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SKELETAL MUSCLE PERFORMANCE IN METABOLIC DISEASE: MICROVASCULAR OR MITOCHONDRIAL LIMITATION OR BOTH?

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Abstract

One of the clearly established health outcomes associated with chronic metabolic diseases (e.g., type II diabetes mellitus) is that the ability of skeletal muscle to maintain contractile performance during periods of elevated metabolic demand is compromised as compared to the fatigue-resistance of muscle under normal, healthy conditions. While there has been extensive effort dedicated to determining the major factors that contribute to the compromised performance of skeletal muscle with chronic metabolic disease, the extent to which this poor outcome reflects a dysfunctional state of the microcirculation, where the delivery and distribution of metabolic substrates can be impaired, versus derangements to normal metabolic processes and mitochondrial function, versus a combination of the two, represents an area of considerable unknown. The purpose of this manuscript is to present some of the current concepts for dysfunction to both the microcirculation of skeletal muscle as well as to mitochondrial metabolism under these conditions, such that these diverse issues can be merged into an integrated framework for future investigation. Based on an interpretation of the current literature, it may be hypothesized that the primary site of dysfunction with earlier stages of metabolic disease may lie at the level of the vasculature, rather than at the level of the mitochondria.

Keywords

mitochondria; microcirculation; fatigue resistance; oxygen limitation; capillary

INTRODUCTION

Metabolic disease can be defined as a developing series of pathological states where the normal control systems for glucose handling, lipid levels and arterial pressure (among others) are no longer able to maintain these parameters within optimal levels. These can include conditions such as type II diabetes mellitus/insulin resistance, obesity and hypertension, with the greatest impact being a chronic elevation in the risk for poor health outcomes. There is little argument that the growing incidence and prevalence of conditions

of elevated cardiovascular, peripheral vascular and cerebrovascular disease risk, and their associated disease sequelae represent one of the most challenging issues facing clinical care and public health across many nations world-wide (107). A defining characteristic of these conditions of elevated risk and ultimately the pathological states that they can create is an evolving mismatch in the ability of microvascular systems in question to deliver and distribute blood flow within tissue and organs that meets changing metabolic demand (30, 40). As a result, a condition of growing ischemia, either across the organ in question, or within sub-sections of the organ or tissue, is created, which can be a major contributor to an impaired function of that tissue or organ. Tracking in parallel with this is a growing impairment to the metabolic efficacy of the tissues which also appears to develop with the growing metabolic disease (26, 87), such that organ function can be impaired as a result of poor metabolic regulation itself, potentially independent of any changes to microvascular function. The purpose of this manuscript is to attempt to integrate some of the recent work addressing metabolic disease and its impact on skeletal muscle function, mediated through the microcirculation, through the skeletal muscle fiber itself and through the communications and interactions between the two. As will be presented below, we believe that a new paradigm has begun to emerge that provides for a more accurate, integrated understanding of the multi-scale impairments that are associated with chronic metabolic disease that lead to the functional or clinical outcomes of peripheral vascular disease. Through a greater understanding of these impairments and interactions, future experiments can be more effectively targeted to improve skeletal muscle/skeletal muscle microvascular function in a manner that improves health outcomes under this challenging chronic disease condition.

