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Endurance, interval sprint, and resistance exercise training: impact on microvascular dysfunction in type 2 diabetes

T. Dylan Olver¹ and M. Harold Laughlin¹,²
¹Department of Biomedical Sciences, University of Missouri, Columbia, Missouri; ²Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, Missouri

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Olver TD, Laughlin MH. Endurance, interval sprint, and resistance exercise training: impact on microvascular dysfunction in type 2 diabetes. Am J Physiol Heart Circ Physiol 310: H337–H350, 2016. First published September 25, 2015; doi:10.1152/ajpheart.00440.2015.—Type 2 diabetes (T2D) alters capillary hemodynamics, causes capillary rarefaction in skeletal muscle, and alters endothelial and vascular smooth muscle cell phenotype, resulting in impaired vasodilatory responses. These changes contribute to altered blood flow responses to physiological stimuli, such as exercise and insulin secretion. T2D-induced microvascular dysfunction impairs glucose and insulin delivery to skeletal muscle and other tissues such as skin and nervous, thereby reducing glucose uptake and perpetuating hyperglycemia and hyperinsulinemia. In patients with T2D, exercise training (EX) improves microvascular vasodilator and insulin signaling and attenuates capillary rarefaction in skeletal muscle. EX-induced changes subsequently augment glucose and insulin delivery as well as glucose uptake. If these adaptations occur in a sufficient amount of tissue, and skeletal muscle in particular, chronic exposure to hyperglycemia and hyperinsulinemia and the risk of microvascular complications in all vascular beds will decrease. We postulate that EX programs that engage as much skeletal muscle mass as possible and recruit as many muscle fibers within each muscle as possible will generate the greatest improvements in microvascular function, providing that the duration of the stimulus is sufficient. Primary improvements in microvascular function occur in tissues (skeletal muscle primarily) engaged during exercise, and secondary improvements in microvascular function throughout the body may result from improved blood glucose control. We propose that the added benefit of combined resistance and aerobic EX programs and of vigorous intensity EX programs is not simply “more is better.” Rather, we believe the additional benefit is the result of EX-induced adaptations in and around more muscle fibers, resulting in more muscle mass and the associated microvasculature being changed. Thus, to acquire primary and secondary improvements in microvascular function and improved blood glucose control, EX programs should involve upper and lower body exercise and modulate intensity to augment skeletal muscle fiber recruitment. Under conditions of limited mobility, it may be necessary to train skeletal muscle groups separately to maximize whole body skeletal muscle fiber recruitment.

endurance; interval and resistance exercise training; type 2 diabetes; microvascular

IN 2015, ~30 million Americans, 20 years and older, have diabetes. Furthermore, it is estimated an additional 86 million Americans, 20 years and older, have prediabetes. Therefore, in excess of 100 million Americans (or ~1 in 3 Americans) live with diabetes or prediabetes conditions (19a). Diabetes is associated with micro- and macrovascular complications, resulting in adult-onset blindness, end-stage renal failure, and nontraumatic limb amputation, as well as coronary artery disease, stroke, and peripheral vascular disease (19a, 35). In adults with diabetes, ~95% have type 2 diabetes (T2D), which begins with and is characterized by insulin resistance in various cell types and tissues (i.e., skeletal muscle, vasculature, adipose, hepatic, skin, and brain) throughout the body. It is generally believed that T2D can be prevented and managed with healthy food choices and routine physical activity (19a). Herein, we will discuss physical activity-induced microvascular adaptations throughout the body in the setting of T2D. Furthermore, we propose a model whereby exercise training
Exercise training (EX) stimulates local microvascular adaptations in tissues engaged directly in EX and systemic microvascular adaptations as a result of improved glycemic status in tissues not involved in EX (Fig. 1).

Inactivity is a recognized risk factor involved in the development of T2D (19a). Among patients with T2D, participation in no or only mild-intensity (i.e., walking, bowling, etc.) physical activity is associated with an increased incidence of cardiovascular events, microvascular complications, and all-cause mortality (17), and a decreased aerobic capacity is associated with the presence of neuropathy, retinopathy, or nephropathy (48). Likewise, in humans, T2D is associated with slowed oxygen kinetics at the onset of exercise (13, 129) and attenuated skeletal muscle blood flow responses to exercise (70, 76, 152), as well as glucose intolerance (19a). Importantly, each of the aforementioned conditions (neuropathy, retinopathy, nephropathy, exercise intolerance, and glucose intolerance) is caused, in part, by microvascular dysfunction. Studies performed in humans with insulin resistance or T2D and in animal models of disease demonstrate that EX mediates local and systemic improvements in endothelial (14, 27, 28, 93, 95, 101–103) and smooth muscle function (14, 27, 95) indicated by improved vasodilator signaling. Exercise training also appears to attenuate microvascular rarefaction in skeletal muscle associated with insulin resistance (23, 52, 67, 90, 126). Collectively, these EX-induced adaptations may help treat T2D-induced microvascular complications in vascular beds throughout the body.

**EX Is Beneficial in Treatment of T2D**

The joint-position stand from the American College of Sports Medicine and the American Diabetes Association recommends that people with a high risk of developing T2D participate in at least 2.5 h/wk of moderate (~40–60% VO\textsubscript{2}) to vigorous (>60% VO\textsubscript{2}) physical activity (30). Likewise, they advise that, for people with T2D, regular physical activity can be undertaken safely and used effectively to manage diabetes. Specifically, guidelines indicate that aerobic exercise should be performed at least 2–3 days/wk and consist of 8–10 exercises involving the major muscle groups, 1–4 sets per exercise, at a moderate to vigorous exercise intensity ranging from 50–80% 1-repetition maximum. For both training modalities (aerobic and resistance), it is recommended that participants increase the volume (i.e., the duration and frequency) and intensity of exercise gradually. In patients with T2D and preexisting peripheral vascular disease, peripheral motor or autonomic neuropathy, retinopathy, or nephropathy, careful screening or medical supervision may be required, and exercise prescriptions may need to be modified to optimize health outcomes. These guidelines are based loosely on observations that similar forms of training have improved blood glucose control, blood lipids, hypertension, cardiovascular events, mortality, and quality of life in patients with T2D (30). An exercise prescription targeting microvascular function in patients with T2D does not appear to exist.

