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

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Submitted 16 September 2015; accepted in final form 8 October 2015

Laughlin MH. Physical activity-induced remodeling of vasculature in skeletal muscle: role in treatment of type 2 diabetes. J Appl Physiol 120: 1–16, 2016. First published October 15, 2015; doi:10.1152/japplphysiol.00789.2015.—This manuscript summarizes and 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 in 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 that 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”.

angiogenesis; arteriogenesis; capillary; diabetes; muscle fibers

THE PURPOSE OF THIS MANUSCRIPT is to summarize and discuss the adaptations to skeletal muscle vasculature induced by physical activity and to apply this understanding to the 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. Figure 1 (top) illustrates the typical branching pattern observed in skeletal muscle, in this case the soleus muscle of the rat. As the feed artery (FA) of the soleus (Fig. 1) enters the epimysium of the muscle it is designated the 1A arteriole. The 1A arteriole branches off into 2A arterioles, and 2A arterioles branch off into 3A arterioles, and so on, out to 10 to 15 branches to the capillaries. As discussed in detail below, current literature indicates that physical activity does not uniformly influence the arterioles of a skeletal muscle arteriolar tree. This is illustrated in Figure 2 where decreased physical activity produced by hindlimb unloading resulted in decreased dilation in response to ACh in the soleus FA and 1A arteriole but not in the 2A arterioles. As is discussed below, an additional source of nonuniformity of adaptation is that the effects of changes in physical activity are also not the same between muscles. For example, in Fig. 1, effects of exercise training on the 2A arterioles of the soleus may not be observed in the 2A arterioles of the red or white portions of the gastrocnemius muscle (Gw, respectively). 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 nonuniform pattern throughout the arteriolar tree in a muscle and that the signals differ with fiber type composition and recruitment

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http://www.jappl.org 8750-7587/16 Copyright © 2016 the American Physiological Society
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 subjects with T2D, but first we will review research 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 American Physiological Society web site “. . . is best known for his research in environmental physiology, particularly in adaptation in hot and cold environments” defined adaptations as “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 maximum 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, although the relationship between oxygen consumption and exercise intensity below maximal oxygen consumption is largely unaffected by training. Substantial literature is available that examines where along the “pathway for oxygen” maximal oxygen consumption is determined or limited between outside air and skeletal muscle mitochondria (127, 133). There are examples of each step in the transport pathway in various disease states that provide 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, by 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, limited skeletal muscle oxidative capacity, or a combination of these (127, 132, 133). It appears that the step that constitutes 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 focused on exercise training-induced adaptations in the skeletal muscle vasculature that increase skeletal muscle blood flow capac-

Fig. 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; the 1A arteriole gives rise to second-order 2A arterioles, which give rise to the third-order arterioles (3A). In the gastrocnemius muscle, RG2A are the 2A arterioles that provide blood flow to the red portion of the muscle and WG2a are the 2A arterioles that provide blood to the superficial, white portion of the muscle (top of the gastrocnemius muscle). [Published with permission (71).]
ity 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 because 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 both young and old men and women (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 wk 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 the values of sedentary individuals (111). Our results from exercise-trained rodents indicate that exercise training increases capillary exchange capacity because 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; fast-twitch glycolytic; 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 three training programs that we hypothesized would induce different spatial patterns of adaptation among skeletal muscles: low-intensity training (1 h/day, 5 days/wk, for 15 wk 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 days/wk, for 9 wks) (68, 121); and high-intensity endurance training (32 m/min, 15% incline, 90 min/day, 5 days/wk, 12 wk) (122). We evaluated vascular transport capacity in these models of exercise training with isolated perfused hindquarters and conscious rats running on a 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 51Cr-labeled EDTA; and 2) gravimetric techniques to measure capillary...
ficient (Kf), precapillary resistance (Ra), postcapillary resistance (Rv), and the pre- and postcapillary 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 FOG muscle tissue (68, 121). These results agree with those reported by Mackie and Terjung (79) who used a similar training program. As shown in Fig. 3, we also found that high-intensity exercise-trained rats had increased maximal capillary filtration coefficients, normal isogravimetric capillary pressures, and decreased pre- and postcapillary resistances (121). These physiologic results indicate that sprint training alters all three components of the microvascular tree: precapillary resistance vessels, postcapillary 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, then 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 postcapillary resistances (Fig. 3) (121). Our hypothesis also predicted that blood flow capacity would increase in muscles of all fiber type compositions. In contrast, regional blood flow data indicated that the primary changes in blood flow capacity occurred in mixed muscle tissue because 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 increase 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 those researchers (46, 47) 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. These exercise training-induced adaptations cause improved muscle microvascular partial pressure of oxygen (PO$_{2mv}$) kinetics in normal active muscle partially through increased nitric oxide (NO)-mediated dilation (46). Of interest, in animals with congestive heart failure when increased non-RBC flowing capillaries compromise oxygen transport, exercise training has similar effects on muscle PO$_{2mv}$ 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