The biophysical limits to maximal muscle performance derive from one of four possible mechanisms either alone or in combination. Exercise performance can be limited by: 1) cardiac output/oxygen delivery, 2) pulmonary diffusive capacity, 3) oxygen carrying capacity, and 4) mitochondrial respiratory capacity (6). Each of these limitations work together to support muscle respiration and therefore the absolute limiting factor is influenced by the type of work being performed, namely: size of muscle mass recruited, mechanical efficiency of contraction, and work rate in its relation to maximum oxygen uptake (VO_{2max}) (6). The effect of the type of work being performed is best characterized in relation to the critical speed/lactate threshold which are established as the best predictors of exercise performance (47). In healthy individuals during intensive whole-body exercise, where oxygen consumption is approaching maximum, it is well accepted that cardiac output/oxygen delivery is the limiting factor to aerobic respiration and thus muscle performance (35, 40, 53, 86, 103). In contrast, at submaximal workloads, as in single limb exercise, oxygen delivery is not challenged to the same extent as whole body exercise and thus oxygen utilization by the mitochondria may become the limiting factor to muscle performance and not oxygen supply (8, 39, 102). This is illustrated in studies of sedentary subjects by Cardus and co-workers demonstrated during cycling that breathing hyperoxic air and elevating arterial PO_2 does not alter VO_{2max} (12). Since oxygen consumption did not increase with the increased oxygen supply this suggests that oxygen utilization by the mitochondria is the limiting factor and not supply. Where oxygen demand versus oxygen supply balance in the limits to muscle performance is lesser known in metabolic diseases

and has been conceptualized as a “tipping point” by Poole and Jones (79). Therefore, measurement of both mitochondrial respiratory capacity and oxygen supply/delivery in exercise deficiencies of metabolic disease (25, 65, 83) is required to operationally define the mechanistic causes of decreased muscle performance.

MICROVASCULAR IMPAIRMENTS AT MULTIPLE LEVELS

Dilator Reactivity:

Of the myriad vascular impairments that accompany metabolic disease, the one that is consistently identified (regardless of model) is an impaired endothelial function that stems from a loss of vascular nitric oxide bioavailability (92). Any discussion of vasculopathy with metabolic disease must, almost by definition, start with this concept as whether one is discussing models of diabetes mellitus, dyslipidemia, type II diabetes, or a combined, multi-pathology state, the loss of nitric oxide bioavailability impacting vascular reactivity is consistent across nearly every vascular bed. This loss of nitric oxide bioavailability with metabolic diseases obviously impacts vascular reactivity in multiple ways, blunting dilator responses to a wide array of agonists/stimuli (77, 99), but also through a loss of any buffering effect for constrictor responses to adrenergic, peptidergic and myogenic stimuli (7, 60, 72).

A focal point of ongoing debate and investigation centers not only on the mechanistic basis of the reduction to nitric oxide (NO) bioavailability; but also on how the different mechanisms contribute to an integrated outcome rather than just on the actual mechanisms themselves (18, 28, 54). Multiple studies have clearly identified mechanistic pathways including oxidant radical scavenging (5), co-factor bioavailability (2), expression of key proteins and enzymatic steps (5), impairments to residue phosphorylation compromising NOS activity (49), among others. While there are variances in the contributions of these pathways depending on the specific model of metabolic disease, the organ in question, and the specific time point within the evolution and progression of the disease state, the loss of vascular NO bioavailability under these conditions is one of the defining elements of the impaired vascular function with metabolic disease.

In addition to the loss of vascular NO levels, a myriad of other metabolites have emerged that are associated with a loss of dilator reactivity and a potential enhancement of constrictor responses. Among these are the increased production of carbon monoxide from heme oxygenase HO –1 (46, 83), an array of alterations to the normal patterns of arachidonic acid metabolism to include reductions to PGI₂, PGE₂, elevations in TxA₂ and PGH₂ as well as altered metabolism through the lipoxygenase, CP450 ω -hydroxylase, epoxygenase and other pathways (28, 34, 108, 110). Additionally, there are studies implicating changes to the generation of oxidative radicals such as peroxynitrite (ONOO⁻) and hydrogen peroxide (H₂O₂) as well as other gaseous mediators such as hydrogen sulfide H₂S (13, 45, 64). However, the extent to which these functionally impact vascular function and the regulation of resistance, as well as the extent to which they act as primary mediators of vascular reactivity or agents which emerge as compensatory processes under conditions of low NO bioavailability remains an active area of investigation and one with considerable potential from the perspective of therapeutic potential (59).

