOLD effects may have intravascular and extravascular components. The intravascular BOLD effect refers to the effect of blood oxyhemoglobin saturation (%HbO$_2$) and hematocrit on the T_2 and T_2^* of water protons in the blood. Free intracellular water protons that exchange with water from deoxyhemoglobin's hydration shell or diffuse through its magnetic susceptibility gradient cause transverse relaxation (42,43,47,48). Because of rapid trans-membrane water exchange, the whole-blood's T_2 or T_2^* is affected. The intravascular BOLD effect changes the whole-tissue's T_2 or T_2^* in proportion to the relative blood volume. The extravascular BOLD effect refers to the effect of magnetic susceptibility differences between blood vessels and the tissue parenchyma on the transverse relaxation of extravascular water. As this water diffuses in and out of the magnetic susceptibility gradients formed around the vessels, they precess at different Larmor frequencies, and therefore undergo a transverse relaxation effect. The extravascular BOLD effect may introduce a vascular structural dependence to BOLD phenomena (49,50). However, several studies have shown that under most reasonably foreseeable experimental conditions, the extravascular BOLD effect is practically unimportant in skeletal muscle at field strengths of 3T and below (51-54).

Recently, muscle BOLD MRI has been used to infer small vessel function following arterial occlusion (55) and the infusion of vasoactive compounds (53), to estimate oxygen extraction during exercise (56,57), and to study vascular function in peripheral artery disease (58). Another application, and the focus of this abstract/presentation, is the use of BOLD MRI to study muscle microvascular function associated with muscles' primary functional state,

contraction (52,59). Brief (<10 s) isometric contractions have been used as a model of the onset of exercise.

A brief isometric contraction places a relatively small metabolic load on a muscle. During the contraction, intramuscular fluid pressure increases. This compresses the arterioles (60) and reduces blood volume in the venous circulation (61). After the contraction, the vessels refill and dilate; blood volume increases transiently (62). Because blood has a greater water content than the muscle tissue it displaces (63,64), the proton density increases. Also, the flow response is generated in anticipation of a longer exercise bout, so the increase in O_2 supply exceeds the increase in O_2 demand (in healthy persons). Thus, the oxy-hemoglobin saturation ($\%HbO_2$) is transiently elevated.