Data from the ADVANCE trial, collected from over 10,000 patients, indicate a protective effect of moderate- and vigorous-intensity (i.e., fast walking, jogging, tennis, swimming, etc.)

**Fig. 1.** In blood vessels supplying active skeletal muscle fibers (or other tissues engaged during exercise, i.e., nervous), chronic microvascular adaptations to exercise training in the setting of T2D include improved vasodilator signaling, possibly improved capillary perfusion, increased microvascular and capillary density, and enhanced insulin/phosphatidylinositol 3-kinase (PI3K) signaling. If the exercise training program engages a large enough amount of skeletal muscle mass and maximizes muscle fiber recruitment (within each muscle), it will induce adaptations in active muscle fibers (and/or other active tissues, i.e., skin or peripheral nerve) and the blood vessels that supply them. Whole body exposure to hyperglycemia and hyperinsulinemia will also be reduced. Improved blood glucose control will reduce the risk of microvascular complications (i.e., in all blood vessels throughout the body) associated with type 2 diabetes (T2D). Gluc, glucose; NO, nitric oxide; ENDO, endothelium; VSM, vascular smooth muscle; BF; blood flow; IRS, insulin receptor substrate; P, phosphorylated; Akt, protein kinase B; eNOS, endothelial NO synthase; GSK3, glycogen synthase kinase 3; HbA1c, glycosylated hemoglobin; BG, blood glucose; micro, microvascular.
but not mild-intensity (i.e., walking, bowling, etc.) physical activity on the incidence of cardiovascular events (stroke or infarction), microvascular complications (in the kidneys or eye), and all-cause mortality in patients with T2D (17). Combined aerobic and resistance training, in line with the aforementioned guidelines, and more so than either training modality alone, can improve insulin sensitivity and reduce hyperglycemia (20). Although improved insulin sensitivity and reduced hyperglycemia should also reduce the risk of T2D-induced microvascular complications (17, 19, 51, 68, 128), this has yet to be established. Furthermore, existing research provides evidence that resistance (27) and aerobic exercise (28), and in particular vigorous-intensity interval aerobic EX (up to 80% VO2 peak) (103), independently and combined (93), exert direct, beneficial effects on endothelial and smooth muscle function indicated by improved vasodilation in the microcirculation of people living with T2D. The mechanisms responsible for the protective effects of moderate- and vigorous-intensity, but not mild-intensity, physical activity on the incidence of cardiovascular events, microvascular complications, and all-cause mortality in patients with T2D are unknown (17). The US physical activity guidelines suggest that the accumulation of 500–1,000 MET-min/wk (MET equivalent of exercise multiplied by total duration of exercise in min) is associated with lower rates of disease (121). For example, an adult could achieve this target by performing mild intensity EX such as walking for ~150 min/wk or moderate to vigorous intensity exercise such as jogging or running for ~50 min/wk. However, the notion that adaptations to EX are governed by total energy expenditure is inconsistent with observations that, when matched for energy expenditure, combined training programs appear to be more beneficial for glycemic status than aerobic or resistance training alone (20), and increasing aerobic EX intensity (from 65–80%) mediates greater improvements in insulin signaling (47, 145) and microvascular adaptations (103). Herein, we review evidence pertaining to the nature of EX-induced microvascular adaptations in various tissues (i.e., skin, nerve, and skeletal muscle, etc.) and propose a strategy to maximize the training effect in all microvascular tissue based on the documented effects of EX on cutaneous microvascular function in humans with T2D and the understanding of interactions of muscle fiber recruitment patterns and spatial patterns of EX-induced microvascular adaptations in skeletal muscle, as illustrated in Fig. 1.

**Endothelium**

In health, the endothelium contributes to vascular homoeostasis through the production of vasodilators (i.e., prostacyclin and nitric oxide) and vasoconstrictors [i.e., angiotensin II, thromboxane, endothelin-1, and reactive oxygen species (ROS)]. Altered metabolic status, such as hyperglycemia or insulin resistance, may perturb the balance between vasodilator and vasoconstrictor signaling, resulting in abnormal vasomotor control (128). Although the relationship between plasma glucose and insulin changes throughout the course of metabolic disease, the progression from healthy to prediabetes to T2D, to some degree, is associated with both hyperglycemia as well as hyperinsulinemia (19a). Long-term exposure to hyperglycemia results in the accumulation of advanced glycated end-products, the increased expression of adhesion molecules, and expression of proinflammatory genes, as well as the activation of protein kinase C and generation of ROS. In endothelial cells, increased ROS, in the form of superoxide, reacts with nitric oxide (NO), thereby inactivating NO and forming peroxynitrite. Peroxynitrite oxidizes the NO synthase cofactor tetrahydrobiopterin and subsequently decreases NO production. Through this, and other pathways, hyperglycemia may reduce NO production and availability (117, 123, 128). Long-term exposure to hyperinsulinemia, leading to insulin resistance, results in an imbalance in the insulin-mediated activation of the anti-inflammatory/vasodilator pathway (via NO) phosphatidylinositol-3-kinase (PI3K) vs. the proinflammatory/vasoconstrictor (via endothelin-1, ET-1) Ras/mitogen-activated protein kinase (MAPK) pathway, which favors MAPK signaling (68, 108). Cumulatively, exposure to hyperglycemia and hyperinsulinemia alters the phenotype of endothelial cells indicated by a decreased production or availability of NO and vasoactive prostaglandins and increased production of ROS, vasoconstrictor prostanooids, and ET-1 (68, 108).

