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Tissue-specific and substrate-specific mitochondrial bioenergetics in feline cardiac and skeletal muscles

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ABSTRACT. No studies have investigated the mitochondrial function in permeabilized muscle fiber from cats. The aim of this study was to investigate tissue-specific and substrate-specific characteristics of mitochondrial oxidative phosphorylation (OXPHOS) capacity in feline permeabilized oxidative muscle fibers. Biopsies of left ventricular cardiac muscle and soleus muscle, a type I-rich oxidative skeletal muscle, were obtained from 15 healthy domestic cats. Enzymatic activity of citrate synthase (CS), a biomarker of mitochondrial content, was measured. Mitochondrial OXPHOS capacity with various kinds of non-fatty-acid substrates and fatty-acid substrate in permeabilized muscle fiber was measured by using high-resolution respirometry. CS activity in the heart was 3 times higher than in the soleus muscle. Mitochondrial state 3 respiration, ADP-stimulated respiration, with complex I-linked and complex I+II-linked substrates, respectively, was significantly higher in the heart than in the soleus muscle when normalized for muscle mass, but not for CS activity, indicating that greater capacity for mitochondrial OXPHOS with these non-fatty-acid substrates in the heart may depend on higher mitochondrial content. In contrast, the soleus muscle had higher mitochondrial state 3 respiration with fatty acids than the heart when normalized for CS activity, indicating greater capacity for fatty-acid oxidation per mitochondrion in the soleus. Our findings suggest that there are tissue- specific and substrate-specific quantitative and qualitative differences in mitochondrial OXPHOS capacity between the different types of oxidative muscles from cats.

KEY WORDS: feline, heart, mitochondria, oxidative phosphorylation, skeletal muscle

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Cardiac muscle and the soleus muscle are biochemically characterized as oxidative muscle, primarily relying on aerobic metabolism and mitochondrial oxidative phosphorylation (OXPHOS) to produce ATP. The soleus muscle is functionally classified as a postural skeletal muscle and consists of 98% type-I fibers in cats [1, 37]. Because of its homogeneous nature, feline soleus muscle is an excellent skeletal muscle for studying mitochondrial physiology [1, 39]. Oxidative muscle displays higher mitochondrial content than non-oxidative muscle. Indeed, 6% of the soleus muscle cell and 27% of the feline cardiac muscle cell are occupied by the mitochondria in cats [2, 13]. Due to the highest mitochondrial content in cardiac muscle among oxidative muscles, it is well established that mass-specific mitochondrial oxidative capacity in the heart is greater than in the oxidative skeletal muscle [7, 32]. However, recent evidence suggests that not only mitochondrial content but also mitochondrial qualitative factors, such as substrate sensitivity and coupling control, can influence oxidative capacity and metabolic flexibility of various muscle types [11, 15, 30].

Most previous studies have assessed mitochondrial function across different muscle types by using isolated mitochondria in animals and humans. However, disadvantages of using isolated mitochondria are the necessity of larger amounts of tissue due to lower mitochondrial yield and absence of interaction between the mitochondria and surrounding structures within the cytosol [27, 31]. To overcome these disadvantages, mitochondrial function can be investigated *in situ* by using saponin-skinned permeabilized fibers, and this has been recently proposed as one of the best methods to compare the levels of mitochondrial respiratory capacity in different types of muscles [15, 16, 28]. Moreover, it has recently been proposed that cats can be a good spontaneous investigation model for human metabolic and cardiac diseases [6, 12, 17], since cats are prone to develop cardiomyopathies with similar clinical and pathophysiological characteristics to humans [38]. However, no previous studies have investigated mitochondrial function in permeabilized muscle fiber from cats.

The purpose of our study was to conduct a thorough investigation of mitochondrial OXPHOS capacity with various substrates including non-fatty-acid and fatty-acid substrates in permeabilized cardiac muscle and skeletal muscle (soleus muscle) fibers from healthy domestic cats. In our study, quantitative analysis of mitochondrial OXPHOS capacity was conducted by normalization for muscle mass, and qualitative analysis by normalization for CS activity (i.e. analysis of mitochondrial OXPHOS capacity per mitochondrion).

