Physical activity-induced remodeling of vasculature in skeletal muscle: role in treatment of type 2 diabetes

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Abstract: This manuscript summarizes/discusses adaptations of skeletal muscle vasculature induced by physical activity and applies this understanding to benefits of exercise in prevention and treatment of type 2 diabetes (T2D). Arteriolar trees of skeletal muscle are heterogeneous. Exercise training increases capillary exchange and blood flow capacities. The distribution of vascular adaptation to different types of exercise training are influenced by muscle fiber type composition and fiber recruitment patterns that produce different modes of exercise. Thus, training-induced adaptations of vascular structure and vascular control in skeletal muscle are not homogeneously distributed throughout skeletal muscle or along the arteriolar tree within a muscle. Results summarized indicate that similar principles apply to vascular adaptation in skeletal muscle in T2D. It is concluded that exercise training induced changes in vascular gene expression differ along the arteriolar tree and by skeletal muscle fiber type composition. Results suggest that it is unlikely that hemodynamic forces are the only exercise-induced signals mediating the regulation of vascular gene expression. In patients with T2D, exercise training is perhaps the most effective treatment of the many related symptoms. Training-induced changes in the vasculature and in insulin signaling in the muscle fibers and vasculature augment glucose and insulin delivery as well as glucose uptake. If these adaptations occur in a sufficient amount of muscle mass, exposure to hyperglycemia and hyperinsulinemia will decrease along with the risk of microvascular complications throughout the body. It is postulated that exercise sessions in programs of sufficient duration, that engage as much skeletal muscle mass as possible and recruit as many muscle fibers within each muscle as possible, will produce the greatest benefit. The added benefit of combined resistance and aerobic training programs and of high intensity exercise programs is not simply “more exercise is better”.
Introduction

The purpose of this manuscript is to summarize and discuss the adaptations of skeletal muscle vasculature induced by physical activity and to apply this understanding to use of exercise in prevention and treatment of type 2 diabetes (T2D). An important concept concerning physical activity-induced adaptation in skeletal muscle is that adaptations do not occur uniformly along the arteriolar tree in skeletal muscle. The top of Figure 1 illustrates the typical branching pattern seen in skeletal muscle, in this case the soleus muscle of the rat. As the feed artery of the soleus (FA in Fig 1) enters the epimysium of the muscle it is designated the 1A arteriole. The branches given off of the 1A arteriole are called the 2A arterioles and the branches given off of the 2A arterioles are called 3A arterioles etc. out 10 to 15 branches to the capillaries. As discussed in detail below, current literature indicates that physical activity does not influence the arterioles of a skeletal muscle arteriolar tree uniformly. This is illustrated in Figure 2 where decreased physical activity produced by hindlimb unloading resulted in decreased dilation in response to acetylcholine in the soleus FA and 1A arteriole but not in the 2A arterioles. As we will discuss below, an additional source of non-uniformity of adaptation is that the effects of changes in physical activity are also not the same between muscles. For example, in Figure 1 effects of exercise training on the 2A arteriole of the soleus may not be observed in the 2A arterioles of the red or white portion of the gastrocnemius muscle. The key concept then is that within skeletal muscle arteriolar networks the relative vasomotor responsiveness of each segment is tuned, through control of vascular cell gene expression, to the predominant signals present that control vascular structure and smooth muscle tone in those arterioles and resistance arteries. Current literature indicates that exercise training shifts these control factors and signaling mechanisms in vascular cells in a non-uniform pattern throughout the arteriolar tree in a muscle
and that the signals differ with fiber type composition and recruitment among muscles. The goal of the discussion below is to focus on recent work related to exercise-induced adaptations in skeletal muscle arteriolar trees of T2D subjects, but first I review work in normal subjects to provide a foundation of understanding vascular adaptation in skeletal muscle as established by data in the current literature.

**Exercise Training Increases Maximal Oxygen Consumption:** Dr. Edward F. Adolph, who according to the APS web site “…is best known for his research in environmental physiology, particularly in adaptation in hot and cold environments”, defined adaptation as: “Adaptations are modifications of organisms that occur in the presence of particular environments or circumstances. Physiological adaptations appear within the single individual, and constitute changes in its functions. The term ‘adaptations’ as here used includes phenomena sometimes labeled acclimatization and/or acclimations.” (4). One key adaptation induced by chronic physical activity/exercise training is an increase in the maximal ability of an animal to consume oxygen (maximal oxygen consumption)(45, 127, 131).

The maximal oxygen consumption of an animal or person is best determined by measuring oxygen consumption with increasing exercise intensity (45, 113, 114, 131). Oxygen consumption increases with increasing exercise intensity until maximal is reached. At this point, oxygen consumption no longer increases with increasing exercise intensity. Prolonged aerobic exercise training can increase an individual’s maximal oxygen consumption while the relationship between oxygen consumption and exercise intensity, below maximal oxygen consumption, is largely unaffected by training. There is substantial literature available relating to where along the “pathway for oxygen” between outside air and skeletal muscle mitochondria, maximal oxygen consumption is determined or limited (127, 133). In disease states there are
examples of each step in the transport pathway providing a limit to maximal oxygen consumption (113, 114). That is, maximal oxygen consumption can be limited by the ability of the respiratory system to maintain alveolar oxygen levels, limited maximal cardiac output, limited capacity of the blood to carry oxygen (i.e. anemia), limited skeletal muscle blood flow capacity, limited skeletal muscle capillary exchange capacity, and/or limited skeletal muscle oxidative capacity (127, 132, 133). It appears that the step that is the major limitation for oxygen transport may differ among normal mammals. In all animals examined, exercise training-induced increases in maximal oxygen consumption could result from increases in any one of these steps in the pathway for oxygen and/or from increased oxidative capacity of the skeletal muscle (45, 127, 131, 133). Over the years our research has been focused on the exercise training induced adaptations in the skeletal muscle vasculature that increase skeletal muscle blood flow capacity and capillary exchange capacity and thereby contribute to increased maximal oxygen consumption.

