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Cardiac, skeletal, and smooth muscle mitochondrial respiration: are all mitochondria created equal?

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Unlike cardiac and skeletal muscle, little is known about vascular smooth muscle mitochondrial respiration. Therefore, the present study examined mitochondrial respiratory rates in smooth muscle of healthy human feed arteries and compared with that of healthy cardiac and skeletal muscles. Cardiac, skeletal, and smooth muscles were harvested from a total of 22 subjects (53 ± 6 yr), and mitochondrial respiration was assessed in permeabilized fibers. Complex I + II, state 3 respiration, an index of oxidative phosphorylation capacity, fell progressively from cardiac to skeletal to smooth muscles (54 ± 1, 39 ± 4, and 15 ± 1 pmol·s^{-1}·mg^{-1}, $P < 0.05$, respectively). Citrate synthase (CS) activity, an index of mitochondrial density, also fell progressively from cardiac to skeletal to smooth muscles (222 ± 13, 115 ± 2, and 48 ± 2 μmol·g^{-1}·min^{-1}, $P < 0.05$, respectively). Thus, when respiration rates were normalized by CS (respiration per mitochondrial content), oxidative phosphorylation capacity was no longer different between the three muscle types. Interestingly, complex I state 2 normalized for CS activity, an index of nonphosphorylating respiration per mitochondrial content, increased progressively from cardiac to skeletal to smooth muscles, such that the respiratory control ratio, state 3/state 2 respiration, fell progressively from cardiac to skeletal to smooth muscles (5.3 ± 0.7, 3.2 ± 0.4, and 1.6 ± 0.3 pmol·s^{-1}·mg^{-1}, $P < 0.05$,

respectively). Thus, although oxidative phosphorylation capacity per mitochondrial content in cardiac, skeletal, and smooth muscles suggest all mitochondria are created equal, the contrasting respiratory control ratio and nonphosphorylating respiration highlight the existence of intrinsic functional differences between these muscle mitochondria. This likely influences the efficiency of oxidative phosphorylation and could potentially alter ROS production.

Keywords:
oxidative phosphorylation capacity; respiratory control ratio; feed arteries

THE INTEGRATED FUNCTION of cardiac, skeletal, and vascular smooth muscles is essential for O_2 delivery and utilization, especially during exercise, when synchronicity can determine capacity. Cardiac muscle produces the driving force to convectively transport blood-borne O_2 to the periphery, where skeletal muscle uses this O_2 for the metabolic requirements of locomotion (35, 36). Smooth muscle, the major component of the arterial system, dictates the distribution of blood flow and O_2 transport, dependent on need (6, 33). Each of these distinct muscle tissues contain mitochondria, which consume O_2 and produce ATP through cellular respiration. Interestingly, al- though mitochondrial respiration of both cardiac and skeletal muscles has been studied extensively in health and disease (4, 5, 31, 32, 39), little is known about smooth muscle mitochondrial respiration (42).

The heart is a vital organ with a high metabolic demand and is subsequently rich in mitochondria, with these mitochondria accounting for ~35% of the volume of cardiac tissue (39) and generating up to 90% of ATP requirements by 13-oxidation at rest (21, 28, 30). Attenuated coupled respiration and an increase in uncoupled respiration in cardiac muscle are common indicators of heart disease (4). Indeed, diseased hearts often exhibit decreased oxidative phosphorylation, secondary to both reduced mitochondrial enzymes and content (27, 47), and excessive mitochondrial free radical production in human and animal models (16, 38). In skeletal muscle, due to the high

metabolic requirements for locomotion, mitochondrial ATP production is also certainly important, with typically 3– 8% of the skeletal muscle volume being mitochondria (23), but this is highly dependent on physical activity. Additionally, studies have revealed that a reduction in skeletal muscle mitochondrial oxidative phosphorylation capacity and/or volume may con- tribute to muscle dysfunction (11–13). Therefore, understanding and characterizing mitochondrial function in both cardiac and skeletal muscles has implications in both health and disease.

Although far less well studied, the mitochondria within smooth muscle are thought to play a role in maintaining vascular tone, facilitating cellular transport, and producing energy for vascular cell secretion (42, 46). These mitochondria typically comprise 3~5% of the smooth muscle cell volume (3). Until recently, little was known about the role of vascular smooth muscle mitochondria in terms of vascular function and disease. Now, new evidence suggests the potential importance of mitochondrial function in the development of vascular diseases (14, 43, 45). However, it is important to note that most of these studies used mitochondrial protein expression or protein activity to estimate mitochondrial function rather than actual measurements of mitochondrial respiratory capacity, and, hence, direct assessments of mitochondrial respiration in vascular smooth muscle are still lacking. Indeed, there has yet to be a comprehensive assessment of mitochondrial respiratory rate in smooth muscle and a comparison of this function with cardiac and skeletal muscles.

Recognizing the lack of data specific to mitochondrial function in smooth muscle of the vasculature, the present study sought to assess mitochondrial respiration in smooth muscle of skeletal muscle feed arteries and compare this with that of cardiac and skeletal muscles. Due to the anticipated differences in mitochondrial density between cardiac, skeletal, and smooth muscles, in conjunction with the vastly different functional requirements of each, it was hypothesized that, on a mitochondrion-to-mitochondrion basis, respiratory function would be very similar. This would indicate that all muscle mitochondria are created equal in terms of respiration, regardless of origin.

METHODS

Subjects. A total of 22 subjects (17 men and 5 women) participated in this study.