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MATERIALS AND METHODS

Animals: The experimental protocol was approved by the Danish Animal Experimental Inspectorate (Authorization No. 2011/561–137) and conformed to EU Directive 2010/63/EU on the protection of animals used for scientific purposes. The study took place at the University Hospital for Companion Animals, University of Copenhagen, and written informed consent was given by the owners of cats prior to the study. Pedigree bred cats were eligible for the study when they were above one year of age and presented for euthanasia for reasons other than cardiac, systemic or endocrine diseases. Cats were excluded, if they had received any medication other than antiparasitic or contraception drugs within the last three months before the study. Information on sex and whether or not the cat was neutered was obtained.

Sample preparation: All cats underwent physical examination, blood tests, electrocardiogram and echocardiography. Only two cats needed sedation before echocardiography. Euthanasia was performed with an intramuscular injection of dexmedetomidine (0.04 mg/kg Dexdomitor, Orion Pharma, Turku, Finland) followed by an intravenous injection of pentobarbitone (150 mg/kg). Immediately following cardiac arrest, the heart was rapidly excised and weighed. The mid-left ventricular free wall was divided into two parts; the first was stored in an ice-cold relaxing and preservation solution (BIOPS; in mM; CaK_2EGTA 2.77, EGTA 7.23, taurine 20, $MgCl_2$ 6.56, ATP 5.77, phosphocreatine 15, dithiothreitol 0.5 and 4- morpholineethanesulfonic acid 50, pH 7.1) for measurements of mitochondrial OXPHOS capacity in permeabilized muscle fibers, and the remaining part was quickly frozen in liquid nitrogen and stored at −80°C for later analyses of enzymatic activities. In parallel, the proximal part of the soleus muscle was excised from the left rear limb and stored in the same way as the heart.

Echocardiographic measurements: Transthoracic echocardiographic measurements were performed to evaluate cardiac function by using a Vivid 7 Dimension ultrasound unit (GE Healthcare, Horten, Norway) with a 10S multifrequency phased array transducer (4–11.5 MHz), as previously described [10].

Mitochondrial enzymatic activities: CS and 3-hydroxyacyl-CoA dehydrogenase (HAD) activities were measured by using a spectrophotometry. Approximately 2 mg of cardiac muscle and 10 mg of soleus muscle were homogenized in 1.5 m*l* of 0.3 M K_2HPO_4 with 0.05% bovine serum albumin (pH 7.7) for 2 min on a Tissuelyzer (Qiagen, Venlo, Limburg, Netherlands). 15 μl of 10% triton was added, and the samples were left on ice for 15 min before they were stored at −80°C for later analysis.

CS: The homogenate was diluted 50 times in a solution containing 0.4 mM acetyl-CoA, 0.6 mM oxaloacetate, 0.157 mM 5,5′-dithiobis- (2-nitrobenzoic acid) (DTNB) and 39 mM Tris-HCl (pH 8.0). The change in DTNB to TNB at 37°C was measured spectrophotometrically at 415 nm [34] on an automatic analyzer, Cobas 6000, C 501 (Roche Diagnostics, Mannheim, Germany).

HAD: The homogenate was diluted 70 times in a solution containing 0.33 mM acetoacetyl–CoA, 180 μM NADH, 41.7 μM EDTA and 27.1 mM imidazole (pH 7.0). The change in NADH at 37°C was measured spectrophotometrically at 340 nm [3] on Cobas 6000, C 501.

All enzymatic activities are expressed as micromoles substrate per min per g wet weight of muscle tissue.

Preparation of permeabilized fibers: After rapid manual dissection of the tissue, fiber bundles were chemically permeabilized by gentle agitation for 30 min in an ice-cold BIOPS solution with saponin 50 μg/m*l*, as previously described [36]. After permeabilization, fibers were rinsed by agitation twice for 10 min in an ice-cold respiration medium MiR05 (in mM; sucrose 110, K-lactobionate 60, EGTA 0.5, 0.1% bovine serum albumin, $MgCl_2$ 3, taurine 20, KH_2PO_4 10 and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES] 20, pH 7.1).