Indeed, the aforementioned shift in endothelial phenotype may contribute to changes observed at the level of the microvasculature. In patients with T2D, forearm blood flow (57) and forearm cutaneous microvascular responses (18, 127) to endothelium-dependent vasodilator acetylcholine (ACh) are blunted. In the study by Caballero and coworkers (18), participants with impaired microvascular responsiveness also had greater plasma concentrations of von Willebrand Factor (%) and elevated concentrations of circulating ET-1, as well as soluble intercellular and vascular cell adhesion molecules, all indicators of altered endothelial cell phenotype. Similar observations have been made by others, and, in their 2013 meta-analysis, Montero and coworkers (105) documented that 17/19 studies reported that T2D is associated with impaired endothelial reactivity in human microvasculature. Importantly, physical inactivity is a risk factor for the development of T2D (19a), and participation in no or only mild-intensity physical activity (17) as well as decreased fitness (48) are associated with microvascular complications throughout the body in patients with T2D. Also, EX increases capillary density and improves microvascular vasodilatory function in the setting of T2D (27, 28, 67, 93, 103, 126). Thus we propose that properly designed EX programs (147) may be a viable treatment option to correct or attenuate the decline in microvascular function in this population.

**EX and Cutaneous Microvascular Endothelial Function in Humans with T2D**

To address the issue of T2D-induced reductions in NO production and availability in humans, Krause and colleagues (72) investigated the effects of two different, unsupervised 16-wk walking programs, performed at either the heart rate estimated to induce the greatest fat oxidation, considered mild intensity, or to replicate the heart rate achieved at the ventilatory threshold, considered moderate- to vigorous-intensity exercise. Neither training program improved body composition, glycemic control, aerobic capacity, or measures of NO production or availability in patients with T2D. However, they reported that the more intense aerobic training program increased plasma catalase activity and decreased circulating protein carbonyls, indicative of reduced ROS production or increased...
circular blood flow during exercise were not examined in these studies but may be greater during and in recovery from vigorous- vs. moderate-intensity aerobic exercise (66). Also, which training modality, between aerobic and resistance training, elicits the greatest cutaneous microvascular adaptations in patients with T2D is unknown because no single study has directly compared the two, and null results have been documented following both types of training (29, 99). In prehypertensive and stage 1 hypertensive patients, forearm reactive hyperemia is greater following 4 wk of moderate to vigorous resistance (exercise for major upper and lower body muscle groups, 3 sets of 10 repetitions, performed at 65% 10-repetition max, 3 days/wk for 4 wk) vs. aerobic (treadmill running at 65% \( \dot{V}O_2 \text{peak} \), 3 days/wk for 4 wk) training (32). The authors speculated that the blood flow shear stress experienced by the arm was likely greater during upper body resistance vs. the lower body endurance exercise and that this contributed to the augmented forearm reactive hyperemic response in the resistance training group. Cutaneous microvascular function, assessed using laser Doppler, is related to glycemic status (31) as well as other microvascular complications (i.e., foot ulcers and neuropathy) associated with T2D (31, 143). Therefore, establishing the nature of EX-induced microvascular adaptations in the skin may be important for skin health (i.e., foot ulcers) and the treatment of microvascular dysfunction in other tissues (i.e., the peripheral nerve).

Of note, the aforementioned studies examined the effects of EX on cutaneous microvascular function in the upper and lower limbs of people with T2D. Although cutaneous microvascular vasomotor function is related to microvascular complications in T2D (31, 143), whether similar microvascular adaptations occurred in skeletal muscle of the arm or legs or in other vascular beds remains unknown. Also, the significance of EX-induced adaptations in cutaneous microcirculation has not been established. Similar exercise intensity-dependent effects have been observed in skeletal muscle and may be explained by greater muscle fiber recruitment and adaptation during and following vigorous- vs. mild-intensity aerobic exercise (12, 42, 54, 153). Given its role in physical activity and the development of T2D, in the following section we will focus on EX-induced microvascular adaptations in skeletal muscle in the setting of T2D.