![Fig. 3. Effects of high-intensity exercise training on capillary filtration coefficient ($K_f$), precapillary resistance ($R_a$), postcapillary resistance ($R_v$), and the ratio of pre- to postcapillary resistance ($R_a/R_v$) of rat hindquarters. Values are means ± SE. *Significant difference from control at $P < 0.05$. [Published with permission (121).]](http://jap.physiology.org/)

`J Appl Physiol · doi:10.1152/japplphysiol.00789.2015 · www.jappl.org`
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 nonuniform 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 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 tissues that have the greatest increase in activity during training sessions (8, 11, 25, 42, 43, 68, 75, 78, 114, 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 mechanisms: 1) vascular structural adaptation (angiogenesis of capillaries, remodeling and enlargement of arteries and arterioles and arteriogenesis), and 2) vascular function adaptation (i.e., altered control of vascular resistance) (66, 67). As shown in Fig. 4, endurance training produced increases in blood flow capacity in FOG (the red portion of the gastrocnemius; GR) and soleus muscle, whereas interval sprint training (IST) produced increases in blood flow capacity throughout the gastrocnemius muscle but 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 (GR) portions of the gastrocnemius muscle (Fig. 4), but not in the soleus. Finally, arteriolar density was increased throughout the gastrocnemius muscle but it was not altered in the soleus by endurance training (ET) (Fig. 4). ET resulted in greater capillary and arteriolar densities in GW, but blood flow capacity was not altered. On the other hand, ET increased the blood flow capacity of soleus muscle but it did not alter capillary or arteriolar density. Thus as shown in Fig. 4, 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. Furthermore, model analysis indicates that changes in the arteriolar network of the gastrocnemius muscle induced by IST 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 the structure of the vascular bed of skeletal muscle induced by exercise training include greater capillary density and 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 the increases in blood flow capacity (75), suggesting that exercise training induces an additional adaptation that may 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 in oxygen transport is the result of modification of spatial and temporal heterogeneities of blood flow and oxygen uptake that are remodeled by exercise training. A better understanding of the matching of oxygen transport (convective and diffusive) with oxygen consumption of the muscle is an important addition to our understanding of the mechanisms of vascular adaptation in skeletal muscle (37, 44, 100). The improved matching of blood flow distribution with capillary exchange capacity and with 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 various exercise performances influence the spatial distribution of adaptations in vascular structure. Because 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 IN CONTROL OF VASCULAR RESISTANCE**

A vast array of mechanisms is believed to be involved in the control of vascular resistance and responsible for exercise hyperemia in striated muscle that 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 smooth muscle of resistance arteries and arterioles in skeletal muscle (50). To allow separation of the effects of skeletal muscle fiber metabolism and neurohumeral 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 ACh or the EDD signaled with intraluminal flow (flow-induced dilation) (50). Jasperse et al. (51) proposed that soleus FAs do not adapt to endurance exercise because soleus blood flow is only modestly increased during endurance exercise training bouts. 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 et al. (50) also 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. 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 behavior 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 to study the arteriolar network of the gastrocnemius muscle. The data are not summarized 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 5 A and 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 IST and, as mentioned above, neither ET nor IST altered arteriolar eNOS expression (Fig. 5, C and 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, wherein 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, and to 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 observed 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 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