Constrictor Responses:

Changes to constrictor responses within the resistance vasculature with chronic metabolic disease have elucidated somewhat more heterogeneous responses between models and organ/tissue types. With models of diabetes (or significant insulin-resistance) alone, these responses are somewhat modest, including mild elevations to adrenergic constriction or constrictor responses to specific agonists such as endothelin-1, angiotensin II or TxA₂ (17, 98, 109). However, to date it is somewhat unclear the extent to which these shifts in constrictor responses reflect actual changes to the signaling mechanisms and sensitivity underlying vasoconstriction or the loss of a buffering pathway through the shifts toward poor endothelial function itself (33, 70, 71). It does seem apparent that, with the exception of models of severe metabolic disease where a fundamental process such as sympathetic activity is significantly elevated causing chronically increased constrictor tone, the changes in constrictor responses have a more modest impact on controlling bulk blood flow to the organ in question and may be of greater importance in terms of the spatial and temporal distribution of perfusion within the organ or tissue (57).

Structural Remodeling:

While it is clear that the reactivity of the resistance vasculature in multiple organs across models of metabolic disease suffers from an imbalance resulting in poor dilator responses (with or without an increase in constrictor influences), the structural alterations to resistance arterioles and microvascular networks themselves represent the second major venue through which a compromised vascular function can be realized with chronic metabolic disease.

At the individual vessel level of resolution, these structural alterations of consequence are manifested as a change to the mechanics of the vascular wall, resulting in a reduced distensibility, an increased wall stiffness, and an overall lowering of deformability (96). The extent to which this is present across models of metabolic disease and across organs can be highly variable. In models of diabetes, these effects are generally mild (if present), but can be more significant with models of atherogenic dyslipidemia and especially so if significant hypertension also develops (4, 15, 88).

The impact of remodeling of the vessel wall on blood flow appears to be dependent on the metabolic demand of the tissue at that time. Under resting conditions and at low levels of metabolic demand, the impact of a loss of distensibility of the resistance vessel wall appears to be minimal as bulk blood flow requirements are not sufficiently elevated to be constrained by the increase in the minimum resistance that would accompany the structural remodeling (33). However, as metabolic demand increases and the functional hyperemic response grows to match that need, the progressive elevation in the minimum resistance arising from the remodeling of the arteriolar wall creates a condition wherein the progressive loss in resistance arteriolar distensibility, particularly in the proximal arterioles, results in a growing constraint on hyperemic responses with continuing elevations in metabolic demand (33). Obviously, this has the potential to create a progressively worsening ischemic condition in the tissue/organ as metabolic demand increases.

While it remains unclear that there are consistent alterations to the overall structure of the microvascular networks by the evolution of metabolic disease (i.e., branch angles, length density, fractal geometry of arteriolar networks), there are certain patterns that have emerged in specific tissues (e.g., retinal microcirculation) that are continuing as active areas of investigation in terms of their impact on perfusion distribution (16, 80). However, one of the more consistent observations in peripheral tissues across models of metabolic disease is a progressive rarefaction of the microvascular network, resulting in a reduced density of terminal arterioles, capillaries and post-capillary venules (29). As with the other sites of impairment discussed above, the severity of the reduction to microvessel density is dependent on the specific model of metabolic disease (28), the time point within the disease process (29), and the organ in question (14, 19). It does seem apparent that the reduction to microvessel density reaches an asymptote at approximately a 25–30% reduction in the total microvessel density before the expression of angiogenic and angiostatic influences equalize to terminate the net loss of microvessels (29).

In some respects, the mechanistic bases of the evolving rarefaction of the microvascular network parallels that for the impairments to vascular tone regulation, as there is compelling evidence that the pro-oxidant and pro-inflammatory conditions that accompanies metabolic disease are associated with changes to vasoactive metabolite bioavailability that may be an initiating factor to microvessel loss (29). Taken in combination with multiple other mechanistic pathways that have been implicated in contributing to the loss of microvessels with metabolic disease (73, 74), this area of research clearly remains one of considerable unknown; but which is also with outstanding potential for therapeutic benefit.