Cardiac muscle was harvested from four subjects (2 men and 2 women), skeletal muscle was harvested from nine subjects (9 men), and skeletal muscle feed arteries were harvested from nine subjects (6 men and 3 women). All subjects were free from overt cardiovascular disease, and there was no evidence of involvement in regular exercise, as determined by interviews and medical records. Although none of the subjects were taking medications recognized to alter mitochondrial function, cardiac and skeletal muscle feed artery donors were anesthetized using a standard protocol that included propofol, fentanyl, benzodiazepines, and vecuronium bromide dosed according to the patient's body weight, whereas lidocaine was used for local anesthesia of subjects donating skeletal muscle. Subjects or their legal representative (cardiac muscle donors) provided informed consent, and study protocols were approved by the University of Utah and Veteran's Affairs Medical Center Institutional Review Boards.

Cardiac muscle. Cardiac muscle was harvested from the left ventricular apex of normal donor hearts, not allocated for heart transplantation due to noncardiac issues (e.g., heart size, incarceration, etc.). Fat and connective tissue were removed from cardiac muscle in precooled *buffer A* [containing (in mM) 2.77 CaK_2EGTA, 7.23 K_2EGTA, 6.56 $MgCl_2$, 0.5 DTT, 50 K-MES, 20 imidazol, 20 taurine, 5.77 Na_2ATP, and 15 phosphocreatine, pH 7.1 at 4°C], and the sample remained in this solution until permeablization.

Skeletal muscle. After local anesthesia (5–10 ml of 1% Lidocaine HCL, Hospira, Lake Forest, IL), skeletal muscle was harvested by the needle biopsy technique from the lateral aspect of the quadriceps muscle. Skeletal muscle was stored and dissected in precooled *buffer A*, and the sample remained in this solution until permeablization.

Skeletal muscle feed arteries. Human skeletal muscle feed arteries (~7 mg wet wt) were harvested as previously reported (20). Briefly, feed arteries (200 –500 μm) were identified and harvested during melanoma-related surgeries in either the axillary and inguinal regions. Specifically, skeletal muscle feed arteries, supplying the serratus anterior, latissimus dorsi, quadriceps femoris, and hip adductor muscles, were harvested for this study. Skeletal muscle feed arteries were stored and dissected in precooled *buffer A*, and the sample remained in this solution until permeablization.

Preparation of permeabilized tissues. All muscle samples were stored in

precooled *buffer A* for <30 min before the commencement of the permeabilizing procedures (22). Specifically, cardiac and skeletal muscle tissues were teased apart by needle tip to increase permeability of the membrane and avoid limited diffusion of the substrates. After mild shaking for 30 min (cardiac and skeletal muscles) and 40 min (vessels) in *buffer A* with saponin (5 mg/ml), the muscle was rinsed twice in *buffer B* [containing (in mM) 2.77 CaK_2EGTA, 7.23 K_2EGTA, 6.56 $MgCl_2$, 0.5 DTT, 20 imidazole, 5.77 ATP, 15 phosphocreatine, 50 K-MES, and 20 taurine, pH 7.0] for 10 min. Note that the optimum duration of permeabilization for vessel mitochondria (longer than cardiac and skeletal muscles) was deter- mined by a series of pilot studies before this investigation. Specifically, four different permeablization time periods (20, 30, 40, and 50 min) were assessed, with the highest respiration rate occurring with the 40-min period, without membrane damage (cytochrme *c*). The optimal substrate concentration for vessel mitochondrial respiration was determined by titrating the ADP concentration in 1-mM increments (final chamber concentration: 1– 6 mM), which revealed that an ADP concentration of 5 mM resulted in the highest respiration rate and that 6 mM did not facilitate any further increase.

Mitochondrial respiration. Mitochondrial respiratory O_2 flux was assessed with a Clark-type high-resolution Oxygraph respirometer (Hansatech, Kings Lynn, UK). Permeabilized muscle fibers (2– 4 mg wet wt) were incubated in the respirometer with 2 ml *buffer B* while being continuously stirred at 37°C. First, baseline muscle respiration was recorded, in the absence of respiratory substrates. To assess the function of each mitochondrial complex, O_2 consumption was assessed with the addition of a series of respiratory substrates and inhibitors in the following order and final concentrations in the chamber: glutamate-malate (2:10 mM), ADP (5 mM), succinate (10 mM), cytochrome *c* (10 μM), rotenone (0.5 μM), antimycin-A (2.5 μM), oligomycin (2 μg/ml), and *N*,*N*,*N*,*N*-tetramethyl- *p*-phenylene- diamine (TMPD)-ascorbate (2:0.5 mM). This allowed the determination of *1*) complex I state 2 respiration, the nonphosphorylating resting state that provides an index of proton leak, assessed in the presence of malate + glutamate; *2*) complex I, state 3 respiration, the ADP- activated state of oxidative phosphorylation, assessed in the presence of glutamate + malate + ADP; *3*) complex I + II, state 3 respiration, assessed in the presence of glutamate + malate + ADP +

succinate; and *4*) complex IV respiration, assessed by blocking complex 3 (antimycin A) and complex 5 (oligomycin) followed by TMPD + ascorbate. O_2 consumption derived from autoxidation of TMPD and ascorbate was measured with the following method: first, complex III respiration was inhibited by antimycin A, and then complex IV respiration was measured. Complex IV respiration was then blocked by cyanide at the very low O_2 tension in the chamber to measure chemical respiration. Chemical and instrument-related respiration (without tissue) was subtracted from total complex IV respiration to calculate complex IV respiration.