Fig. 5. Effects of IST (10 wk 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 ET (10 to 12 wk 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 arterioles and arteries of rats in ET and IST groups expressed relative to the eNOS content of paired samples from sedentary (Sed) animals. eNOS protein content was quantified by scanning densitometry with ImageJ (National Institutes of Health) 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). [Published with permission (83).] B: effects of IST on eNOS protein content of gastrocnemius muscle arteries. GFA data are combined from two sets of IST and Sed animals. Gastrocnemius 1A-5A data are from four different groups of Sed and IST rats, thus means and SE are presented. Each group of Sed and IST animals consisted of 5–10 rats, so the data are from 10 to 30 different rats. *IST value is different from that of Sed, P < 0.05 (by one-way Student’s t-test). [Published with permission (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. [Published with permission (83).]
training-induced adaptations in vasomotor function indicates that the nonuniform 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 T2D?**

The importance of this question is established by the ~30 million Americans age 20 yr and older who have diabetes (nearly 95% T2D), and the estimated additional 86 million Americans 20 yr and older who have prediabetes (116). Diabetes is associated with micro- and macrovascular complications resulting in adult-onset blindness, end-stage renal failure, nontraumatic limb amputation, coronary artery disease, stroke, and peripheral vascular disease (22, 29, 44, 95, 110). Inactivity is a recognized risk factor 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 and to 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 (10, 27, 81) function 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 FAs of gastrocnemius, but not soleus FAs of OLETF rats (Fig. 6, top) (10). When OLETF rats were physically active using voluntary running wheels in their cages we found that the endothelial dysfunction of the gastrocnemius FA was prevented and, as has been true in normal animals, wheel running exercise did not alter soleus FA 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 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.

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**Fig. 6. Concentration-response curves to ACh and endothelin-1 GFA (left) and soleus feed arteries (right). Data are presented as percent maximal dilation (ACh) and percent possible constriction (endothelin-1). Values are means ± SE; sample size appears in parentheses. OSED, Otsuka Long-Evans Tokushima Fatty rats that are sedentary; LSED, Long-Evans Tokushima Otsuka rats. *P < 0.05. [Published with permission (10).]**
In our most recent studies we isolated FAs and arterioles from the soleus and gastrocnemius muscles of OLETF rats after two different exercise training programs. We studied OLETF rats assigned to one of three groups: 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 gastrocnemius feed arteries (GFAs) and of 2A arterioles isolated from the red (RG2a; G2A-R in Fig. 7) and white (WG2a; G2A-W in Fig. 7) 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 that exercise training restores EDD in a fiber type-dependent manner (10, 81, 88, 89) because exercise training improves EDD nonuniformly in the arterial tree of skeletal muscle (10, 52, 81, 88, 89). As shown in Figure 7, both SPRINT and EX rats produced an increase in ACh-induced EDD in the GFA and the RG2a, but improved vasodilation of the WG2a occurred only in EX rats. Neither training program altered responses of the soleus FA. As shown in Figure 8, insulin produced vasodilation of the RG2a in EX animals only. When ET-1 receptors were blocked with tezosentan (a nonselective endothelin-1 receptor antagonist), RG2a from all three groups exhibited vasodilation to insulin. Similar results were observed in WG2a. Thus these results led us to conclude that EDD is blunted in T2D skeletal muscle arterioles in a muscle fiber type-dependent manner (10, 81, 88, 89). Indeed, it is striking that exercise training improves EDD nonuniformly 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; GFA, G1a, RG2a, RG3a, WG2a, and WG3a; soleus FA, S1a, S2a, and S3a; and 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 that were used by Martin et al. (81) for vasomotor function experiments. We used transcriptome-wide RNA sequencing (RNA-Seq) analysis to provide a better understanding of the molecular events involved in exercise-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 IST 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 rats in the SPRINT group would produce greater vascular transcriptional changes compared with rats in the EX group in arterioles isolated from G\( _{W} \) 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 (FA through 3A) 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 obesity genes in the two limb skeletal muscles (Fig. 9) (96). Because we observed nearly opposite