Mitochondrial OXPHOS capacity with non-fatty-acid substrates and fatty-acid substrates: Mitochondrial OXPHOS capacity with non-fatty-acid and fatty-acid substrates in permeabilized fibers was measured at 37°C by using high-resolution respirometry (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria), as previously described [20, 35]. Samples of 1.5–2.5 mg of permeabilized cardiac muscle fiber and 2.5–3.5 mg of permeabilized skeletal muscle fiber each were transferred to the respiration chambers. All respiratory measurements were carried out in duplicate after hyperoxygenation to avoid any potential oxygen limitation for respiration. The integrity of the mitochondrial outer membrane was tested by addition of cytochrome *c*, and the data were eliminated if oxygen consumption rate increased by more than 10% as a sign of damaged outer mitochondrial membrane. Three substrate-uncoupler-inhibitor-titration (SUIT) protocols were applied in parallel to cardiac and skeletal muscles for the assessment of respiratory capacities of individual complexes of the electron transport system (ETS) with saturating concentrations of non-fatty-acid substrates, a medium-chain fatty-acid and a long-chain fatty-acid, respectively (Table 1).

Protocol I (including non-fatty-acid substrates): Resting non-ADP-stimulated respiration ($LEAK_N$) was obtained after supplying complex I-linked substrates, malate and glutamate (GM). Then, complex I-linked, ADP-stimulated state 3 respiration (GM3) was assessed, followed by addition of succinate for measurement of complex I+II-linked state 3 respiration (GMS3). Oligomycin was added to the respiratory chambers, thereby inhibiting the ATP synthase for obtaining the $LEAK_{omy}$ steady-state [8]. After addition of antimycin A (AA) for inhibition of complex III, N,N,N’,N’-tetramethyl-p-phenylenediamine (TMPD) and ascorbate (Asc) were finally added to measure the capacity of complex IV (TMPD/Asc). A chemical background correction of the flux was performed in order to correct for autooxidation of the reduced compounds Asc and TMPD [9].

Protocol II (including fatty-acid substrates): A medium-chain fatty-acid (octanoyl-l-carnitine; Oc) and a long-chain fatty-acid (palmitoyl-l-carnitine; Pa) were used as substrates in protocols IIa and IIb, respectively. Before the study, optimal concentrations of each fatty-acid for the heart and

Table 1. Substrate-uncoupler-inhibitor-titration (SUIT) protocols

Protocol I		Protocol II	
Malate	2 mM	Malate	2 mM
Glutamate	10 mM	Octanoyl-l-carnitine (IIa) or Palmitoyl-l-carnitine (IIb)	Heart: 0.625 mM, SOL: 1.25 mM or Heart: 0.1 mM, SOL: 0.2 mM
ADP	5 mM	ADP	5 mM
Cytochrome c	10 μM	Cytochrome c	10 μM
Succinate	10 mM	Rotenone	0.5 μM
Oligomycin	2 μg/m*l*	Succinate	10 mM
Antimycin A	2.5 μM	FCCP	0.25 μM Steps
Asc/TMPD	2/0.5 mM		

Substrates, inhibitors, ADP and FCCP were added in this order from the top in each protocol. Protocols IIa and IIb are identical, except for fatty-acids. Values indicate final concentration. SOL, soleus muscle; Asc, ascorbate; TMPD, N,N,N',N'-tetramethyl-p-phenylenediamine; FCCP, carbonylcyanide-4-trifluoromethoxy-phenylhydrazone.

skeletal muscle were determined by titration protocols in pilot studies. Non-adenylated resting respiration ($LEAK_N$) was assessed with malate and octanoyl-l-carnitine (MOc) or palmitoyl-l-carnitine (MPa). This was followed by state 3 respiration (MOc3 or MPa3), obtained after adding ADP. After addition of rotenone, an inhibitor of complex I, succinate was supplied to evaluate complex II-linked state 3 respiration (MOc[Rot]S3 or MPa[Rot]S3). Finally, titration with an uncoupling agent (carbonylcyanide-4-trifluoromethoxy-phenylhydrazone; FCCP) was carried out to evaluate maximal capacity for the ETS.