In each condition, the respiration rate was recorded for 3 min, and the average of the last minute was used for data analysis. Mitochondrial membrane integrity was evaluated by cytochrome *c* induction. The rate of O_2 consumption was measured as picomoles of O_2 per second and then expressed relative to muscle sample mass (in pmol·s^{-1}·mg wet wt^{-1}). These respiration rates were further normalized by either citrate synthase (CS) activity or complex IV respiration. The respiratory control rate (RCR) was calculated by state 3/state 2 respiration, normalized for CS activity. The substrate control ratio for succinate was calculated as complex I + II state 3/complex I state 3.

CS activity. After the respiration measurements, the same muscle samples (3.5–4.5 mg wet wt) were homogenized with homogenization buffer [containing (in mM) 250 sucrose, 40 KCl, 2 EGTA, and 20 Tris·HCl] (Qiagen, Hilden, Germany). The CS activity assay was performed as previously described (31) and read with a spectrophotometer (Biotek Instrument).

Statistical analysis. One-way ANOVA was performed using SPSS (version 18, SPSS, Chicago, IL). If significance was detected, a Tukey post hoc test was used to identify the significant difference. For all analyses, a P value of <0.05 was considered significantly different. All data are expressed as means ± SE.

Table 1. Subject characteristics

	Cardiac Muscle	Skeletal Muscle	Smooth Muscle
Total number of subjects	4	9	9
Age, yr	52±3	54±3	52±1
Height, cm	161±15	174±3	177±4
Body mass, kg	57±10	77±10#	104±7*†
Systolic blood pressure, mmHg		120±2	121±3
Diastolic blood pressure, mmHg		78±2	77±2
Medication			
Over-the-counter analgesics		0/9	2/9
Ibuprofen or excedrin		0/9	2/9
Cardiovascular	0/4	0/9	0/9
Diabetic	0/4	0/9	0/9

Data are expressed as means ± SE or number of subjects (out of the total number). Note that users of cardiovascular (statin, 13-blocker, angiotensin- converting enzyme inhibitor, diuretic, Ca^{2+} channel blocker, etc.) and diabetic (insulin, metformin, etc.) medications were excluded from the study. *$P < 0.05$, smooth muscle vs. cardiac and skeletal muscles; †$P < 0.05$, smooth muscle vs. skeletal muscle; #$P < 0.05$, skeletal muscle vs. cardiac muscle.

RESULTS

Subject characteristics. The ages of the subjects who pro- vided cardiac muscles (52 ± 3 yr), skeletal muscles (54 ± 3 yr), and smooth muscles (52 ± 1 yr) were well matched across the groups. However, cardiac muscle donors were significantly shorter in stature (height: 161 ± 15 cm) and lighter (weight: 57 ± 10 kg) than skeletal muscle donors (height: 174 ± 3 and weight: 77 ± 10 kg) and smooth muscle donors (height: 177 ± 4 cm and weight: 104 ± 7 kg), who were also different from each other in terms of body weight (Table 1).

Oxidative phosphorylation. As shown in Fig. 1, when state 3 respiration rates for both complex I as well as complex I and II were expressed per unit of wet weight, oxidative phosphorylation capacity fell progressively from cardiac to skeletal to smooth

muscles (complex I: 43 ± 0.1 vs. 32 ± 3 vs. 13 ± 2 skeletal to smooth muscles (222 ± 13, 115 ± 2, and 48 ± 2 μmol·min^{-1}·mg^{-1}, $P < 0.05$, respectively; Fig. 2*A*) and was therefore well correlated with both complex I ($r = 0.8$) and complex I + II, state 3 (Fig. 2*A*) respiration rates. When state 3 respiration for complex I as well as complex I and II was normalized by CS activity, mitochondrial respiration in these three muscle types, expressed simply as wet weight (Fig. 1), were no longer apparent, with cardiac, skeletal, and smooth muscles all exhibiting similar rates of oxidative phosphorylation (Fig. 2*B*). Complex IV was highly correlated with CS activity (Fig. 3*A*), and, thus, when state 3 respiration, assessed at either complex I or complex II, was normalized for complex IV respiration, sometimes considered an index of respiratory efficiency, there were no differences in this ratio across cardiac, skeletal, and smooth muscles (Fig. 3*B*). Also, the substrate control ratio was not different across cardiac, skeletal, and smooth muscles (1.3 ± 0.1, 1.3 ± 0.1, and 1.2 ± 0.3, respectively).