The progressive loss of capillaries has clear negative implications for tissue and organ function under resting conditions, and this becomes increasingly concerning as metabolic demand increases due to impairments to mass transport and exchange through multiple mechanisms. This decay in the efficiency and efficacy of mass transport and exchange is not only a central contributor to poor tissue/organ performance under “normal physiological” conditions, but is also a critical aspect in terms of poor outcomes for wound healing (23) and following such challenges as ischemia:reperfusion injury under conditions of stroke or myocardial infarction (24), as examples.

While there have been reports of alterations to venular and lymphatic function with the development of chronic metabolic disease (58, 89), the impact of these effects on tissue/organ function with alterations to metabolic demand and the regulation of blood flow are unclear at present. Additional investigation will be required to determine if alterations in venular function are sufficiently significant to impact hemodynamics in the upstream capillaries and compromise mass transport and exchange to a meaningful extent.

HEMODYNAMIC IMPLICATIONS OF VASCULAR ALTERATIONS

The cumulative impact of these alterations to vascular reactivity, vascular structure and microvessel network density, likely in combination with additional alterations such as endothelial glycocalyx degradation (36, 97) and any rheological alterations associated with metabolic disease (93) appear to have significant implications for hemodynamics within the

distal microcirculation and the ability of the microvascular networks to adapt to imposed challenges.

Notably, a previous study has demonstrated that not only are the number of anatomically present capillaries reduced with metabolic disease, the numbers of these capillaries that are perfused with a significant number of erythrocytes at any time are also reduced, leading to overall reductions to capillary tube hematocrit and O₂ delivery to regions of tissue within the *in situ* skeletal muscle (12, 75, 78). This compromised outcome likely reflects the combination of the dysregulation of the control of vascular tone (and resistance) within the arteriolar networks, leading to an increasingly heterogeneous distribution of erythrocytes at arteriolar bifurcations (the “network Fahraeus effect”; Ref. 82) and the lowered capillary hematocrit and number of RBC-perfused capillaries overall with metabolic disease.

We have recently provided some evidence that this impairment to the spatial distribution of erythrocytes and perfusion that develops in the microcirculation with metabolic disease may serve as a central link between poor perfusion/blood flow responses and poor skeletal muscle fatigue resistance (31). Specifically, as the distribution of erythrocytes at successive arteriolar bifurcations becomes increasingly heterogeneous, this leads to an iterative effect culminating at the pre-capillary level characterized by a significant increase in not only blood flow distribution heterogeneity into the capillaries, but also in terms of the distribution of red blood cells entering into these networks.

The effect of this increasingly heterogeneous spatial distribution of perfusion and erythrocytes will be to compromise virtually all processes of mass transport and exchange in a manner extremely similar to that identified by Poole and colleagues (40, 52, 75, 78, 85). These impairments include reductions to the percentage of erythrocyte-perfused capillaries, capillary hematocrit, erythrocyte flux and the oxygen delivery per unit of muscle in the diabetic condition, leading to reductions in microvascular oxygen pressures (75). Further this can be exacerbated by the rarefaction of the capillary networks outlined above and fails to consider the loss of system flexibility and adaptability in the microcirculation, discussed below, that can accompany metabolic disease of progressively increased severity (10, 28).

Biological systems, when faced with challenges that compromise critical outcomes, attempt to compensate and adjust their behavior in an attempt to maintain these outcomes to the extent possible. In this case, the most relevant system compensation for an increasingly heterogeneous spatial distribution of erythrocytes is an increased temporal activity at bifurcations (also known as temporal switching or “flicker”). In this condition, the heterogeneous distribution of flow or erythrocytes is at least partially reversed, such that the original “high flow” pathway loses perfusion and the “low flow” pathway receives a greater proportion of the perfusion entering the bifurcation. This would reflect the temporal changes to the downstream resistance in the “low flow” branch as a result of the greater stimuli for dilation through multiple potential mechanistic pathways (10, 28).