Statistical analysis: All values are presented as means ± standard error of the mean (SEM). Student's T-test was performed to compare means between heart and skeletal muscle. All statistical analyses were performed by using GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA, U.S.A.), and $P<0.05$ was considered statistically significant.

RESULTS

Baseline characteristics: Fifteen cats of the breeds Maine Coon cat (n=10) or Norwegian Forest cat (n=5) were enrolled in the study (Table 2). All male cats and two out of 11 female cats were neutered. Body condition ranged from four to seven on a nine-point body condition scoring scale (BCS), on which five is the ideal body condition [18]. Echocardiographic data showed that cardiac size and function were normal in all cats, according to a previously described classification [10].

Mitochondrial enzymatic activities: CS activity was 3 times higher in the cardiac muscle than the skeletal muscle, indicating greater mitochondrial content in heart (Fig. 1A). Furthermore, enzymatic activity of HAD, one of the fatty acid β-oxidation enzymes, was 2.3 times higher in the cardiac muscle (Fig. 1B).

Mitochondrial OXPHOS capacity with non-fatty-acid and fatty-acid substrates: In all respiratory states with non-fatty-acid substrates measured in protocol I, mass-specific mitochondrial respiratory capacity was significantly higher in the heart than in the soleus muscle (Fig. 2A). However, when normalized for CS activity, there were no differences in mitochondrial respiratory capacities between the oxidative muscles, except for complex IV capacity, which was significantly higher in the soleus muscle than in the heart (Fig. 2B).

Table 2. Baseline characteristics and echocardiographic variables

Variable	
Baseline parameters	
Age, years	4.9 ± 0.7
Gender (male/female)	4/11
Body weight, kg	5.3 ± 0.3
Heart weight, g	17.1 ± 0.1
Heart weight/Body weight, %	0.33 ± 0.04
Body condition score	5.4 ± 0.2
Echocardiographic data	
Left atrium/Aorta	1.20 ± 0.03
Interventricular septum thickness, mm	4.0 ± 0.1
LV free wall thickness, mm	3.9 ± 0.1
LV end-diastolic diameter, mm	14.8 ± 0.5
Fractional shortening, %	42.0 ± 2.6

Data are presented as means ± SEM (n=15). LV, left ventricular.

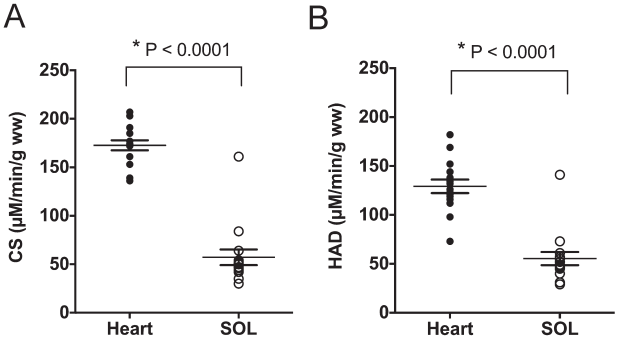

Fig. 1. Mitochondrial enzymatic activities. (A) Citrate synthase (CS) activity. (B) 3-hydroxyl-CoA-dehydrogenase (HAD) activity. Closed circles indicate the left ventricle (Heart), and open circles indicate the soleus muscle (SOL). The transverse bars indicate means ± SEM (n=15).