**EX and Skeletal Muscle Microvascular Adaptations in T2D**

Skeletal muscle consists of three general phenotypes of muscle fibers classified according to both their contractile and metabolic properties (133): slow-twitch oxidative (SO), fast-twitch glycolytic (FG), and fast-twitch oxidative glycolytic (FOG). Skeletal muscle fiber type composition and muscle fiber recruitment patterns during exercise have a major influence on vascularization, capillary exchange capacity, vascular structure, mechanisms of vasomotor control, and regional distribution of blood flow within and among muscles during exercise (10, 16, 63, 79, 87, 97). Importantly, EX alters relationships among muscle fiber type, recruitment patterns, and blood flow (11, 82, 110, 139) by modifying vascular structure, endothelium (37, 38, 40, 71, 81, 85, 96, 98, 107, 120, 137, 142, 150), and vascular smooth muscle (VSM) of skeletal muscle arteries/arterioles (77, 80, 81). Although many EX-induced vascular adaptations are concentrated in the muscle...
tissue, having the greatest relative increase in activity during training sessions (11, 15, 22, 55, 56, 82, 84, 92, 131, 138, 139), the relative amount of adaptation is not distributed uniformly for any of these parameters (84), and these adaptations are not the same for different types of EX (11, 81, 82, 109, 110, 139). Thus different intensities and types of exercise activities require different fiber-recruitment patterns, which subsequently influence the spatial distribution of adaptations within skeletal muscle induced by the EX. We postulate that the EX program that engages the most skeletal muscle and the most muscle fibers within each skeletal muscle (i.e., greatest increase in fiber recruitment from rest to exercise), given that the stimulus is applied for a sufficient duration of time, will generate the most widespread adaptations, leading to improvements in microvascular function and insulin sensitivity. Thus intensity and mode should be modulated to maximize fiber recruitment (i.e., maximize the number of skeletal muscle fibers recruited and meet/exceed the duration of recruitment required to induce adaptation). EX will impact the skeletal muscle and associated microvasculature directly involved in the training session. Similar principles may apply to other tissues whose activity can be altered by exercise bouts (i.e., cardiac muscle, skin, central and peripheral nervous tissue, etc.). Also, the EX-induced improvements in insulin sensitivity, predominantly in skeletal muscle tissue, will mediate reductions in systemic hyperglycemia and hyperinsulinemia, which will subsequently influence the spatial distribution of adaptations within skeletal muscle induced by the EX. We postulate that the EX program that engages the most skeletal muscle and the most muscle fibers within each skeletal muscle (i.e., greatest increase in fiber recruitment from rest to exercise), given that the stimulus is applied for a sufficient duration of time, will generate the most widespread adaptations, leading to improvements in microvascular function and insulin sensitivity. Thus intensity and mode should be modulated to maximize fiber recruitment (i.e., maximize the number of skeletal muscle fibers recruited and meet/exceed the duration of recruitment required to induce adaptation). EX will impact the skeletal muscle and associated microvasculature directly involved in the training session. Similar principles may apply to other tissues whose activity can be altered by exercise bouts (i.e., cardiac muscle, skin, central and peripheral nervous tissue, etc.). Also, the EX-induced improvements in insulin sensitivity, predominantly in skeletal muscle tissue, will mediate reductions in systemic hyperglycemia and hyperinsulinemia, which will subsequently reduce the risk of microvascular complications in vascular beds throughout the body that are uninvolved directly in EX (141) (Fig. 1). Data from our laboratory, using the Otsuka Long Evans Tokushima fatty (OLETF) rodent model of T2D, indicate that endothelium-dependent dilation (EDD), examined using pressurized myography, is blunted in a muscle fiber type-dependent manner (14, 64, 95). As illustrated in Fig. 2 (14, 64, 95), both moderate to vigorous aerobic and vigorous interval sprint training resulted in increased ACh-induced EDD in second-order arterioles of red gastrocnemius (G2A-R) (Fig. 2A), whereas only aerobic training improved ACh-induced EDD in second-order arterioles of white gastrocnemius (G2A-W) in rats with T2D (Fig. 2B) (95). The latter finding, pertaining to EDD in G2A-W, was surprising because we expected that interval sprint training would have greater effects on G2A-W, as interval sprint training increases recruitment of FG/FOG fibers (i.e., white portion of gastrocnemius). Accordingly, both training modalities increased citrate synthase in the vastus lateralis, but the training effect was more pronounced in the white vastus lateralis in the vigorous interval sprint training group (95). Of note, in T2D, there is a skeletal muscle fiber type shift toward FG/FOG fibers, and this may alter fiber recruitment and skeletal muscle blood flow during exercise (94, 154). Copp and colleagues (33) reported that, at submaximal exercise intensity, steady-state skeletal muscle blood flow to predominantly FG/FOG fibers was increased in the Goto-Kakizaki T2D rat vs. Wistar controls. Interestingly, the moderate to vigorous aerobic running speeds were similar in the aforementioned studies (i.e., 20 m/min, 10–15% grade). Although exercising skeletal muscle blood flow in the white portion of the gastrocnemius was not different between control

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**Endothelium dependent dilation (EDD) in skeletal muscle arterioles in untrained and trained T2D rats**

**Fig. 2.** A and B: acetylcholine (ACh) dose response curve in gastrocnemius second-order arterioles from red (G2A-R) and white (G2A-W) portion of the gastrocnemius in 32-wk-old, Otsuka Long-Evans Tokushima (OLETF) sedentary (Sed, •), endurance exercise-trained (EndEx, □), and interval sprint-trained (IST, ◇) rats. *EndEx > Sed (P < 0.05); ¤IST > Sed (P < 0.05); 5EndEx > IST (P < 0.05). C and D: ACh dose response curve in the gastrocnemius feed artery (GFA) in 20-wk-old Long-Evans Tokushima Otsuka (lean control) sedentary (LSed, ⊕), OLETF sedentary (OSED, ⊘), and OLETF wheel-running (OPA, ◊) rats (C) and in 32-wk-old, OLETF Sed (◊), EndEx (□), and IST (◇) rats (D). *OSED < LSed (P < 0.05), †OSED > OPA (P < 0.05), ‡OSED > OPA (P < 0.05). E and F: sodium nitroprusside dose response curve in the soleus feed artery (SFA) in 20-wk-old LSed (◊), OSED (◇), and OPA (⊘) rats (E) and in 32-wk-old, OLETF Sed (◊), EndEx (□), and IST (◇) rats (F). *IST > Sed (P < 0.05); 6IST > EndEx (P < 0.05). All data are presented as means ± SE. BL, baseline. A, B, D, and F were adapted from Martin et al. (95), and E and F were adapted from Bender et al. (14) with permission from the American Physiological Society.
and T2D, these data highlight an important point, namely that, during EX skeletal muscle fiber recruitment, blood flow and therefore microvascular adaptations to EX are likely different between control and T2D conditions. Combined with differences in fiber recruitment, the nature of the exercise stimulus (i.e., continuous vs. interval) and subsequent differences in the magnitude and duration of the blood flow shear stress may have contributed to differences in microvascular adaptations (95). Differential vasomotor responses to training also occur at the level of the feed artery, as ACh-induced EDD in the feed artery of the gastrocnemius was increased by voluntary wheel running (Fig. 2C) (14) but not by aerobic or interval sprint training performed on a motorized treadmill (Fig. 2D). Thus EX-induced microvascular adaptations in the setting of T2D differ between training modalities and among skeletal muscle arterioles. Interestingly, similar training- and arterial-dependent differences have been observed with regard to microvascular insulin signaling in T2D (34, 36, 101, 102).