Taking advantage of this transient elevation in $\%HbO_2$, Meyer *et al.* were able to observe a transient, positive change in BOLD-dependent signal intensity (SI) following a 1 s isometric contraction (52). The authors suggested that BOLD contrast in skeletal muscle may be used to reflect microvascular function. Indeed, a subsequent study by Towse *et al*. showed that the post-contraction muscle BOLD contrast in chronically physically active subjects is ~3-fold higher than the responses in sedentary subjects. Also, they developed a tissue-specific metabolic and vascular model and used it to show that post-contraction BOLD SI changes depend on the balance between O_2 delivery and O_2 consumption, with a strong dependence on blood flow and volume changes. A final study from this group showed that there were no differences in post-contraction BOLD responses among T1DM and T2DM patients and age, body-mass index (BMI), and physical activity matched controls that were detectable by this approach. However, they did report age-dependent variations in post-contraction BOLD contrast (65).
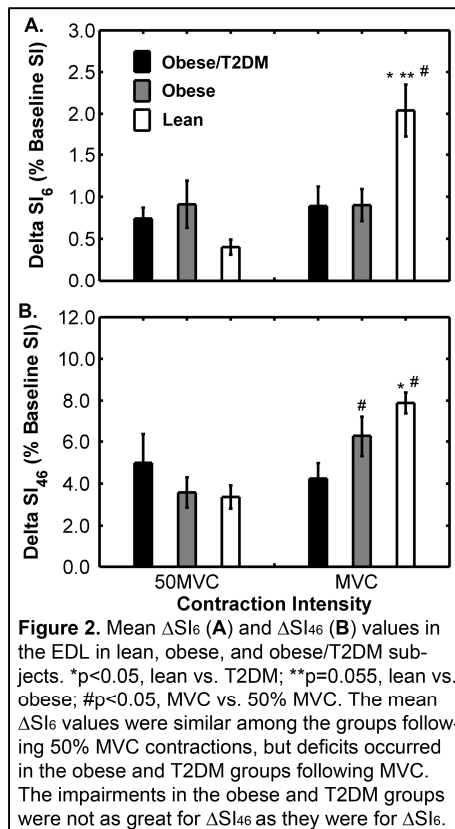
Based on the studies from the Meyer group, Damon and colleagues used a dual gradient-recalled echo (GRE) MRI sequence to study blood volume and $\%HbO_2$ changes in the muscle microvascular bed following isometric contractions (66). As implemented in this context, the dual-GRE sequence acquires signals with a repetition time (TR) of 1 s and with echo times (TEs) of 6 ms and 46 ms. Figure 1 shows sample SI time course data from their 2011 paper on obesity and T2DM (67). Panels A and D show data from a healthy subject and illustrate how the amplitudes of the SI transients, ΔSI_6 and ΔSI_{46}, are measured. In their 2007 paper (66), they used two experiments to test the hypotheses that ΔSI_6 reflects blood volume changes and that ΔSI_{46} reflects $\%HbO_2$ changes. First, they measured ΔSI_6, ΔSI_{46}, [THb], and $\%HbO_2$ before, during,
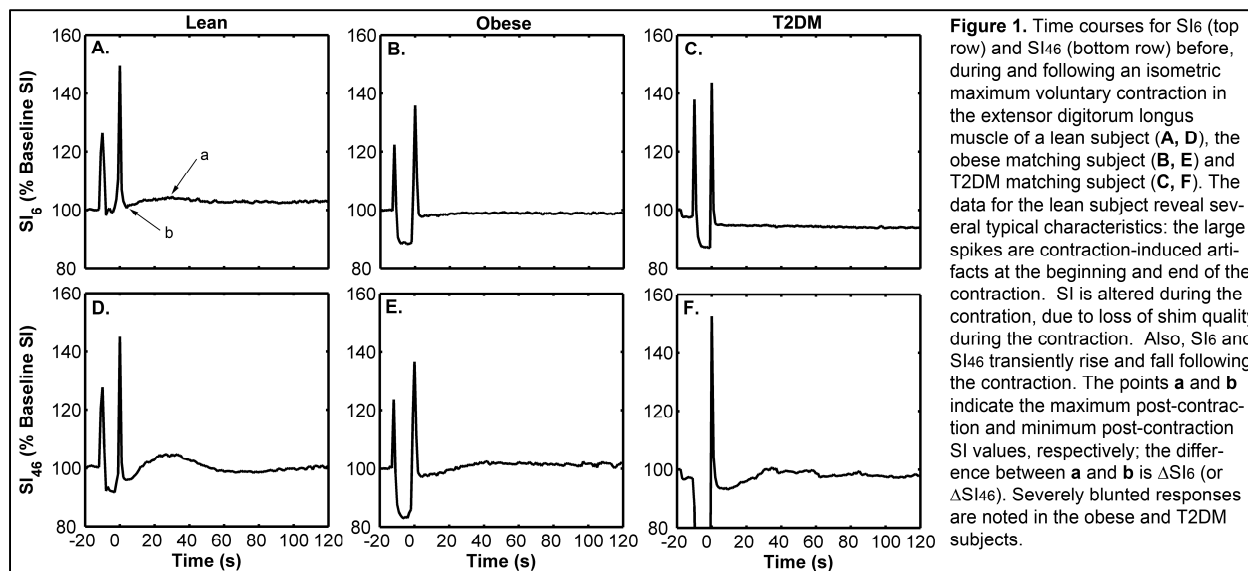
Figure 1. Time courses for SI_6 (top row) and SI_{46} (bottom row) before, during and following an isometric maximum voluntary contraction in the extensor digitorum longus muscle of a lean subject (**A, D**), the obese matching subject (**B, E**) and T2DM matching subject (**C, F**). The data for the lean subject reveal several typical characteristics: the large spikes are contraction-induced artifacts at the beginning and end of the contraction. SI is altered during the contration, due to loss of shim quality during the contraction. Also, SI_6 and SI_{46} transiently rise and fall following the contraction. The points **a** and **b** indicate the maximum post-contraction and minimum post-contraction SI values, respectively; the difference between **a** and **b** is ΔSI_6 (or ΔSI_{46}). Severely blunted responses are noted in the obese and T2DM subjects.