**Exercise training increases skeletal muscle vascular transport capacity; both capillary exchange capacity and blood flow capacity are increased:** Convective transfer of blood to (blood flow) and distribution of blood flow among skeletal muscle capillaries represents the first step in the process of transport in skeletal muscle vasculature as it brings nutrients into the exchange vessels. Diffusional transcapillary exchange from blood to tissue is the final step in oxygen transport from air to muscle mitochondria. Current literature establishes that exercise training increases skeletal muscle blood flow capacity in male and female, young and old humans (82, 124) and both capillary exchange capacity and blood flow capacity in various other mammals (66, 67). Transcapillary exchange in microvascular exchange vessels and delivery of matched blood flow are equally important to the supply of oxygen for muscle tissue. Studies in
several animal models reveal that training increases skeletal muscle capillary diffusion capacity (63, 111, 121, 123). In addition to these findings from nonhuman species, Roca et al. (112) reported that 9 weeks of endurance exercise training of human subjects increased oxygen-diffusing capacity in skeletal muscle by 33.5% during maximal exercise. Roca et al. (111) used the ratio of maximal oxygen consumption divided by mean capillary PO2 as an index of tissue diffusion capacity and determined that exercise training reduces diffusional limitations to oxygen transport. Thus, tissue diffusion capacities in exercise trained individuals who have higher maximal oxygen consumptions were nearly twice values of sedentary individuals (111). Our results from exercise trained rodents indicate that exercise training increases capillary exchange capacity in that both capillary filtration coefficient for water, and capillary diffusion capacity for lipid insoluble substances are increased (68, 74, 121, 122).

Skeletal muscle consists of three general phenotypes of muscle fibers classified according to both their contractile and metabolic properties (115); Slow-twitch oxidative (SO), fast-twitch, glycolytic (FG), and fast-twitch, oxidative, glycolytic (FOG). Skeletal muscle fiber type composition has a major influence on vascularization, capillary exchange capacity, vascular structure, and mechanisms of vasomotor control within and among muscles (5-9, 12, 48, 65-67). We have examined adaptation of vascular transport capacity in rat skeletal muscle using 3 training programs that we hypothesized would induce different spatial patterns of adaptations within and among skeletal muscles: low-intensity training (1 hr/day, 5 d/wk, for 15 wks at a treadmill speed of 30 m/min, 0 incline) (8, 74), interval sprint training (6 bouts of 60 m/min up a 15 % incline for 2.5 min each followed by 4.5 min rest, 5 d/wk, for 9 wks) (68, 121) and high-intensity endurance training (32 m/min, 15 % incline, 90 min/day, 5 d/wk, 12 wks)(122). We evaluated vascular transport capacity in these models of exercise training with isolated perfused
hindquarters and conscious rats running on the treadmill. Capillary exchange capacity and total and regional blood flows were measured in isolated perfused hindquarters (74, 121, 122) and total and regional skeletal muscle blood flows were measured in rats during treadmill exercise (8, 64, 68). Capillary exchange capacity was measured with two techniques: 1) The single-injection indicator dilution technique to measure the capillary diffusion capacity for $^{51}$Cr-labeled EDTA, and 2) Gravimetric techniques to measure capillary filtration coefficients, isogravimetric capillary pressures, and pre- and post-capillary resistances (121). Low-intensity training resulted in: 1) increased blood flow capacity, primarily in the red high-oxidative muscle tissue, 2) no significant changes in maximal capillary filtration coefficients or isogravimetric capillary pressures, and 3) increased capillary diffusion capacity for EDTA (67). We conclude that low-intensity training increased blood flow capacity specifically in the skeletal muscle tissue that had the greatest relative increase in activity during training bouts. Results of the interval sprint training study demonstrate that blood flow capacity is increased and that primary changes in blood flow capacity occur in fast-twitch glycolytic (FOG) muscle tissue (68, 121). These results agree with those of Mackie and Terjung who used a similar training program (79). As shown in Figure 3, we also found that high intensity exercise trained rats had increased maximal capillary filtration coefficients, normal isogravimetric capillary pressures, and decreased pre- and post-capillary resistances (121). These physiologic results indicate that sprint training alters all 3 components of the microvascular tree: pre-capillary resistance vessels, post-capillary resistance vessels and exchange vessels.

We also examined high-intensity endurance training to further test the hypothesis of regional specificity of training-induced adaptations. We reasoned that if this hypothesis was correct, this training program would produce increases in vascular transport capacity throughout the extensor...
muscles. This seemed appropriate because all fibers in extensor muscles should have increased activity at some point during training bouts of this intensity and duration (35). Results demonstrate that high-intensity endurance training produced increases in blood flow capacity of total hindquarters, increases in maximal capillary filtration coefficients, normal isogravimetric capillary pressures, and decreases in pre- and post-capillary resistances (Fig 3)(121). Our hypothesis also predicted that blood flow capacity would increase in muscles of all fiber type composition. In contrast, regional blood flow data indicated that the primary changes in blood flow capacity occurred in mixed muscle tissue as the relative training-induced change in blood flow capacity was linearly related to the % FOG composition of the muscles (123).