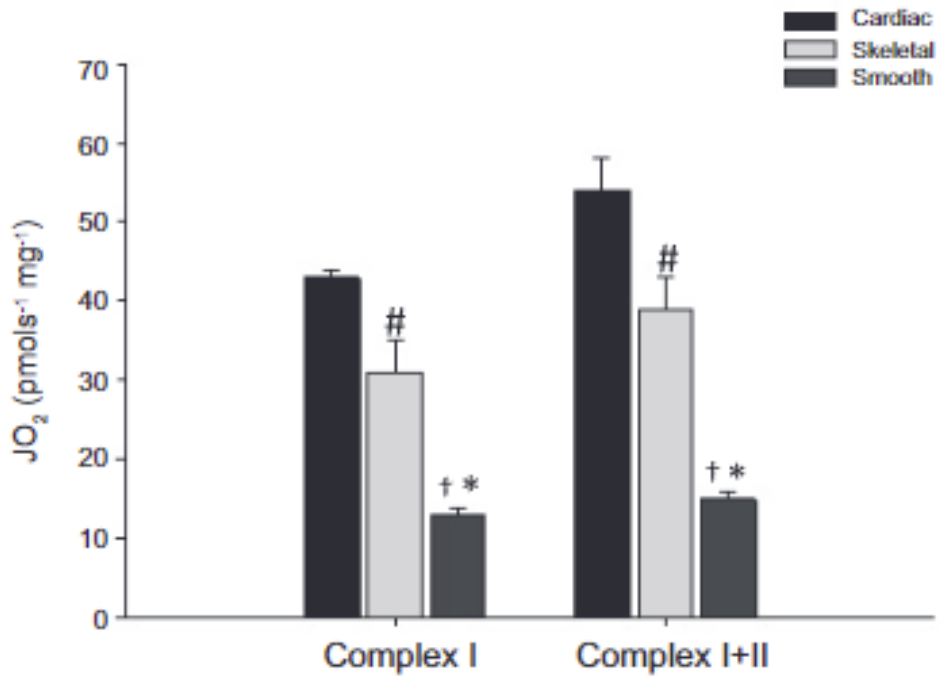

Fig. 1. Oxidative phosphorylation capacity in cardiac, skeletal, and vascular smooth muscles. JO2,O2flux. *P<0.05, smooth muscle vs. cardiac and skeletal muscles; †P<0.05, smooth muscle vs. skeletal muscle; #P<0.05,skeletal muscle vs. cardiac muscle. Complex I, complex I state 3 respiration; complex I+II, complex I+II, state 3 respiration.

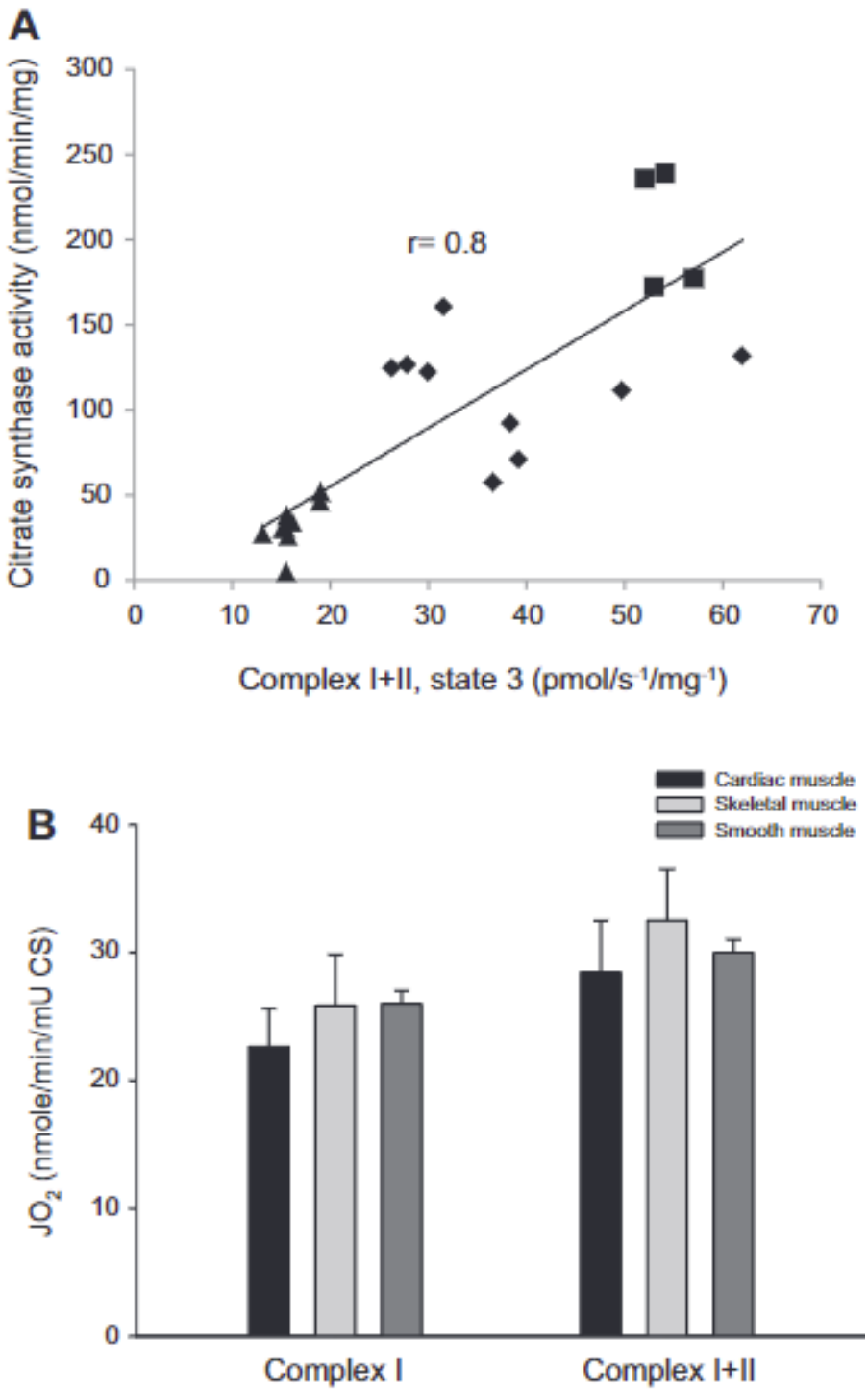

Fig. 2.A: relationship between citrate synthase (CS) activity and oxidative phosphorylation capacity (complex I+II, state 3) in cardiac muscle (squares),skeletal muscle (diamonds), and vascular smooth muscle (triangles).P<0.001.B: oxidative phosphorylation capacity normalized by CS activity, a marker of mitochondrial density, in cardiac, skeletal, and vessel smooth muscles.