Fig. 7. Concentration-response curves to ACh in GFAs (A), soleus feed arterioles (SFA) (B), red gastrocnemius 2A (G2A-R) arterioles (C), and white gastrocnemius 2A (G2A-W) (D) from Otsuka Long-Evans Tokushima Fatty rats that were cage-confinied (Sed), or underwent endurance exercise training (EndEx) or IST. Data are presented as percent possible dilation. Values are means \( \pm \) SE. *Sed vs. EndEx (\( P < 0.0167 \)). \( \delta \)Sed vs. IST (\( P < 0.0167 \)). \( \delta \)EndEx vs. IST (\( P < 0.0167 \)). [Published with permission (81).]
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 arterioles may also be different than that observed in the soleus and gastrocnemius muscles. We contrasted the EX- and SPRINT-induced changes observed in diaphragm arterioles to those observed 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 Figure 9 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. Furthermore, 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 carrying out RNA-Seq on this number of samples per animal, we chose to study only 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 on vascular gene expression in limb muscles and diaphragm, we proposed that the effects of exercise training on gene expression in diaphragm arterioles may also be different than that observed in the soleus and gastrocnemius muscles. We contrasted the EX- and SPRINT-induced changes observed in diaphragm arterioles to those observed 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.

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T2D and exercise training (97). As shown in Figure 10, expression of a small number of genes was altered by obesity/T2D, whereas expression was partially or entirely restored by EX and/or SPRINT training.

Further analysis of results indicated that whereas rats in the EX group exhibited few to no changes in gene expression in the GFA, but there were many gene expression changes in the S2a and WG2a arterioles. The effects of exercise in the SPRINT and EX groups were much different; we observed substantial changes in soleus FA and GFA gene expression. Overall, we were surprised that SPRINT exercise 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 11. First note that for genes related to contractile proteins and arterial structure that (Actin related, Arterial structure, Myosin related, Tnn related, and Tubb related; Fig. 11) RG2a indicates changes that are nearly the opposite of those in GFA, whereas 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 and RG2a 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 Figures 6 and 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). Among the three FAs, 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 FAs (71, 72).

CONCLUSIONS

On the basis of current literature we 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 nor 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.

On the basis of 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 on 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). Furthermore, 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. Although cardiovascular health is important in T2D, exercise training can also restore metabolic health, we believe, through its ability to induce phenotypic changes in skeletal muscle fibers.

On the basis of these observations and considerations, we postulate that an 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
Fig. 11. Illustration of the effects of IST (10 wk 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 changes in gene expression relative to the expression level of OLETF rats confined to cage activity. Categories of genes are listed across the top, specific genes are listed across the bottom. From top to bottom: WG2a, RG2a, GFA, S2a, and SFA. Notice that for Actin related, Myosin related, and troponin (Tnn) genes of the GFA (middle) how gene expression is increased, whereas expression of these same genes is decreased in S2a and RG2a and relatively unchanged in WG2a and SFA. [Published with permission (71).]
most widespread adaptations leading to greater improvements in microvascular function and insulin sensitivity.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant ROI-HL-036088.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.H.L. conception and design of research; M.H.L. performed experiments; M.H.L. analyzed data; M.H.L. interpreted results of experiments; M.H.L. prepared figures; M.H.L. drafted manuscript; M.H.L. edited and revised manuscript; M.H.L. approved final version of manuscript.

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