Thus, overall results from our studies of regional blood flow capacity indicate that exercise training-induced adaptations are localized to the areas within skeletal muscles that have the greatest relative increase in muscle fiber activity during exercise training bouts (66, 67). This specificity of training-induced changes suggests that adaptations of muscle oxidative capacity and blood flow capacity are linked in skeletal muscle (3).

Optimal capillary exchange requires that regional capillary perfusion be matched to exchange capacity. A recent review by Poole et al. (100) clearly summarizes how important it is to recognize that most capillaries in resting skeletal muscle have moving red blood cells (RBCs) and that during exercise the increased in RBC flux is the result of a number of changes including increased RBC velocity, capillary hematocrit and altered flow path through the capillaries that increases the “effectiveness” of the exchange area of the capillaries. More recent observations from this group confirm that exercise training increases capillary diffusion capacity through a number of adaptations in the microcirculation that improve matching of oxygen transport and oxygen utilization (46, 47). These exercise training-induced adaptations cause improved muscle


microvascular partial pressure of oxygen (PO$_2$mv) kinetics in normal active muscle partially through increased NO-mediated dilation (46). Of interest, in animals with congestive heart failure, where increased non-RBC flowing capillaries compromise oxygen transport, exercise training has similar effects on muscle PO$_2$mv kinetics but the adaptation does not appear to be NO-mediated (47).

There is growing appreciation of the importance of heterogeneities of blood flow and oxygen uptake in skeletal muscle because it is clear that if blood flow and capillary exchange area are not matched, oxygen transport will be limited due to poor diffusion (56, 66, 67, 69). Importantly, there is solid evidence that these heterogeneities are conserved across humans and animals (55, 101). We must keep in mind that variations in capillary blood flow not matched to exchange area can produce limitation of oxygen transport (108, 109). However, the presence of microvascular blood flow heterogeneity is not necessarily a sign of poor vascular function (56, 69). Indeed, because oxygen consumption of muscle tissue is not uniform during exercise, a heterogeneous distribution of blood flow is the most efficient mechanism to fulfill oxygen transport to a heterogeneous mass of muscle. Also, exercise training induces vascular adaptation heterogeneously within and among skeletal muscles (66, 67). This non-uniform vascular adaption in skeletal muscle results from a number of mechanisms including the heterogeneity of fiber type composition of skeletal muscle and the heterogeneous muscle fiber recruitment patterns generated by different modes and intensities of exercise. Also, as discussed below, muscle fiber recruitment patterns during exercise have a major influence on the regional distribution of adaptations in vascularization, capillary exchange capacity, vascular structure, mechanisms of vasomotor control and regional distribution of blood flow within and among muscles during exercise (12, 48, 65-67, 83). Importantly, relationships among muscle fiber type,
recruitment patterns and blood flow are altered by exercise training (8, 68, 123) through changes in vascular structure as well as functional changes in endothelium (31-33, 57, 66, 67, 73, 84, 85, 93, 98, 120, 130, 134) and vascular smooth muscle (VSM) of skeletal muscle arteries and arterioles (14-21, 59-62, 66, 67, 70, 92, 128, 129). Our results in rodents indicate that, although many exercise training-induced vascular adaptations are concentrated in the muscle tissue having the greatest increase in activity during training sessions (11, 25, 75, 114) (8, 42, 43, 68, 78, 121, 123), the relative amount of adaptation is not distributed uniformly for any of these parameters (66, 75) and these adaptations are not the same for different types of exercise training (8, 66-68, 94, 123). Thus, different intensities and types of exercise activities require different fiber recruitment patterns, which subsequently influence the spatial distribution of exercise training-induced adaptations of muscle fibers and the associated microvasculature.

**Exercise training-induced structural vascular adaptation in skeletal muscle:** Exercise training induces vascular adaptation in skeletal muscle through two general types of adaptation or mechanisms; 1) structural vascular adaptation (angiogenesis of capillaries, remodeling and enlargement of arteries and arterioles and arteriogenesis) and 2) adaptation of vascular function i.e. altered control of vascular resistance (66, 67). As shown in Figure 3, endurance training produced increases in blood flow capacity in FOG (GR) and SO (Soleus) muscle while interval sprint training (IST) produced increases in blood flow capacity throughout the gastrocnemius muscle, not the soleus, and these changes in blood flow capacity seemed to be linked with changes in muscle oxidative capacity. In contrast, both types of training produced increased capillary density in the white (GW) and red portions (GR) of the gastrocnemius muscle (Figure 3), not the soleus. Lastly, arteriolar density was increased throughout the gastrocnemius muscle but not altered in the soleus by endurance training (ET in Fig 3). Endurance training (ET in Fig 3)
increased capillary and arteriolar densities in Gw but blood flow capacity was not altered. On the other hand, endurance training increased blood flow capacity of soleus but did not alter capillary or arteriolar density. Thus, as shown in Figure 3 neither changes in capillary density nor arteriolar density explain the increases in skeletal muscle blood flow capacity. Our work has revealed that it is important to think of the arteriolar networks within the skeletal muscle, not just arteriolar density. Further, model analysis indicates that changes in the arteriolar network of the gastrocnemius muscle induced by interval sprint training could explain the increases in blood flow capacity we measured in this muscle of interval sprint trained rats (12). So the results summarized above and the results of others indicate that adaptations in structure of the vascular bed of skeletal muscle induced by exercise training include increased capillary density, arteriolar density (network analysis indicates that this is focused in larger arterioles)(12), and that these adaptations are not homogeneous within or among skeletal muscles. Muscle fiber type composition and muscle fiber recruitment patterns influence the spatial distribution of adaptations in capillary and arteriolar density. Importantly structural adaptation of the skeletal muscle arteriolar networks only partially explains increases in blood flow capacity (75) suggesting that exercise training induces an additional adaptation which is suggested to be vasomotor reactivity, another determinant of vascular resistance and control of blood flow.