Effects of EX on Microvascular Insulin Signaling in T2D

In addition to reduced sensitivity to ACh-induced EDD, patients with insulin resistance and T2D display impaired vasoreactivity to insulin (65, 75). In the endothelium, insulin signaling functions through the insulin receptor substrate-1/PI3K/protein kinase B (IRS-1/PI3K/Akt) pathway, resulting in the production of vasodilator NO, as well as the MAPK pathway, resulting in the production of vasoconstrictor ET-1 (68, 108). Thus insulin signaling involves a balance between vasodilator (NO) and vasoconstrictor (ET-1) pathways. Under healthy conditions, operating through the PI3K pathway, acute increases in circulating insulin stimulate the production of endothelial NO and increase vascular conductance in the lower limb (3, 140). The vasodilatory effects of insulin appear to operate following insulin infusion (140) as well as glucose-stimulated insulin secretion (100, 113). Whether insulin is infused or secreted in response to glucose, the vasodilation is NO dependent (114). Insulin-induced vasodilation may contribute to the maintenance of normoglycemia by augmenting insulin and glucose dispersal in skeletal muscle (21, 26). Insulin-stimulated increases in skeletal muscle blood flow and lower limb vascular conductance are blunted in patients with T2D (65, 75). On the basis of data from our laboratory, insulin-stimulated vasodilation is impaired in skeletal muscle arterioles in T2D because NO production is diminished, whereas vasoconstriction through ET-1 release is augmented (i.e., an imbalance in PI3K/NO vs. MAPK/ET-1 signaling) (14, 64, 95). In addition, because insulin augments sympathetic nerve activity and sympathetic nerve activity is elevated in individuals with insulin resistance, attenuated vasodilation to insulin may occur because of reduced NO production coupled with normal or elevated sympathetic-mediated vasoconstriction (149). Lastly, decreased vasodilator responses to insulin and increases in insulin resistance may occur on account of decreased capillary perfusion (118, 119) and microvascular rarefaction (90). Decreased insulin-stimulated vasodilation and skeletal muscle perfusion may reduce insulin and glucose delivery to skeletal muscle and contribute to impaired glucose tolerance observed commonly in people with insulin resistance and T2D (21, 62, 74).

Impaired vasodilation in response to insulin infusion (36, 62) as well as glucose-stimulated insulin secretion (100) in patients with T2D can be improved with EX. For example, Dela and colleagues (36, 62) conducted a series of studies comparing lower limb blood flow during a euglycemic hyperinsulinemic clamp in patients with T2D, before and after different EX programs. The training consisted of either vigorous one-legged cycling exercise (36) (30 min/day at 70% of max heart rate, 6 days/wk, for 10 wk) or one-legged resistance exercise (62) (leg press, knee extension, and hamstring curls; 3–4 sets of 8–12 repetitions at ~80% 1-repetition max, performed 3 days/wk, for 6 wk). Training had no effect on blood glucose or HbA1c but did increase GLUT4 content. Also, aerobic training increased VO2peak, and resistance training increased muscular strength in the trained vs. untrained limb.

As expected, glucose clearance increased in the trained limb following training. Of note, insulin-mediated vasodilation was greater, but glucose extraction was unchanged in the trained limb from before to after training. Thus the beneficial effects of EX on lower limb insulin sensitivity and glucose clearance are, in part, related to improved vasodilation. Interestingly, similar observations were not made in the untrained limb, suggesting that the effects of EX on insulin-mediated vasodilation were restricted to the vascular supply of the active skeletal muscle. Similar improvements in forearm cutaneous microvascular reactivity to insulin were observed in patients with metabolic syndrome following a 6-mo lifestyle intervention that included diet counseling and a combined mild-to-moderate endurance (aquagym, walking, or cycling) and resistance (8 free-weight/machine exercises; 3 sets of 10 repetitions) training program (90 min/day, 4–5 days/wk, for 6 mo) (148). Tjonna and coworkers (145) compared moderate to vigorous-intensity continuous vs. vigorous-intensity interval aerobic EX, matched for energy expenditure, in individuals with prediabetes and documented superior training effects on aerobic capacity, conduit artery flow-mediated dilation, and skeletal muscle PGC-1α content and insulin signaling following the more vigorous-intensity exercise program. More recently, Eskelinen and coworkers (47) compared insulin-stimulated glucose uptake in the upper and lower limbs following either moderate-intensity endurance or vigorous-intensity interval sprint training and reported that skeletal muscle glucose uptake improved in lower, but not upper, body limbs. Furthermore, both training programs improved aerobic capacity and increased glucose uptake in the vastus lateralis, intermedius, and medialis, but only vigorous interval sprint training increased glucose uptake in the rectus femoris (47). In line with the aforementioned findings, results from our laboratory indicate that improvements in EDD produced by EX in skeletal muscle arterioles from T2D rats are dependent on skeletal muscle fiber recruitment during the exercise bouts. Specifically, we established that insulin signaling in arteriolar endothelium differs among types of skeletal muscle among different branch orders in skeletal muscle arteriolar trees (14, 64, 95) and that different types of EX (wheel running, endurance, and interval sprint training performed on a treadmill) impact the arterioles differentially and improve insulin-induced EDD nonuniformly in the arterial tree of skeletal muscle (14, 95, 101, 102). Insulin-mediated vasodilation was improved by wheel running and vigorous interval sprint training in G2A-W (Fig. 3, A, B, and D) (95, 102), whereas moderate to vigorous endurance training...
improved insulin-induced EDD in the G2A-R (Fig. 3C) (95). Thus EX-induced improvements in EDD are distributed heterogeneously throughout skeletal muscle arteriolar networks, and the pattern of distribution of these effects depends on types of training and signal for EDD (i.e., ACh vs. insulin) (95, 102). These observations add importantly to the growing body of evidence that highlight differences among vasomotor properties of arteries of different tissues (89), within tissue (8, 41, 79, 89, 135, 136, 151), and along the length of the arterial network (39, 46, 58, 73, 77, 78, 81, 83). The relative importance of vascular control mechanisms also differs among muscles (1, 2), and endothelial phenotype varies among and within vascular beds (4, 5, 7, 49, 50, 81, 85, 87, 97, 111, 112, 122).