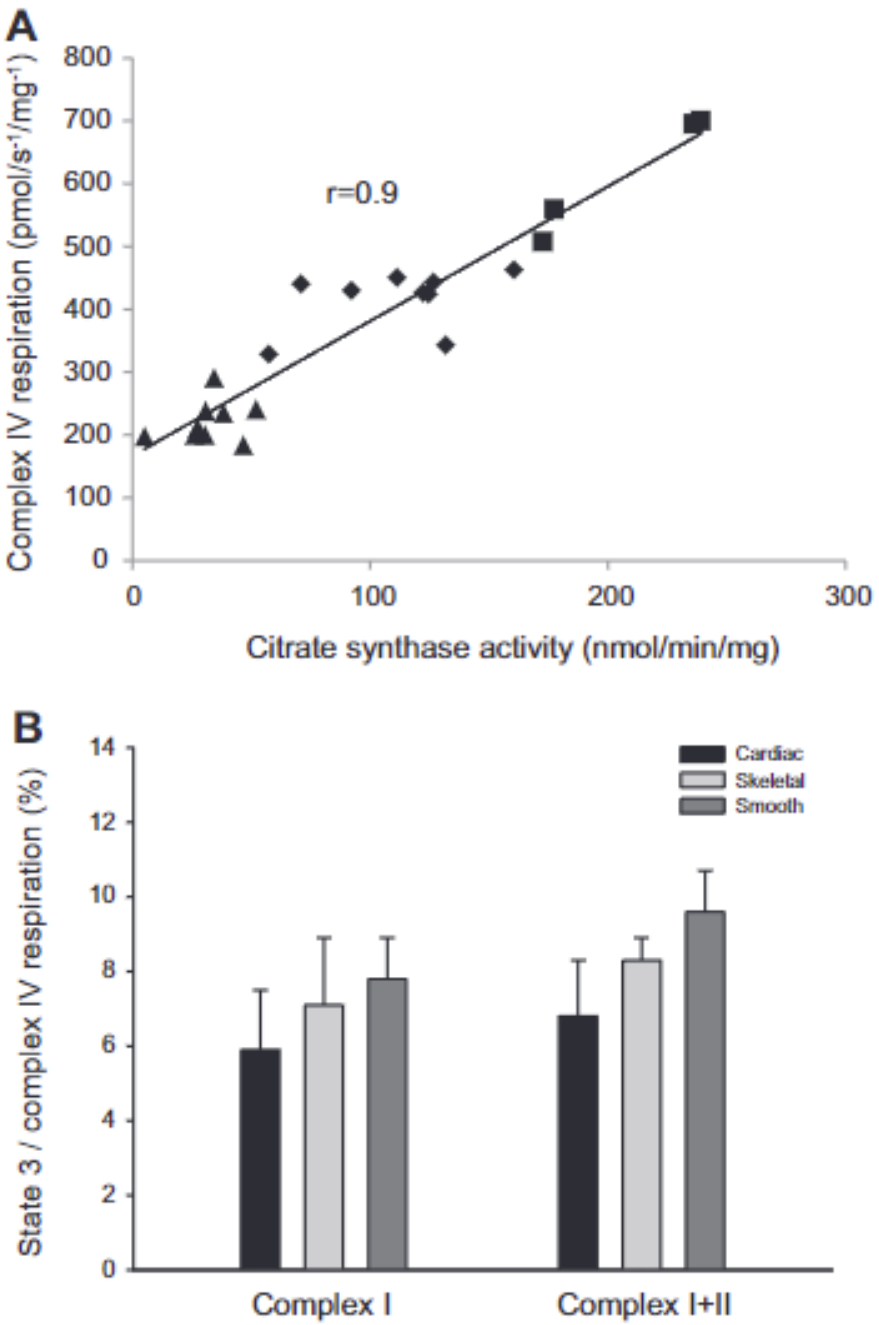

Fig. 3. *A*: relationship between CS activity and complex IV respiration (*N*,*N*,*N*,*N*-tetramethyl- *p*-phenylenediamine + ascorbate) in cardiac muscle (squares), skeletal muscle (diamonds), and vascular smooth muscle (triangles).r = 0.9, $P < 0.001$. *B*: oxidative phosphorylation capacity normalized by electron transport capacity.

Nonphosphorylating respiration and RCR. Complex I state 2 respiration, an index of proton leak, when expressed as wet weight, was not significantly different in cardiac,

skeletal, and smooth muscles (Fig. 4*A*). When mitochondrial density was accounted for by normalizing these wet weight data by CS activity, there was a progressive rise in nonphosphorylating respiration or greater proton leak from cardiac to skeletal to smooth muscles (5.5 ± 0.5, 10.4 ± 0.8, and 16.4 ± 1.6 pmol·mg^{-1}·CS activity^{-1}, respectively; Fig. 4*B*). Thus, when the RCR (complex I + II, state 3 respiration/complex I, state 2 respiration) was calculated, there was a progressive fall from cardiac to skeletal to smooth muscles (5.3 ± 0.7, 3.2 ± 0.4, and 1.6 ± 0.3, respectively; Fig. 4*C*).