Available evidence suggests that exercise training increases capillary exchange capacity and/or microvascular oxygenation by adaptations beyond structural increases in capillary density/angiogenesis (66, 67). Thus, Hirai and colleagues (46, 47) have shown that exercise training can reverse the derangements in skeletal muscle oxygen transport resulting in greater peak oxygen uptake in skeletal muscle of rats with congestive heart failure. The improvement of oxygen transport is the result of modification of spatial and temporal heterogeneities of blood
flow and oxygen uptake that are remodeled by exercise training. Improved understanding of the
matching of oxygen transport (convective and diffusive) to oxygen consumption of the muscle is
an important addition to our understanding of mechanisms of vascular adaptation in skeletal
muscle (37, 44, 100). The improved matching of blood flow distribution to capillary exchange
capacity and to regional muscle oxygen consumption after exercise training appears to result in
better microvascular oxygenation in contracting skeletal muscle, and suggests that exercise
training induces changes in vascular control.

To summarize, in normal mammals exercise training increases vascular transport capacity
(total and regional blood flow capacity and capillary exchange capacity) and causes structural
vascular adaptation in skeletal muscle. Exercise training-induced increases in regional blood
flow capacity are associated with increased capillary density and increased arteriolar density.

Importantly, results indicate that vascular adaptations are not homogeneous throughout
skeletal muscle or along the arteriolar tree. Equally important, results indicate that muscle
fiber type composition and muscle fiber recruitment patterns, that produce the different
exercise performances, influence the spatial distribution of adaptations in vascular structure.

As the increases in vascular transport capacity cannot be explained from measured changes in
vascular structure our discussion will next examine the novel hypothesis that adaptations in the
total and/or regional control of vascular resistance in skeletal muscle contribute to the increases
in transport capacity.

**Exercise training-induced adaptation of control of vascular resistance:** There is a vast array of
mechanisms believed to be involved in the control of vascular resistance and responsible for
exercise hyperemia in striated muscle which may be adapted by exercise training (66, 67). Our
approach to testing the hypothesis that exercise training alters control of blood flow and vascular
resistance in skeletal muscle vascular beds has been to evaluate the hypothesis that exercise
training induces adaptive changes in the function of endothelial cells and/or smooth muscle of
resistance arteries and/or arterioles in skeletal muscle (50). To allow separation of effects of
skeletal muscle fiber metabolism and neuro-humeral control factors we examined vasomotor
function of cannulated arterioles and resistance arteries isolated from skeletal muscle of
sedentary and trained animals (1, 2, 49, 50, 76, 83, 117, 118, 136, 137). In a major resistance
artery of the soleus muscle, the soleus feed artery (FA), we were initially surprised to discover
that endurance exercise training did not alter endothelium-dependent dilation (EDD) induced by
acetylcholine or the EDD signaled with intraluminal flow (flow-induced dilation) (50). Jasperse
proposed that soleus FAs do not adapt to endurance exercise because soleus blood flow is only
modestly increased during endurance exercise training bouts (51). Specifically, the soleus
resistance vasculature is already adapted because the soleus muscle has a high level of activity in
just maintaining posture even in untrained subjects so endurance exercise does not generate a
sufficient signal to induce adaptations in the soleus or its arteriolar tree. Jasperse further
proposed that if this concept were correct then decreased activity of the soleus muscle caused by
chronic hindlimb unloading would result in decreased EDD in the soleus FA (50). As shown in
Fig 2, results of his experiments supported this hypothesis and further demonstrated that
endothelial nitric oxide synthase (eNOS) expression was decreased in the soleus FA of hindlimb
unloaded rats. Similar changes in vasomotor function and eNOS expression were found in
soleus 1A arterioles of hindlimb unloaded rats (117). In contrast, soleus 2A arterioles did not
exhibit adaptation of EDD behaviour or eNOS expression in response to hindlimb unloading
(117, 118). It is also of interest that hindlimb unloading decreased the response of vascular
smooth muscle to the vasodilator actions of adenosine in the gastrocnemius 1A arteriole but did
not alter the response of soleus 1A arterioles to adenosine (87).

We used a similar strategy for the study of the arteriolar network of the gastrocnemius muscle. I will not summarize all of the data here but suffice it to say that we found that exercise training-induced functional vascular adaptation differed along the arteriolar tree within skeletal muscle as well as between skeletal muscles of the same animal (76, 83). As illustrated in Figure 4 A & B, endurance exercise training produced changes in eNOS expression along the arteriolar tree of the gastrocnemius muscle that were different from the changes induced by interval sprint training and, as mentioned above, neither endurance or sprint training altered arteriolar eNOS expression (Fig 4 C&D) or vasomotor function of soleus arterioles.