In addition to the relationship between skeletal muscle fiber recruitment and microvascular adaptations to EX discussed above, increasing the amount of skeletal muscle involved (i.e., multiple large muscle groups) and the number of skeletal muscle fibers recruited within each muscle during EX may contribute directly to increased insulin sensitivity (36, 47, 62, 145) as well as more pronounced reductions in blood glucose observed following vigorous intensity training (145) or combined aerobic and resistance training programs (20). By extension, improved glycemic status may also improve microvascular function indirectly in tissues beyond active skeletal muscle (17, 103, 141). However, Dela and colleagues (36) reported that the effects of single-limb EX on insulin-mediated vasodilation were restricted to the trained limb. We observed that metformin treatment in OLETF rats exerted favorable effects on body composition and HbA1c but did not improve insulin-induced EDD in any skeletal muscle arteriole (34). Similarly, withholding food to match body weights of sedentary OLETF rats with OLETF rats that exercise trained caused a reduction in body weight, percentage of body fat, fasting plasma glucose, and HbA1c but did not improve insulin-induced EDD in any skeletal muscle arteries (34, 101, 102). Furthermore, a study by Olver and coworkers (115) compared insulin-mediated vasodilation in the vasa nervorum (blood vessels supplying the sciatic nerve) in sedentary and moderate to vigorous aerobic exercise-trained rats with insulin-treated T1D and found that, although the groups were matched for levels of hyperglycemia, EX prevented the decline in insulin-mediated vasodilation observed in the sedentary group with T1D. Thus available evidence indicates that EX, and not glycemic status, mediates systemic changes in metabolic status. Therefore, in patients with orthopedic or other physical limitations, it may be necessary to exercise train small muscle masses (to induce adaptations in as much skeletal muscle and the associated microvasculature as possible), for example one arm and one leg at a time, to provide optimal treatment. If our hypothesis is correct, the key to designing effective EX programs is to assure that as much tissue, skeletal muscle mass in particular, and corresponding microvasculature as possible undergoes exercise-induced adaptation. As a result of widespread adaptations in tissues engaged directly in EX, glycemic status may improve and thereby benefit vascular beds throughout the body that are not involved in EX directly.

**EX, Smooth Muscle and Capillary Function, and Vascular Remodeling**

In addition to blunted EDD, T2D is associated with impaired VSM function (18). In fact, in their 2013 meta-analysis, Montero and coworkers (105) noted that 12/17 studies that documented blunted EDD also reported impaired, endothelium-independent VSM relaxation. This observation was most apparent in the studies investigating the cutaneous microcirculation (105). Attenuated VSM responsiveness to NO is problematic, as impaired micro-VSM
function may reduce vasoreactivity to endothelium-dependent and -independent stimuli. For example, a decreased vascular responsiveness to ACh and insulin may be due, in part, to blunted VSM responses to NO. Thus improving endothelial NO production and release alone may not restore vascular homeostasis completely. Furthermore, altered VSM function in patients with T2D can impair other aspects of vasomotor control, such as myogenic tone (134) and autoregulation of blood flow, both of which can affect tissue perfusion and organ health (88). Reductions in micro-VSM relaxation in patients with T2D may be explained by an altered VSM cell phenotype, characterized by increased expression of proinflammatory genes, changes in VSM cell morphology favoring a rhomboid vs. a hill-and-valley shape, disorganized actin fiber arrangement, enlarged intracellular organelles, resistance to apoptosis, and differences in cell proliferation and migration (124). In conjunction with an altered endothelial phenotype, such changes may also contribute to vascular remodeling (94, 130), a reduced capacity to undergo arteriogenesis (106, 132, 146), and perturbed skeletal muscle capillary hemodynamics (118, 119), as well as capillary rarefaction in individuals with insulin resistance or T2D (9, 53, 90, 94).