and after 2 and 8 s isometric dorsiflexion contractions. The post-contraction [THb] and ΔSI_6 responses were similar following 2 and 8 contractions. However, the $\%HbO_2$ and ΔSI_{46} responses were larger following 8 s contractions than following 2 s contractions (66). Second, they obtained NIRS and MRI data before, during, and after 5 min of arterial occlusion. [THb] did not change during occlusion; however, $\%HbO_2$ decreased. Following cuff release, [THb] and $\%HbO_2$ increased over baseline. Therefore in both experiments, similar behaviors were observed in the SI_6 and [THb] data and also in the SI_{46} and $\%HbO_2$ data. These experiments demonstrate the correspondences between [THb] and SI_6 and between $\%HbO_2$ and SI_{46}. This study (66), as well as the preceding study by Meyer *et al.* (52), also showed that the magnitude of the SI transients does not vary with TR, supporting the idea that flow-induced changes in the apparent T_1 do not contribute significantly to the SI changes. A final study in the development of the dual-GRE approach established the reproducibility of the protocol (68).

The dual GRE protocol was then used to test for differences in post-contraction blood volume and $\%HbO_2$ responses in groups of obese/T2DM, obese, and lean persons (67). Eight T2DM patients were individually matched by age, gender, and race to non-T2DM persons with similar body mass index (BMI; 6/8 subjects in this "obese" group had BMI>30 kg/m^2) and lean subjects (BMI< 25 kg/m^2). The groups' mean physical activity levels, resting heart rate (RHR), systolic and diastolic blood pressures (SBP and DBP), and ankle-brachial indices (ABI) did not differ significantly. The T2DM subjects had glycosylated Hb (Hb$_{A1C}$)=7.1±0.4%, indicating good diabetic control. ΔSI_6 and ΔSI_{46} were measured from the tibialis andterior (TA) and extensor digitorum longus (EDL) muscles before, during, and after 10 s isometric dorsiflexion

Figure 2. Mean ΔSI_6 (**A**) and ΔSI_{46} (**B**) values in the EDL in lean, obese, and obese/T2DM subjects. *$p<0.05$, lean vs. T2DM; **$p=0.055$, lean vs. obese; #$p<0.05$, MVC vs. 50% MVC. The mean ΔSI_6 values were similar among the groups following 50% MVC contractions, but deficits occurred in the obese and T2DM groups following MVC. The impairments in the obese and T2DM groups were not as great for ΔSI_{46} as they were for ΔSI_6.

contractions performed at 50% and 100% of maximum voluntary contraction (MVC) force.

Figure 1 shows severely attenuated ΔSI_6 and ΔSI_{46} responses in obese and obese/T2DM subjects. Also, note the absence of overshoot of the baseline in the SI_{46} response in these subjects. This reflects a blood flow response so reduced that it failed to cause the normal excess of %HbO$_2$. Figure 2 shows the group-mean data for the 50% MVC and MVC conditions in the EDL; the TA data had similar trends, but fewer among-group differences (67). The post-MVC ΔSI_6 response in the lean group was larger than in the other two groups (Fig. 2A). Across all groups, there were significant or near-significant correlations between the post-MVC ΔSI_6 values in the EDL and: RHR ($r^2=0.171$); Hb$_{A1C}$ ($r^2= 0.172$); and BMI ($r^2=0.387$). Multiple regression analysis revealed BMI to be the only significant predictor of ΔSI_6. The lean group had greater mean post-MVC ΔSI_{46} values than the T2DM group (Fig. 2B).

The ΔSI_6 data reveal small vessel dysfunction that is related to obesity, with no additional effect of well controlled T2DM. The ΔSI_{46} data suggest impaired O$_2$ supply-demand matching in the obese/ T2DM subjects. Finally, the larger effects in the EDL than in the TA indicate that these deficits occur in muscle-specific manners. These conclusions are consistent with the proportionality of a muscle's vascular response to fiber type and capillary density (69), fitness (59), and insulin sensitivity (70). The relationship of ΔSI_6 to RHR suggests that sympathetic tone may be altered in obese subjects, consistent with published reports (5,15,16,29). Attenuated NO signaling (71) or sensitivity may also have caused the ΔSI_6 deficits.

Evidence thus exists to support the idea that differences in the time course and magnitude of the post-contraction BOLD contrast may provide powerful insights into muscle microvascular function. This technique has been used to study physically active subjects with supra-normal muscle vascular function and aging persons and T2DM patients with suspected and diagnosed peripheral vascular complications. Future studies will hopefully continue to address the sensitivity of these measurements to physiological regulators of muscle vascular function.

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