Aging-associated reductions in EDD have been shown to be different in skeletal muscles of differing fiber type composition in that Ach-induced EDD is blunted in arterioles and feed arteries from highly oxidative muscle but not from low oxidative muscle whereas, in contrast, flow-induced EDD is impaired in both gastrocnemius and soleus muscle arterioles (91, 125, 126). Metabolic syndrome/T2D and heart failure have also been reported to result in muscle specific decreases in EDD and other changes in skeletal muscle vascular structure/function that differ along the arteriolar tree within skeletal muscle as well as between different muscles (80, 81, 89, 99). In these disease states, exercise training has been shown to reverse blunted EDD (81, 99) and restore vascular structure as well as restore myogenic constrictor responses in skeletal muscle arterioles (40).

The fact that exercise training-induced functional vascular adaptations differ along the arteriolar tree within skeletal muscle is interesting given the intrinsic variation in functional phenotype of vascular cells seen along the arteriolar vascular tree (30). These observations indicate that within the arteriolar network the relative responsiveness of each segment is tuned to
the predominant signals present that control smooth muscle tone in those arterioles and resistance arteries. Available results indicate that exercise training shifts these control factors and signaling mechanisms in vascular cells in a non-uniform pattern throughout the arteriolar tree. As a result there does not appear to be any one branch arteriole that can be sampled to reflect the adaptive changes induced by exercise training throughout the arteriolar network of any skeletal muscle so far examined. Analysis of what is known about exercise training-induced adaptations in vasomotor function indicates that the non-uniform adaptations along the arteriolar tree are the result of differential adaptation of gene expression in vascular cells.

**Do these concepts relating to interactions of exercise, adaptations of vascular structure and function in striated muscle apply in type 2 diabetes (T2D)?** The importance of this question is established by the approximately 30 million Americans, 20 years and older, who have diabetes (nearly 95 % T2D) and it is estimated an additional 86 million Americans, 20 years and older, have pre-diabetes (116). Diabetes is associated with micro- and macro-vascular complications resulting in adult onset blindness, end-stage renal failure and non-traumatic limb amputation as well as coronary artery disease, stroke and peripheral vascular disease (22, 29, 44, 95, 110). Inactivity is a recognized risk factor involved in the development of T2D (110) and participation in no or insufficient physical activity is associated with an increased incidence of cardiovascular events, microvascular complications and all-cause mortality (13) and a decreased aerobic capacity is associated with the presence of neuropathy, retinopathy or nephropathy among patients with T2D(36).

There is evidence that the beneficial effects of exercise training are at least partially related to changes in skeletal muscle vascular beds. For example, T2D is associated with attenuated skeletal muscle blood flow responses to exercise as well as glucose intolerance (54, 135). Studies
performed in humans with insulin resistance or T2D and in animal models of disease demonstrate that exercise training mediates local and systemic improvements in endothelial (10, 27, 28, 80, 81, 88-90) and smooth muscle function (10, 27, 81) indicated by improved vasodilator signaling. Exercise training also appears to attenuate capillary rarefaction, in skeletal muscle, associated with insulin resistance (26, 39, 77, 99, 102). Therefore, collectively these exercise training-induced adaptations may play important roles in optimal treatment of T2D.

Using the same strategy outlined above for normal rats, we examined the functional vascular adaptations along the arteriolar tree of skeletal muscle of differing fiber type in Otsuka Long Evans Tokushima Fatty (OLETF) rats. OLETF rats are hyperphagic and develop obesity and T2D and they have been established as a model of obesity and T2D (103-107). We focused on vasomotor function because recent results make it clear that vascular cells are insulin resistant in T2D subjects and that insulin can contribute importantly to control of blood flow in muscle by signaling to vascular cells (24). In our first experiment with this animal model we found dysfunction of endothelial dependent vasomotor reactivity in the feed artery of gastrocnemius, but not soleus FAs of OLETF rats (Fig 5, top) (10). When OLETF rats were physically active using voluntary running wheels in their cages we found that the endothelial dysfunction of the gastrocnemius feed arteries was prevented and, as has been true in normal animals, wheel running exercise did not alter soleus feed artery vasomotor function(10). The improved endothelial function in gastrocnemius FA was associated with increased phospho-eNOS (10).

There is now a substantial body of evidence that T2D is associated with decreased endothelium dependent dilation (EDD) and vascular rarefaction in skeletal muscle microcirculation (54, 58, 81, 88, 89, 110), which suggests that exercise training restores both vascular structure and function through reversing the effects of T2D on EDD and perhaps due to changes in NO.
availability.

In our most recent studies we isolated feed arteries and arterioles from the soleus and gastrocnemius muscles of OLETF rats after two different exercise training programs. We studied three groups of OLETF rats: endurance exercise trained (EX; n=13), interval sprint trained (SPRINT; n=14), and sedentary (Sed; n=12). The experiments were designed to measure vasomotor function in GFA and of 2A arterioles isolated from the red (RG2a; G2A-R in Fig 6) and white (WG2a; G2A-W in Fig 6) portion of the gastrocnemius muscle and the soleus FA (81). Importantly, we demonstrated that EDD is blunted by T2D differentially in muscle with different muscle fiber type composition and exercise training restores EDD in a fiber type dependent manner (10, 81, 88, 89) as exercise training improves EDD non-uniformly in the arterial tree of skeletal muscle (10, 52, 81, 88, 89). As shown in Figure 6 both SPRINT and EX produced an increase in ACH-induced EDD in the gastrocnemius FA and the RG2a but only EX improved vasodilation of the WG2a. Neither training program altered responses of the soleus FA. As shown in Figure 7 insulin produced vasodilation of the RG2a in EX animals only. When ET-1 receptors were blocked with tezosentan RG2a’s from all three groups exhibited vasodilation to insulin. Similar results were seen in the WG2a. Thus, these results led us to conclude the EDD is blunted in T2D skeletal muscle arterioles in a muscle fiber type dependent manner. EX and SPRINT increased EDD in some arterioles but not all. Results also indicate that insulin signaling in arteriolar endothelium differs among types of skeletal muscle and among different branch orders in skeletal muscle of arteriolar trees (81, 88, 89). Also, EX improved insulin-induced EDD non-uniformly in the arterial tree of skeletal muscle. Thus, results indicate that the blunting of EDD induced by T2D differs with muscle fiber type composition of skeletal muscle and that different exercise training programs reverse this dysfunction differently in arterioles
from skeletal muscles of differing fiber type composition (10, 81, 88). Indeed, it is striking that exercise training improves EDD non-uniformly even within the arteriolar tree of a given muscle, the gastrocnemius (81).