Currently, literature renders it is difficult to form meaningful conclusions regarding the effects of EX on micro-VSM function in people with T2D because data are sometimes inconsistent (27, 93). Some research supports the notion that EX-induced adaptations are more pronounced in the endothelium (vs. VSM) in both conduit and resistance arteries (93, 104). The effects of EX on micro-VSM responses to NO donors have not been established, as some report increased responsiveness (27) and others observe no change (93) in patients with T2D. Both the aforementioned studies included upper body resistance training performed predominantly in the vigorous-intensity domain although training programs were different, documented training-induced reductions in HbA1c and measured forearm vascular responses to NO donors (i.e., endothelium-independent dilation). However, the study by Cohen and colleagues (27) used laser Doppler [vs. strain gauge plethysmography (93)], the EX intervention was 1 yr longer in duration, and they documented improvements in sodium nitroprusside (SNP)-induced cutaneous microvascular dilation. Hodges and coworkers (61) also used laser Doppler to examine forearm cutaneous microvascular responses to ACh and SNP but in obese, non-T2D postmenopausal women, before and at 12, 24, 36, and 48 wk during a progressive mild/moderate- to vigorous-intensity endurance exercise program. They reported increased vasoreactivity to ACh at 24 wk, whereas SNP-induced dilation did not improve until 36 wk (61). Thus the duration of an EX treatment and the intensity domain of the exercise may influence the degree of endothelial and/or VSM adaptation. Other divergent results in human studies may relate to the severity of disease, the exercise prescription, and the study duration. Furthermore, rodent work in the skeletal muscle vasculature reveals that discrepancies may relate to regional differences within a vascular network and arterial branch examined, as we have documented improved vasodilation to NO donors following vigorous interval sprint training (Fig. 2F), but not moderate to vigorous endurance training (Fig. 2F) or wheel running (Fig. 2E) (95), in soleus muscle (predominantly SO fibers) feed arteries (Fig. 2, E and F), but not gastrocnemius feed or G2A-R or G2A-W arteries.

Although the effects of EX on capillary hemodynamics (59, 69), microvascular remodeling, and capillarity (45, 125) have been characterized under normal circumstances, fewer studies have been conducted in patients with T2D. Observations of slowed oxygen kinetics at the onset of exercise and decreased skeletal muscle blood flow during exercise in patients with T2D (13, 70, 76, 129, 152) may be explained, in part, by decreased capillary perfusion. In a series of studies using the Goto-Kakizaki T2D rat model, Padilla and colleagues (118, 119) demonstrated that reductions in the microvascular partial pressure of oxygen at rest and during skeletal muscle contraction may owe to reduced capillary perfusion (i.e., reduced percentage of capillaries perfused with moving red blood cells, lower red blood cell flux, and decreased O2 delivery per unit of muscle). Although total hind limb blood flow at rest and during submaximal exercise was not different between control and T2D rats (regional differences were present) (33), differences in capillary perfusion at rest may contribute to impaired matching of oxygen delivery with utilization resulting in exercise intolerance and decreased glucose delivery/uptake in skeletal muscle. Hirai and colleagues (59) reported that, in healthy rats, progressive moderate to vigorous downhill running endurance EX improved microvascular oxygenation profiles following the onset of skeletal muscle contraction, partially through an NO mechanism. Furthermore, similar improvements were observed in rats with chronic heart failure but did not appear to be NO mediated (60). Interestingly, in the aforementioned studies, EX augmented soleus and red gastrocnemius citrate synthase content, but only in the latter study did citrate synthase content increase in the spinotrapezius muscle, the skeletal muscle where microvascular oxygenation was examined. Thus disease status must be considered when evaluating the mechanisms of EX-induced microvascular adaptations, particularly in skeletal muscle. In humans, moderate to vigorous combined aerobic and resistance training improves oxygen uptake kinetics in patients with T2D (91), but whether aerobic or resistance EX can restore T2D-induced deficits in the microvascular oxygenation or capillary perfusion remains unknown. In line with longstanding observations made in athletes (144), aerobic exercise (67, 126) may be superior to resistance EX (62) at increasing capillary density or attenuating the decline in capillarity observed in individuals with T2D as well as sedentary individuals without T2D (23–25). This should be expected, as resistance exercise tends to induce skeletal muscle fiber hypertrophy without increasing the rate of angiogenesis (25, 62, 144).

Moderate to vigorous aerobic training increases capillary contacts per fiber (this measure of capillary density quantifies the number of capillaries surrounding a muscle fiber) in the vastus lateralis in old, nonobese men with impaired glucose tolerance (Fig. 4A; treadmill running at 60–70% heart rate reserve for 60 min/day, 3 days/wk, for 12 wk) (67), as well as in old, obese men and women with impaired glucose tolerance (Fig. 4B; treadmill walking/running at 50–85% heart rate reserve for 20–45 min/day, 3 days/wk, for 24 wk) (126). With regards to exercise intensity, in non-T2D obese men, both moderate- to vigorous-intensity endurance (Fig. 4C; cycling at 65% \( \text{VO}_{2}\text{peak} \) for 40–60 min/day, 5 days/wk, for 4 wk) and vigorous-intensity interval sprint training (Fig. 4C; 4–7 30-s
cycling intervals, performed at ~200% work rate max achieved during an incremental \( \dot{V}O_2 \) peak test, interspersed by 120 s of active recovery cycling at 30 W, 3 days/wk, for 4 wk) (23) increase capillary contacts per fiber to a similar extent in the lateral portion of the vastus lateralis. Although the depth and fiber composition of the muscle biopsy will impact the results (43, 44), it appears that vigorous-intensity interval sprint training is equal and perhaps superior to endurance training at increasing capillary contacts per fiber in the vastus lateralis (Fig. 4D). Both moderate-intensity endurance and vigorous-intensity interval sprint training increase capillary density in humans (23) and rodents (86), and, similar to adaptations in endothelial function, changes in capillarity occur in a fiber-specific manner. That is, increases in capillary density following endurance vs. interval sprint training occur predominantly in SO vs. FOG/FG muscle fibers, respectively (55, 56, 79, 86).