DISCUSSION

There are several novel findings of this study. First, vascular smooth muscle mitochondrial respiration can be successfully assessed using the same permeablized fiber approach routinely used with both cardiac and skeletal muscles. Second, likely a consequence of very different functional roles, oxidative respiratory capacity, measured as complex I + II, state 3 respiration and expressed as muscle weight, fell progressively from cardiac to skeletal to to smooth muscles. However, when respiration rates were normalized by CS activity, an index of mitochondrial content (23), allowing cardiac, skeletal, and smooth muscles to be compared in terms of mitochondrial content, complex I + II, state 3 respiration was very similar. Third, complex IV respiration across all muscles was very well correlated with CS activity, supporting previous suggestions that this is a good marker of mitochondrial content (23). Thus, normalization for complex IV respiration also annulled the differences in oxidative phosphorylation capacity between the three types of muscle. Finally, there were significant muscle- specific differences in both nonphosphorylating respiration and RCR. Therefore, although this study provides evidence that cardiac, skeletal, and smooth muscle mitochondria appear to be very similar in terms of oxidative phosphoylation capacity, RCR and nonphosphorylating respiration are certainly not similar. This highlights the existence of intrinsic functional differences between these muscle mitochondria, which likely influences the efficiency of oxidative phosphorylation and could potentially alter ROS production.

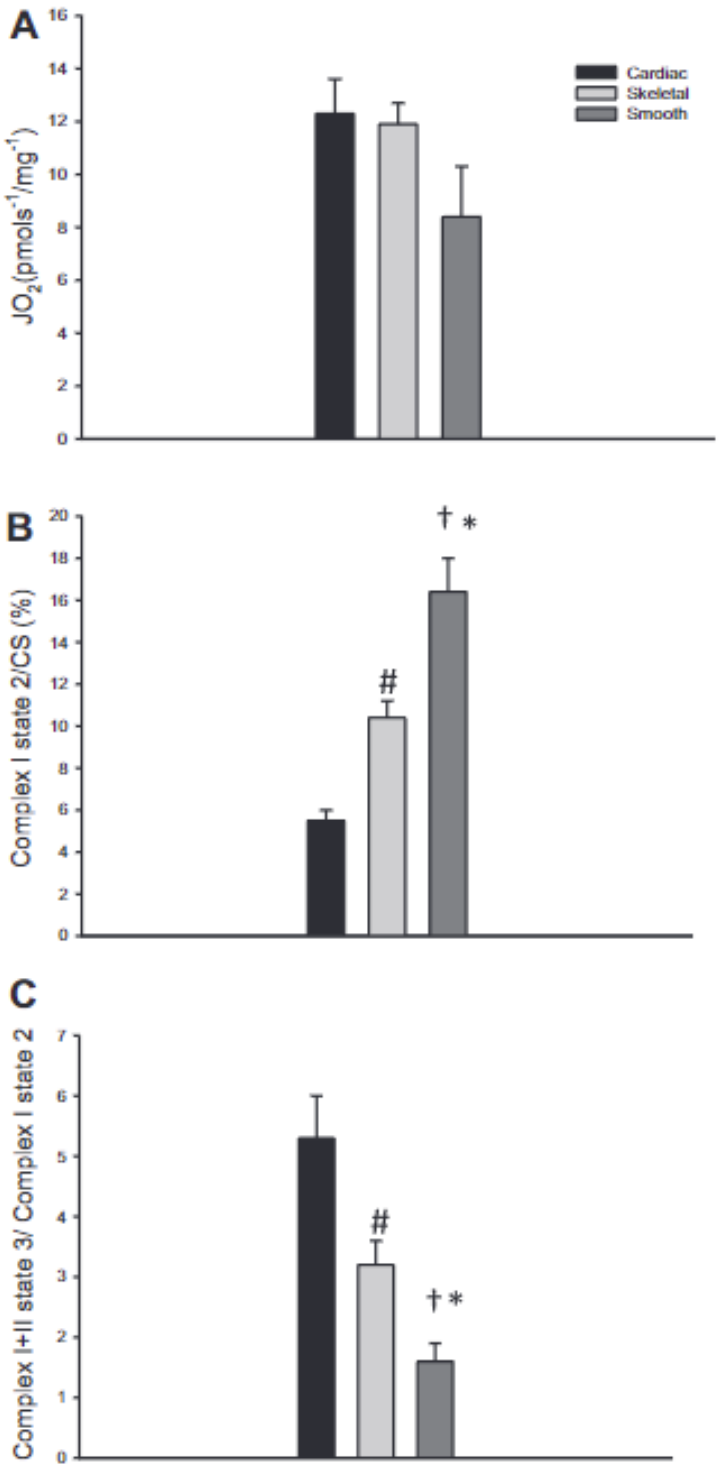

Fig. 4. *A*: complex I state 2 respiration. *B*: complex I state 2 respiration normalized by CS activity, a marker of mitochondrial density. *C*: respiratory control ratio, complex I + II, state 3 normalized by complex I state 2 in cardiac, skeletal, and smooth muscles. *$P < 0.05$, smooth muscle vs. cardiac and skeletal muscles; †$P < 0.05$, smooth muscle vs. skeletal muscle; #$P < 0.05$, skeletal muscle vs. cardiac muscle.