Applying techniques described previously (53, 97) we determined transcriptional profiles for samples of arterioles/arteries from the same rats we used for the function experiments described above. We harvested aorta, iliac, gastrocnemius FA, G1a, RG2a, RG3a, WG2a, WG3a, soleus FA, S1a, S2a, S3a, diaphragm feed artery (DFA) diaphragm 1a (D1a), D2a, and D3a for isolation of total RNA for RNA sequence analysis of gene expression (53, 96) from the same rats as used by Martin et al. (81) for the vasomotor function experiments. We used transcriptome-wide RNA sequencing (RNA-Seq) analysis to provide improved understanding of the molecular events involved in exercise-training induced skeletal muscle vascular adaptations in rats with obesity and T2D. One group of OLETF rats underwent an endurance exercise training program (EX), a second group underwent an interval sprint training program (SPRINT), and a third group was restricted to cage activity (Sed). Our hypothesis was that the greatest effects of exercise training on the transcriptome would be in the gastrocnemius arterioles compared with soleus arterioles. Furthermore we reasoned that SPRINT would produce greater vascular transcriptional changes compared to EX in arterioles isolated from white gastrocnemius muscle because of greater increases in skeletal muscle fiber recruitment of this muscle during sprinting (7, 8, 10, 38, 64, 81, 83, 86, 88, 89). We analyzed arterioles of similar branch order (Feed artery through 3rd order arteriole) from both muscles from the same rats used for the vasomotor function experiments (81). In the initial analysis of these RNA-Seq results we found that with increasing branch order of arterioles, the number of genes differentially expressed with obesity decreased in the diaphragm whereas we found the opposite pattern of alterations in gene
with obesity in the two limb skeletal muscles (Fig 8)(96). Because we observed nearly opposite
effects of obesity/T2D on vascular gene expression in limb muscles and diaphragm, we proposed
that the effects of exercise training on gene expression in diaphragm arteries/arterioles may also
be different than that seen in the soleus and gastrocnemius muscles. We contrasted the EX- and
SPRINT-induced changes observed in diaphragm arterioles to those seen in soleus and
gastrocnemius. Because soleus blood flow increases during exercise to a similar extent as in
diaphragm, we proposed that exercise training would have similar effects on gene expression in
the arterioles of these two muscles. The basis of this hypothesis was the assumption that shear
stress is a primary signal for the effects of exercise on gene expression in arterioles of both
muscles so if blood flow changes during exercise are similar, then alterations in gene expression
should also be similar.

The results summarized in Fig 8 demonstrate that the number of genes whose expression
was altered by obesity increased with increasing branch order in the arteriolar trees of
gastrocnemius and soleus muscles whereas the opposite effect of branch order was observed in
the diaphragm. This figure also illustrates an important principle; a systemic intervention such
as obesity/T2D can have differential effects on gene expression along/throughout the
arterial/arteriolar tree. Further, these effects may not be the same from one muscle to the next.
Our training study was designed with a translational focus. Because of the cost of doing RNA-
seq on this number of samples/animal we chose to only study the effects of EX and SPRINT on
gene expression of the arterioles in OLETF rats (obese/T2D), not in normal animals. However,
in a subset of arteries we examined the interaction of obesity/T2D and exercise training (97). As
shown in figure 9 there were a small number of genes whose expression was altered by
obesity/T2D whose expression were partially or entirely restored by EX and/or SPRINT training.
Further analysis of results indicated that while EX caused little to no changes in gene expression in the GFA, the S2a and WG2a arterioles exhibited the largest number of changes in gene expression following EX. The effects of SPRINT were much different than EX as we observed substantial changes in SFA and GFA gene expression. Overall, we were surprised that SPRINT caused so few changes in gene expression in the WG2a because our hypothesis predicted the largest changes in these arterioles. The contrasts between SPRINT training induced changes in gene expression among WG2a, RG2a, and GFA can be appreciated by examination of the data summarized in Figure 10. First note that for genes related to contractile proteins and arterial structure that (Actin related, Arterial structure, Myosin related, Tnn related and Tubb related on the left of Fig 10) the RG2a has changes that are nearly the opposite of those in the gastrocnemius FA while the WG2a shows only modest changes in these genes. Similar observations are true of the changes in gene expression produced by EX in the soleus and gastrocnemius arteriolar trees (71). S2a’s and RG2a’s exhibit similar changes in gene expression caused by EX but the magnitude of changes is different among the other arteries/arterioles and which branches of the soleus and gastrocnemius arteriolar trees exhibit the largest changes in gene expression are not the same between EX and SPRINT exercise training (71).