However, this compensation system does not appear to be functional within the arteriolar networks following the development of metabolic disease (10, 28). Rather, the temporal activity, or flexibility, of the individual bifurcations appears to be damped down with

evolution of metabolic disease such that the increased spatial heterogeneity of blood flow and erythrocyte distribution becomes increasingly entrenched and is more difficult to overcome, requiring a greater than normal degree of stimuli to cause significant changes to the perfusion distribution at the bifurcation. The end result of this combination of increased spatial heterogeneity of erythrocyte and flow distribution and a decreased temporal flexibility of perfusion control at arteriolar bifurcations, when iterated repeatedly to the level of the capillary networks, is that there are a greater number of pathways through the distal microcirculation that experience low levels of flow, hematocrit and O₂ delivery (at the expense of a small number of regions of very high flow, hematocrit and O₂ delivery) and that these regions are increasingly stable and more difficult to modify. Obviously, the skeletal muscle cells that are almost completely dependent on the convective and diffusive delivery and removal of metabolic substrates and end-products, respectively, are in a position where they are either experiencing an excessive or wasted level of perfusion beyond what they can utilize or are subject to an increasingly entrenched level of ischemia, with the obvious implications for their ability to maintain metabolism and, thereby, muscle cell function (i.e., the ability to maintain tension development).

MITOCHONDRIAL FUNCTION AND SKELETAL MUSCLE METABOLISM IN METABOLIC DISEASE

As previously stated skeletal muscle performance is limited by either oxygen supply to or oxygen consumption through mitochondrial oxidative phosphorylation (MOP). Determining whether oxygen supply (limitations previously described) or mitochondrial respiratory capacity limits MOP can be performed in a broad array of metabolic (dys)functional states (37–39). In healthy human subjects one can test where these limits arise with a simple experiment by altering arterial oxygen concentrations and measuring oxidative phosphorylation rate following exercise perturbations. If arterial oxygen concentration is increased or decreased *and* oxidative phosphorylation rate goes unchanged, this suggests that oxygen availability *is in excess* of the mitochondrial capacity. Conversely if increasing oxygen concentration also increases MOP rates it must mean oxygen availability is *limiting* the system. Using this simple experimental perturbation by breathing gases of differing oxygen concentrations Richardson et al. were able to show that mitochondria were the limiting factor in healthy human subjects (39) while oxygen supply was the limiting factor in exercise trained (38) and peripheral arterial disease patients (37). These studies support the contention that not only can limitations be measured during dynamic exercise, but alterations can be made to reveal the true determinant of muscle respiratory capacity. Taken together, it is clear that in diseases where muscle performance is compromised mitochondrial function and oxygen supply/delivery must each be considered to conclude where limits to performance exist for each metabolic (dys)functional state (67).

Mitochondria and Metabolic Disease:

Mitochondrial function is composed of two factors: mitochondrial respiratory capacity for the organelle itself; and the absolute volume fraction of mitochondria within a given cell. Furthermore, specifically relevant to muscle one must also consider the muscle fiber type composition since the mitochondrial fraction varies considerably between cell types (50, 55,