Cardiac, skeletal, and smooth muscle structure and function. Although both cardiac and skeletal muscles are striated, skeletal muscle is composed of slow (type I) and fast (type II) fibers, whereas cardiac muscle consists of a single fiber type, most similar to type I skeletal muscle fibers. In contrast, smooth muscle cells are not striated but rather consist of dense bodies and intermediate filaments. Smooth muscle contraction and relaxation are slower than both skeletal and cardiac muscle because contraction is accomplished by Ca^{2+}-regulated phosphorylation of myosin rather than the Ca^{2+} and troponin system. This mechanism, which can be activated by stretch, is highly efficient, requiring less ATP for contraction and allowing smooth muscle to maintain tension for prolonged periods of time with a relatively low energy cost compared with cardiac and skeletal muscles (18).

In addition to being of great importance for cellular redox homeostasis, O_2 sensing, and intracellular signaling, mitochondria are the major source of muscle ATP production (2). In fact, as the amount of ATP stored within cardiac muscle (=10 mmol/kg) and skeletal muscle (20–25 mmol/kg) is typically insufficient to meet even the short-term dynamic energy de- mands of these muscles, the mitochondria are largely responsible for the continued resynthesis of this ATP (19, 25). The same is true for smooth muscle; however, due to a combination of a reduced requirement to perform work and greater efficiency of contraction, smooth muscle would be expected to contain fewer mitochondria than skeletal and cardiac muscles. Indeed, the present evaluation reveals that smooth muscle mitochondrial content, as assessed by CS activity, is only ~42% and ~22% that of skeletal and cardiac muscles, respectively. Of note, as factors that alter mitochondrial density, such as individual differences in physical activity and skeletal muscle fiber type composition, these relative mitochondrial con- tents may fluctuate significantly. For example, Lemieux et al. (24) previously reported that mitochondrial density in skeletal muscle was ~33% of cardiac muscle, whereas the present data revealed closer values, with skeletal muscle exhibiting ~52% of the mitochondrial density of cardiac muscle.

Interestingly, in qualitative agreement with the CS activity data and therefore mitochondrial content, the present study revealed that complex IV respiration, an indirect indicator of electron transport capacity, fell from cardiac to skeletal to smooth muscles. Consequently, CS activity and complex IV respiration were well correlated (Fig. 3*A*). Collectively, these findings confirm that electron transport capacity is dictated by mitochondrial content in skeletal and cardiac muscles but extend this finding to include smooth muscle. Additionally, the present study revealed that mitochondrial content in these three distinct muscle types is tightly linked to oxidative phosphorylation capacity (complex I + II, state 3; Fig. 2*A*) and the expected level of aerobic work based on in vivo function, with a progressive fall in capacity evident from cardiac to skeletal to smooth muscles.

Oxidative phosphorylation capacity: muscle- versus mitochondrial-specific content. Muscle-specific phosphorylation capacity (wet wt) was highest in cardiac muscle, falling progressively from skeletal to smooth muscles (Fig. 1). In terms of cardiac

and skeletal muscles, these findings are well aligned with the limited studies that have revealed that the oxidative phosphorylation capacity of cardiac muscle exceeds that of skeletal muscle (24, 26) but extends these findings to include the, now documented, even lower oxidative phosphorylation capacity of smooth muscle. As would be expected, because of the strong relationship between CS activity and oxidative phosphorylation capacity (complex I + II, state 3; Fig. 2*A*), the difference in oxidative phosphorylation capacity in these three distinct tissues was negated when normalized to CS activity (Fig. 2*B*). Thus, in terms of mitochondrial content, oxidative phosphorylation capacity did not vary significantly from cardiac to skeletal to smooth muscles (Fig. 2*A*). This was also the case for the efficiency of oxidative phosphorylation capacity per mitochondrial content, which was not statistically different across the three muscle types (Fig. 3*B*).

Vessel smooth muscle mitochondrial respiration has not been comprehensively studied (15, 42, 44), with this work being not only the first to examine smooth muscle mitochondrial respiratory function with real conviction but also the first to compare human vascular smooth muscle mitochondrial respiration with that of human cardiac and skeletal muscles. Vascular smooth muscle mitochondria derived ATP production is thought to be mainly required for the maintenance of myogenic tone and facilitating cellular transport (42). In contrast, mitochondria in cardiac and skeletal muscles produce ATP to facilitate contraction and relaxation and subsequently perform considerable, measurable work. For the heart, this results in the maintenance of cardiac output and blood pressure, whereas for skeletal muscle, the task is predominantly posture and locomotion. The differing role of each of these muscles requires varied amounts of mitochondria-derived ATP production. Specifically, based on in vivo function, smooth muscle does not likely require the same level of oxidative capacity as cardiac and skeletal muscles due to the lower ATP demand required for the maintenance of myogenic tone and cellular function compared with active force generation. Thus, the reduced tissue mass-specific smooth muscle oxidative capacity, compared with both cardiac and skeletal muscles, in conjunction with a similar oxidative phosphorylation capacity per mitochondrial content, and less of a need for ATP production, teleogically seems appropriate. Therefore, although oxidative phosphorylation capacity in these

three distinct muscle types is different, similar mitochondrion respiratory function but differing mitochondrial content enable each muscle tissue to match cellular energy demand with supply.