As one might expect from the results shown in Figs 6 & 7, SPRINT training and EX did not induce the same adaptive changes in gene expression among the 2a arterioles of the diaphragm, soleus and gastrocnemius muscles (71, 72). In the three feed arteries, SPRINT training induced similar increased expression of two genes (Wisp2 and Tubb2b) but there were no genes whose expression was altered similarly by EX in all there feed arteries (71, 72).

Conclusions: Based on current literature I draw the following conclusions. First, exercise training induced changes in vascular gene expression differ along the arteriolar tree and
by muscle fiber type composition of the muscle in which the arteriolar tree is located. Second, vascular cell gene expression changes signaled by exercise training appear to be relatively unrelated to the spatial location of skeletal muscle adaptations. Given the complex nature of changes in vascular gene expression reported in obesity/T2D (53, 96) and with EX and SPRINT training (71, 97), it seems unlikely that hemodynamic forces are the only exercise-induced signals mediating the regulation of vascular gene expression. Also, I conclude that neither EX or SPRINT have similar effects on the transcriptome of diaphragm and soleus arteries/arterioles, even though these muscles have similar blood flows at rest and during exercise.

Based on these results we propose that exercise prescription for patients with T2D should be designed to cause adaptations throughout the skeletal musculature (all fiber types) to produce the greatest benefit systemically and on vascular health. Both aerobic training and resistance training have beneficial effects of health and fitness (23). A meta-analysis of randomized controlled trials comparing aerobic training, resistance training and combined aerobic and resistance training revealed that aerobic training was more effective in reducing HbA1c and fasting glucose than was resistance training (119). Further, the analysis indicated that combined aerobic and resistance training interventions are the most efficacious exercise training prescription for improvement of glycemic control and blood lipids (119). Equally important, Gordon et al. (41) concluded that vigorous intensity exercise is associated with more favorable T2D risk profiles and greater insulin sensitivity, in both youth and adults, than is low intensity exercise training (physical activity). We consider that both combined aerobic and resistance training and higher intensity aerobic training may be most advantageous because they induce adaptations in a larger number of muscle fibers than does moderate intensity aerobic training. Moderate intensity training is clearly beneficial for cardiovascular health. While cardiovascular
health is important in T2D, exercise training can also restore metabolic health we think through its ability to induce phenotypic changes in skeletal muscle fibers.

Based on these observations and considerations, we postulate that the exercise training program that engages the most skeletal muscle and the most muscle fibers within each skeletal muscle during training sessions (i.e. greatest increase in fiber recruitment from rest to exercise) will generate the most widespread adaptations leading to greater improvements in microvascular function and insulin sensitivity.
ACKNOWLEDGMENTS

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Figure Legends.

Figure 1. Drawings of arteriolar trees of the soleus muscle and gastrocnemius (medial head) muscles. In both muscles, the feed artery (FA) is the last artery entering the muscle prior to the epimysium and becomes the 1A arteriole under the epimysium and the 1A arteriole gives rise to second branch order 2A arterioles which give rise to the third order arterioles (3A). In the gastrocnemius muscle the RG2A arterioles, which provide blood flow to the red portion of the muscle. WG2a are the 2A arterioles that provide blood to the superficial, white portion of the muscle (top of the gastrocnemius muscle). Redrawn from (71) with permission of the American Physiological Society.

Figure 2. Effects of hindlimb unloading (HLU) on acetylcholine-induced endothelium dependent vasodilation of rat soleus feed arteries and arterioles. * indicates the value is significantly different from Control (CON). Data are from (51) for the feed arteries, (117) for 1A arterioles and (118) for 2A arterioles.

Figure 3. Effects of high intensity exercise training on capillary filtration coefficient (Kf), precapillary resistance (Ra), postcapillary resistance (Rv), and the ration of pre- to postcapillary resistance (R_a/R_v) of rat hindquarters. Values are means ± SE. * Significant difference from control at p < 0.05. Reproduced from (121) with permission of the American Physiological Society.
Figure 4. Figure showing the effects of interval sprint training (IST) (10 wks of 6 training bouts/day, 5 days/wk, with each rat running 60 m/min up a 15% incline for 2.5 min with 4.5 min of rest between bouts) and endurance exercise training (ET) (10 to 12 wks of treadmill running at 30 m/min, 60 min/day, 5 days/wk) on oxidative capacity (cytochrome c concentrations), blood flow capacity, capillary density and arteriolar density in the white (Gw) and red (GR) portions of the gastrocnemius and the soleus muscles of rats. These results demonstrate that training-induced changes in arteriolar density do not explain changes in blood flow capacity. Data expressed as a % increase above respective values of sedentary rats. Results demonstrate that IST increases Gw oxidative capacity and blood flow capacity most and these changes correlated with increases in capillary density and small increases in arteriolar density. Results indicate that ET increased oxidative capacity and blood flow capacity most in the soleus and GR muscle. In contrast, to IST, changes in oxidative and blood flow capacity were not correlated with changes in capillary or arteriolar density in the soleus muscle. Data taken from (34, 42, 43, 65, 68, 74).

Figure from Laughlin et al. (66) with permission of the American Physiological Society.