56) and has been suggested as an adaptive mechanism in some metabolic diseases (43, 69). Mitochondrial function is important in metabolic disease since MOP drives substrate utilization and skeletal muscle can account for up to 80% of glucose uptake (22). Mitochondrial function has been the subject of intense investigation in type II diabetes since the work of Kelley and of Shulman in the early 2000s. First, Kelley and colleagues (2002) demonstrated less, smaller, and lower functioning mitochondria in muscle biopsies of individuals with type II diabetes as measured by electron microscopy, activity of the mitochondrial marker citrate synthase, and oxygen consumption of the respiratory enzyme NADH:O₂ oxidoreductase respectively. These findings of different mitochondria led to the conclusion that individuals with type II diabetes had an “impaired bioenergetic capacity of skeletal muscle mitochondria” (51). Subsequently, in a short review, Shulman (2005) expanded upon this and other works arguing that mitochondrial dysfunction was not only present in type II diabetes but a major contributor to the development of insulin resistance (62). However, what is not often considered in the study of mitochondria and diabetes is the impact of the potentially altered metabolic state on apparent mitochondrial function. For example, if a reduction in blood flow were present (as suggested by microcirculation discussed above) this would directly influence interpretation of mitochondrial respiratory capacity by slowing phosphocreatine recovery due to limiting oxygen supply to MOP. Slowed phosphocreatine recovery could falsely lead to a conclusion of mitochondrial dysfunction. In fact, reduced blood flow has been shown to also reduce phosphocreatine recovery rate linearly with flow restriction by using cuff restriction of blood flow to the exercising leg during ³¹Phosphorus-magnetic resonance spectroscopy (³¹PMRS) (65). For this reason, mitochondrial function must be measured *in vivo* while considering/controlling for the altered metabolic state or studied *in vitro* removing the influence of the cellular environment.

Mitochondrial Function in Vivo:

Mitochondrial function *in vivo* is routinely assessed by measuring phosphocreatine recovery time following exercise. This technique has been used broadly in studies performed on human subjects and animal models and is a well-accepted measure of mitochondrial respiratory capacity (76, 106). In individuals with type II diabetes there have been several studies measuring phosphocreatine recovery time following an exercise bout with varying confounding outcomes. Scheuermann-Freestone and colleagues (2003) reported decreased phosphocreatine recovery and concluded that the mechanism must have involved mitochondrial dysfunction (90). However, this study did not control for confounding factors of age, activity, or body mass index (BMI) commonly found in individuals with type II diabetes and each of these variables has been independently shown to influence mitochondrial respiratory capacity (9, 42). The importance of stringent control between subjects is highlighted by a subsequent work by Praet et al. (2006) where similar phosphocreatine recovery times were found in individuals with type II diabetes and compared to the control subjects in the Scheuermann-Freestone study (81). Similarly, others have concluded mitochondrial dysfunction in individuals with type II diabetes while controlling for BMI but without considering the activity levels of their research subjects (66, 91). Once confounding factors such as age, BMI, and activity levels are carefully controlled the apparent difference in mitochondrial function between controls and individuals with type

II diabetes is no longer evidenced as measured from phosphocreatine recovery rates in these subjects (20).

The vast array of conflicting observations within the human literature is likely due to subject variability thus many influential studies in metabolic disease must rely upon well-controlled inbred animal models particularly in the case of type II diabetes. Further, human *in vivo* studies are mostly performed using voluntary contractions which involves muscle recruitment (95) in contrast to the complete recruitment that occurs during sciatic nerve stimulation when using animal models. Measurement of mitochondrial function *in vivo* has been performed in common animal models of metabolic disease including the obese Zucker rat, Zucker diabetic fatty rat (ZDF), and Goto-Kakizaki (GK) rat however the results do not always yield consistent outcomes due to the manner in which experiments were performed. For example, in work by Wessels et al. (2015) ZDF rats studied by ³¹P MRS spectroscopy showed a slowed phosphocreatine recovery rate compared to their matched controls following a tetanic contraction suggesting mitochondrial dysfunction (105). In contrast, in a study performed under the same experimental conditions, De Feyter et al. (2008) demonstrated no difference in phosphocreatine recovery between ZDF diabetic and their matched controls thereby concluding there was not a difference in mitochondrial function (22). Further, using the non-obese GK rat model of type II diabetes no difference in phosphocreatine recovery was shown following exercise at over three times the reported muscle respiratory capacity of 1 Hz twitch stimulation (44) with the authors concluding no mitochondrial dysfunction relative to proper controls (63). Importantly, interpretation of phosphocreatine kinetics at exercise intensities beyond the muscle respiratory capacity is suspect because of the elevated metabolic demands. If there are limitations to oxygen supply, particularly in metabolic diseases, then the large metabolic demands imposed in working muscle preparations may exceed MOP function at an even lower intensity than in control preparations and an acidic muscle pH will develop proportionate to the lactate production from compensatory activity of glyco(geno)lysis (27). Under such circumstances the phosphocreatine recovery kinetics no longer describe MOP function but are constrained by either direct effects of pH on the creatine kinase reaction, or, indirect effects on MOP function by the limited oxygen availability. The inherent variability and comorbidities within human populations necessitate use of many animal models to advance the understanding of mechanism at least initially. Using a rat model of type II diabetes and measuring mitochondrial function within the muscle respiratory capacity where oxygen supply and muscle pH are not influencing interpretation is crucial. For this reason, an experiment performed at several workloads below the muscle respiratory capacity where ATP consumption driven by contraction can achieve a steady state with MOP ATP production is ideal to cogently answer the question of *in vivo* mitochondrial (dys)function in type II diabetes.