Inferences from distinct nonphosphorylating respiration and RCR across muscle types. The proton gradient between the matrix and intermembrane space of the mitochondrion (the proton motive force) facilitates ATP production, and, in com- bination with nonphosphorylating proton conductance (proton leak), these processes regulate the kinetics and efficiency of mitochondrial respiration (40). Proton leak also plays a role in determining the level of ROS, although it is controversial as to whether an increased proton leak reduces ROS production via decreased proton motive force and a subsequent attenuation of the coupled oxidative phosphorylation (34) or if an increased proton leak increases O_2 consumption leading to increase ROS production (37, 41). Interestingly, in the present study, com- plex I state 2 respiration, an indicator of proton leak, was not different across cardiac, skeletal, and vascular smooth muscles when the respiration rate was normalized for tissue wet weight (Fig. 4*A*). This suggests that ROS production per unit of cardiac, skeletal, and smooth muscle tissues may be similar. However, when complex I state 2 respiration was normalized to CS activity, proton leak per mitochondrial content rose progressively from cardiac to skeletal to smooth muscles. This difference in proton leak per mitochondrial content between these three distinct muscle types could, potentially, be due to the different proton permeability of mitochondrial membranes in cardiac, skeletal, and smooth muscles (Fig. 4*B*) (9, 10).

In terms of ROS production and the vasculature, it should be noted that mitochondria of endothelial cells are also an important source for ROS. Recently, Ungvari and colleagues (42) suggested that increased vascular ROS production may be due to reduced mitochondrial content in endothelial cells. How- ever, it should be noted that, in preparation for the present study, pilot data were collected in feed arteries with and without endothelium (denuded with air bubbles). Mitochondrial respiration from endothelial cells makes up an immeasurable portion of the respiration assessed with the present experimental design (state 3 respiration, complex I + II: 13 ± 5 vs. 12 ± 7 $pmol \cdot s^{-1} \cdot mg^{-1}$, respectively). This is likely due both to the very low mitochondrial content and very small overall volume of endothelial cells in the preparation. However,

it is still important to acknowledge the potentially very important interaction between mitochondria-derived ROS and nitric ox- ide from the endothelium, which may play a significant role in the regulation of cellular respiration (29). Therefore, further investigations in this area are warranted.

The RCR, which encapsulates the main respiratory function of mitochondria inasmuch as it documents the ability to idle at a low respiratory rate but responds to ADP by making ATP at a high rate, has been suggested to be a good indicator of mitochondrial respiratory dysfunction when the electron trans- port chain capacity is intact (7, 8, 17). Therefore, the values of RCR in the present study can be interpreted as an index of intrinsic mitochondrial respiratory function. RCR was the highest in cardiac muscle, intermediate in skeletal muscle, and the lowest in smooth muscle (Fig. 4*C*). In this case, differing RCRs in cardiac, skeletal, and smooth muscles may be an indicator of an altered physiological role of endogenous proton leak and ADP oxidation across muscle types rather than dysfunction. This interpretation is well aligned with conclusions from previous studies that have revealed that pancreatic and liver cells have stronger control of ATP/ADP by proton leak than skeletal muscle (1, 8). Thus, based on RCR and proton leak, the present study has identified that different muscle tissues have distinct intrinsic mitochondrial respiratory functions, which likely influences the efficiency of oxidative phosphorylation and could potentially alter ROS production.

Experimental considerations. A fundamental concern in a study that examines several different muscle types, but har- vests each of these muscles from different subjects, is the issue of subject heterogeneity. Indeed, the tissue used in the present study was not harvested from the same person because both logistically and ethically this would be very difficult to achieve. Therefore, it must be acknowledged that a limitation of the present study was the variability of the subjects in terms of lifestyle (e.g., diet, physical activity, etc.). However, presumably, these lifestyle differences were randomly distributed among the groups, as subjects were not selected for any particular habits. In contrast, the potential influence of anesthesia was not the same in all groups. Specifically, cardiac and smooth muscle donors were exposed to a routinely used set of anesthetics, whereas skeletal muscle donors received only topical lidocaine, and the impact of these exposures on the present results is unknown. However, the good and

intuitively expected agreement between both CS activity and complex IV respiration and state 3 (compex I + II) respiration across all muscles (Figs. 2*A* and 3*A*) suggests that this was not a major confounding factor in terms of the assessment of mitochondrial respiration.

Additionally, in the present study, there were no direct measurements of redox balance or ROS, but there was speculation that ROS production may be different between the three muscles examined. This certainly warrants further studies to confirm this speculation; however, it has been well documented that increased proton leak, as documented here (Fig. 4*B*), is associated with the regulation of ROS production (34, 37, 40).

Summary. This study used the permeabilized fiber approach to assess smooth muscle mitochondrial respiration in human arteries and contrasted these novel data with findings in human cardiac and skeletal muscles. Although complex I + II, state 3 respiration rates, when normalized by CS activity, were similar in cardiac, skeletal, and smooth muscles, there were significant muscle-specific differences in both nonphosphorylating respiration and RCR. Therefore, the present study has identified that different muscle tissues have distinct intrinsic mitochondrial respiratory functions, which likely influences the efficiency of oxidative phosphorylation and could potentially alter ROS production.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.-Y.P., F.D., S.L., and R.S.R. conception and design of research; S.-Y.P., J.R.G., R.H.A., J.D.T., J.R.H., R.S.G., N.A.D., S.J.I., S.D., and R.S.R. performed experiments; S.-Y.P., J.R.G., R.S.G., and R.S.R. analyzed data; S.-Y.P. and R.S.R. interpreted results of experiments; S.-Y.P. prepared figures; S.-Y.P., F.D., S.L., S.D., and R.S.R. drafted manuscript; S.-Y.P., R.H.A., J.D.T., J.R.H., F.D., S.L., and R.S.R. edited and revised manuscript; R.S.R. approved final version of manuscript.

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