Figure 5. Figure showing the effects of interval sprint training (IST) (10 wks of 6 training bouts/day, 5 days/wk, with each rat running 60 m/min up a 15% incline for 2.5 min with 4.5 min of rest between bouts) and endurance exercise training (ET) (10 to 12 wks of treadmill running at 30 m/min, 60 min/day, 5 days/wk) on endothelial nitric oxide synthase (eNOS) protein content in arterioles of rat skeletal muscle. Average eNOS protein content in arteries and arterioles of ET and IST rats expressed relative to the eNOS content of paired Sed samples. eNOS protein content was quantified by scanning densitometry with NIH Image software. Values are means ±
SE. A. ET n = 4 groups of pooled vessels each. GFA = gastrocnemius feed artery. G1A = first order; 2A = second order; 3A = third order; 4A = fourth order; 5A = fifth order. * = differences between Sed and ET are significant (P < 0.05); + = significant differences (0.05 < P < 0.10).

Data from McAllister et al. (83) with permission of the American Physiological Society. B. Effects of IST on eNOS protein content of gastrocnemius muscle arteries. GFA are data from 2 sets of IST and Sed animals. Gastrocnemius 1A–5A data are from 4 different groups of Sed and IST rats so means and SE are presented. Each group of Sed and IST animals consisted of 5–10 rats, so there are data from 10 to 30 different rats included. * = IST value is different from Sed, P < 0.05 (by 1-way Student's t-test). Data are from Laughlin et al. (76). C. Effects of ET on eNOS protein content in soleus (S) arterioles. SFA, soleus feed artery. D. SOD-1 protein content in soleus resistance arterioles. Data from McAllister et al. (83) with permission of the American Physiological Society.

Figure 6. Concentration-response curves to acetylcholine and endothelin-1 gastrocnemius feed arteries (GFAs; on the left) and soleus feed arteries (SFAs; on the right). Data are presented as percent maximal dilation (acetylcholine) and percent possible constriction (endothelin-1). Values are means ± SE; sample size in parentheses. Otsuka Long-Evans Tokushima fatty rats that are sedentary (OSED) and Long-Evans Tokushima Otsuka (LSED) rats. *P < 0.05. Data redrawn from Bender et al. 2011 (10) with permission of the American Physiological Society.

Figure 7. Concentration-response curves to acetylcholine (ACh) in gastrocnemius feed arteries (GFAs; A), soleus feed arteries (SFA; B), red gastrocnemius 2A (G2A-R; C) arterioles, and white gastrocnemius 2A (G2A-W; D) from Otsuka Long-Evans Tokushima fatty rats that were
cage confined (Sed), endurance exercise training (EndEx) or interval sprint trained (IST). Data are presented as percent possible dilation. Values are means ± SE. that are sedentary (OSED) and physically active (OPA) are shown. * = Sed vs. EndEx (P < 0.0167). φ = Sed vs. IST (P < 0.0167). δ = EndEx vs. IST (P < 0.0167). Data redrawn from Martin et al. 2012 (81) with permission of the American Physiological Society.

Figure 8. Vascular reactivity to insulin alone (left) and to insulin in the presence of the nonselective endothelin-1 receptor antagonist tezosentan (right) for red gastrocnemius 2 A arterioles (top; G2A-Red and RG2a) and white gastrocnemius 2A arterioles (bottom; WG2a) across all doses of insulin in 32-wk-old Sed, EndEx, and IST OLETF rats. Values are expressed as mean percent possible dilation ± SE. Within each group and vessel, n = 9–12. δ EndEx vs. IST (P < 0.0167). Data redrawn from Martin et al. 2012 (81) with permission of the American Physiological Society.

Figure 9. Illustration showing the effects of obesity on gene expression in 15 arteries. GFA = gastrocnemius feed artery; G1A = gastrocnemius 1st branch order arteriole; WG2A and WG3A = white gastrocnemius 2nd and 3rd branch order arteriole; RG2A and RG3A = red gastrocnemius 2nd and 3rd branch order arterioles; SFA = soleus feed artery; S1A, S2A, S3A = soleus 1st, 2nd, and 3rd branch order arteriole; DFA = diaphragm feed artery; D1A, D2A, and D3A = diaphragm 1st, 2nd, and 3rd branch order arteriole. Figure reproduced from Padilla et al. (96) with permission of the American Physiological Society.
Figure 10. Figure showing the numbers of genes whose expression was altered by obesity that were partially or entirely restored by exercise training. Genes whose expression was significantly changed by hyperphagia-induced obesity were obtained from Jenkins et al. (53). Figure reproduced from Padilla et al. (97) with permission of the American Physiological Society.

Figure 11. Illustration of the effects of interval sprint training (10 wks of 6 training bouts/day, 5 days/wk, with each rat running 60 m/min up a 15% incline for 2.5 min with 4.5 min of rest between bouts) on gene expression in gastrocnemius and soleus arterioles of OLETF rats. Bar graphs present changes in gene expression as fold change in gene expression relative to expression level of OLETF rats confined to cage activity. Categories of genes are listed across the top of the figure and the specific genes are listed across the bottom of the figure. From top to bottom results are presented for the WG2a = white gastrocnemius 2nd a branch order arteriole; RG2a = red gastrocnemius 2nd branch order arteriole; GFA = gastrocnemius feed artery; S2a = soleus 2\textsuperscript{nd} branch order arteriole; and SFA = soleus feed artery. Notice that for actin related, myosin related and troponin (Tnn) genes of the GFA (middle panel) how gene expression is increased whereas expression of these same genes is decreased in S2a and RG2a and relatively unchanged in the WG2a and SFA. Figure reproduced from Laughlin et al. (71) with permission of the American Physiological Society.


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Feed Arteries

Schrage et al. *JAP*, 1999

1A Arterioles

Schrage et al. *JAP* 89, 2000

2A Arterioles

Schrage et al. *JAP*, 2002

![Graphs showing the response of different arterial types to acetylcholine](image-url)