Mitochondrial Function in Vitro:

The prevailing method for mitochondrial functional assessment is by measuring mitochondrial ATP production when isolated from the cellular environment, removing confounding influences such as oxygen and substrate supply or muscle pH. The most quantitative approach for mitochondrial respiration *in vitro* is High-resolution respirometry

(Oroboros Instruments). High-resolution respirometry determines mitochondrial function by measuring mitochondrial oxygen consumption after a bolus injection of ADP. Since the experiment is performed in a sealed chamber, all oxygen consumed after a bolus of ADP is directly related to the production of ATP through complex IV of the mitochondrial respiratory chain (100, 101). While this measure is performed in non-physiological conditions (i.e. saturating O₂), it permits comparison of maximal aerobic function without confounding influences present in the cellular environment.

High-resolution respirometry is routinely used to measure mitochondrial function in biopsies from individuals with type II diabetes and samples harvested from animal models. When removed from the cellular environment, mitochondria in both human diabetes patients and rat models of type II diabetes consistently demonstrate similar respiratory capacity per mitochondrial mass when compared to their respective controls. Specifically, in two separate works individuals with type II diabetes showed similar mitochondrial respiratory capacity to BMI matched controls determined from changes in oxygen consumption using high-resolution respirometry after mitochondria were isolated from vastus lateralis muscle (1, 68). Similar results were shown in skeletal muscle mitochondria isolated from the ZDF (93, 103, 104) and GK (48) rat models of type II diabetes. Mitochondrial respiratory capacities were comparable in both diabetic and control samples determined from high-resolution respirometry and some studies even showed slightly higher fat oxidation capacity in the diabetic mitochondria.

Taken together, results within the metabolic disease state of type II diabetes suggest mitochondrial function is not impaired when isolated from the cellular environment. For this reason alone, conclusion of mitochondrial dysfunction in type II diabetes should be suspect to careful interpretation. This measurement would be very powerful if performed in parallel with *in vivo* measurements in the same animal. This would allow for little discrepancy between what is seen *in vivo* versus *in vitro* to concisely determine the limit to mitochondrial oxidative phosphorylation during muscle performance when accounting for potentially confounding factors in metabolic disease.

Integration and Interpretation:

Integrating multiple sources of information and a diverse body of previous work leads to a reasonable speculation as to the relationships between microvascular dysfunction and metabolic derangements that develop in skeletal muscle with the evolution of metabolic disease. It seems plausible that the initial dysfunction resides predominantly within the microcirculation and microvascular networks, and this is defined by an increasingly heterogeneous spatial distribution of intramuscular perfusion. This reflects a variety of contribution mechanisms, but ultimately compromises not only the effectiveness of mass transport and exchange, but also the flexibility in the patterns of blood flow distribution in the networks on which effective microvascular function is so critically dependent. This blunting of microvascular function leads to the creation of an increasingly constrained environment for mitochondria to function (*in vivo*), thus impairing their ability to function effectively with increasing metabolic demand.