In Refs. 23 and 126, EX increased skeletal muscle capillarity and aerobic capacity and improved glucose tolerance. As previously mentioned, increasing skeletal muscle perfusion augments insulin-mediated glucose uptake, and capillary density is correlated with in vivo insulin sensitivity (6, 23, 90). These effects may be potentiated by increasing capillary blood flow in previously perfused and underperfused capillaries. Therefore, in patients with insulin resistance or T2D, from a vascular perspective, EX may improve insulin signaling by enhancing microvascular responses to insulin (36, 62, 95, 101, 102, 115), attenuating microvascular rarefaction (Fig. 4, A–D) (23, 67, 126) and augmenting capillary blood flow (59, 118, 119), all of which will augment perfusion and increase glucose and insulin delivery in skeletal muscle (21).

**Exercise Prescription Revisited**

Moderate- and vigorous-intensity, but not mild-intensity, physical activity protect against cardiovascular events, microvascular complications (stroke, infarction, retinopathy, nephropathy), and all-cause mortality in patients with T2D. In fact, patients that participate in moderate- or vigorous-intensity physical activity have healthier risk profiles than sedentary patients with lower diastolic blood pressure, lower BMI, lower triglycerides, and better renal function and have been diag-
nosed with T2D for a shorter duration (17). The mechanisms responsible for the protective effects of exercise on microvascular function in skeletal muscle, as well as other tissues, are not understood fully but appear to relate to the direct effects of exercise on microvascular function in tissues (i.e., skeletal and cardiac muscle, skin, and peripheral and central nervous tissue) engaged (undergo exercise-induced increase in activity) during exercise and the indirect effects of improved glycemic status on microvascular function in vascular beds less influenced/ altered during exercise (17, 141) (Fig. 1). However, it is important to note that the effects of EX on insulin-mediated vasodilation in T2D occur exclusively in trained vs. untrained skeletal muscle (36) and are independent of reductions in body weight or blood glucose alone (34, 101, 102). Thus EX and improved glycemic status in the absence of EX help treat microvascular dysfunction in patients with T2D, but likely to a different degree, through different mechanisms and differentially in microvascular beds that supply tissues that undergo exercise-induced increases in physiological activity. Evidence we have reviewed here, from humans (20, 23–25, 36, 47, 62, 67, 72, 103, 126, 145) and rodents (11, 14, 42, 55, 56, 79, 86, 95, 101, 102, 115), is consistent with the notion that EX-induced adaptations in microvascular structure, as well as vasodilatory and insulin signaling, occur predominantly in the skeletal muscle tissue that undergoes sufficient exercise-induced increases in activity from rest to exercise. Therefore, EX that engages the most skeletal muscle mass and recruits the most muscle fibers within each muscle (i.e., greatest increase in fiber recruitment from rest to exercise), for a sufficient duration, will induce adaptations in the greatest amount of skeletal muscle microvascular tissue. Furthermore, providing that insulin sensitivity is enhanced in a sufficient amount of vascular and skeletal muscle tissue, the EX will have systemic benefits attributable to reductions in hyperglycemia and hyperinsulinemia (141). Improvements in glycemic status may impact vascular beds positively throughout the body, even in tissues not engaged directly in EX (Fig. 1).

To determine whether vigorous-intensity EX programs or the combination of different training modalities (i.e., combined aerobic and resistance training) are more effective than mild-intensity EX programs or single modes of training at improving microvascular function, and to evaluate the mechanisms involved, it is important that experiments control for the energy expenditure associated with each training program (20, 103, 145). However, as it pertains to exercise prescription, this consideration is of less importance, as prescriptions are intended to maximize specific health outcomes that may not be caused directly by manipulating energy expenditure alone. If our hypothesis that the greatest microvascular benefit results from producing adaptations in as much tissue/skeletal muscle as possible (primary adaptations occurring in the tissues engaged during EX and secondary systemic adaptations resulting from improved glycemic status) is correct, it does not matter whether vigorous intensity or combined aerobic and resistance training requires more or less energy expenditure each week, providing that the treatment is more efficacious at improving microvascular function and glycemic status. For example, mild-intensity walking performed 2 h/day, 5 days/wk may be less effective than moderate- to vigorous-intensity combined lower and upper body resistance + endurance EX performed 0.5 h/day, 3 days/wk at inducing local and systemic microvascular adaptations and reductions in hyperglycemia, despite that the walking program may result in greater energy expenditure. On the basis of available results, we propose that moderate- to vigorous-intensity aerobic training will produce greater benefit than mild- to moderate-intensity aerobic training because the increased intensity requires recruitment of more skeletal muscle fibers. Similarly, adding resistance training or upper body aerobic training (swimming, rowing, cross-country skiing) can induce adaptations in skeletal muscles only recruited modestly during upright locomotor exercise (i.e., walking, jogging, or running). Thus, in both instances, moderate- to vigorous-intensity and combined lower and upper body EX will signal adaptations in more skeletal muscle mass than mild- to moderate-intensity, single-mode EX programs, even when matched for energy expenditure. This concept is not limited to the skeletal muscle and may apply to skin, peripheral nervous tissue, and other tissues engaged directly during EX. We propose that research is needed to determine whether greater treatment outcomes will result when these mechanistic concepts are integrated into EX prescriptions for patients with metabolic syndrome and T2D.

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AUTHOR CONTRIBUTIONS

Author contributions: T.D.O. and M.H.L. conception and design of research; T.D.O. and M.H.L. drafted manuscript; T.D.O. and M.H.L. approved final version of manuscript; M.H.L. analyzed data; M.H.L. interpreted results of experiments; H.L. prepared figures; M.H.L. edited and revised manuscript.

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