The idea that measures of metabolic demand indicated by PCr kinetics or oxygen consumption cannot by themselves have a unique identifying limitation on muscle respiratory capacity without additional information is illustrated in Figure 2. Phosphocreatine (A) and oxygen consumption (B) kinetics are shown at a fixed workload or oxygen cost (C) under three different metabolic conditions. Black lines represent the control condition where mitochondrial function and oxygen delivery are adequate to match work demand. PCr hydrolysis reaches a steady state and its recovery post-exercise reflects the muscle respiratory capacity. Furthermore, inspection of panel (B) shows how the PCr kinetics mirror the VO_2 kinetics and matches the oxygen cost of contraction. The green dashed lines represent the exercise trained condition where the mitochondrial volume fraction *and* oxygen delivery capacity have increased from control. As a consequence, exercise training results in less PCr hydrolysis and a faster recovery post-exercise (A) and a correspondingly faster increase in VO_2 (B). These changes reflect influences of both enhanced mitochondrial function *and* oxygen delivery. In Contrast, the red dotted lines represent the condition where *either* mitochondrial function *or* oxygen delivery is reduced. Reduced mitochondrial density generally increases PCr hydrolysis and recovery time (A) and has been separately shown to also slow VO_2 kinetics (B). In a metabolic disease such as peripheral arterial disease reduced oxygen delivery could also increase level of PCr hydrolysis, slow PCr recovery and VO_2 kinetics. In the absence of additional data from this Figure one cannot ascertain the mechanistic cause of the red traces in either panels A or B. This example demonstrates the ambiguity derived from insufficient sampling of the system parameters. Consequently, when performing measurements *in vivo* we argue additional measures of mitochondrial function or microvascular function must be performed simultaneously or in parallel to derive a mechanistic conclusion regarding what is actually limiting the system as conceptualized here. Whether this can be validated will rely on future work in this area taking into consideration the functional state of the system as a whole rather than the isolated organ or organelle itself.

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Impacts of Metabolic Disease/Type II Diabetes Mellitus

• Reduced microvessel density (rarefaction) • Increased perfusion heterogeneity • Reduced bulk blood flow • Reduced arteriolar wall distensibility • Reduced convective O₂ delivery	• Increased tissue cylinder radius • Reduced NO bioavailability (oxidant scavenging) • Reduced PGI₂/TxA₂ ratio • Reduced diffusive O₂ delivery • Altered adrenergic tone • Reduced tissue PO₂	• Reduced peri-mitochondrial PO₂ • Reduced PCr/Pi ratio • Altered membrane potential • Reduced mitochondrial density • Impaired O₂ utilization

Figure 1.

A schematic representation of the impacts of chronic metabolic disease on the microcirculation and surrounding parenchymal (i.e., skeletal muscle) tissue at the whole microvascular network, blood/tissue exchange unit, and mitochondrial levels of resolution. The three columns provide a listing of the impacts of chronic metabolic disease on structure and function at each level of resolution.

Figure 2.

Representative phosphocreatine (A) and oxygen consumption transients (B), (re-created in part from Figure 2 of Poole and Jones (79)) at three varying metabolic conditions performing the same amount of work (O_2 Cost) (C). Black lines represent the control condition where mitochondrial function and oxygen delivery are adequate for work. The green dashed lines represent the exercise trained condition. The red dotted lines represent the condition where *either* mitochondrial function *or* oxygen delivery is reduced. For example, reduced mitochondrial density has been shown to increase PCr hydrolysis and recovery time (A) and separately shown to slow VO_2 kinetics (B). Likewise, in a metabolic disease such as peripheral arterial disease reduced oxygen delivery could also increase PCr hydrolysis, slow PCr recovery and VO_2 kinetics.