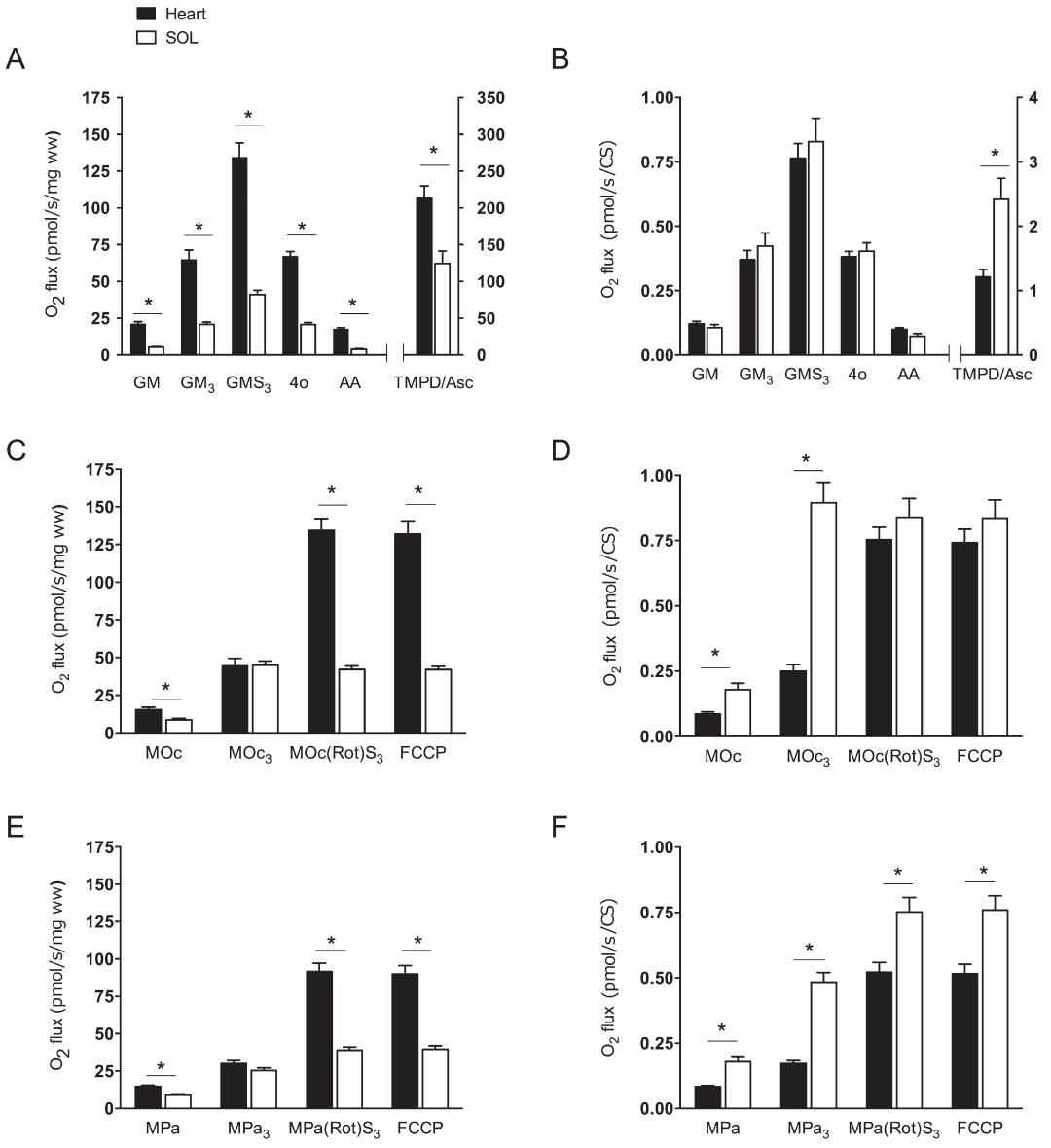

Fig. 2. Mitochondrial OXPHOS capacity with non-fatty-acid and fatty-acid substrates. Three substrate-uncoupler-inhibitor-titration (SUIT) protocols were used to evaluate mitochondrial OXPHOS capacity with various substrates in the left ventricle (Heart; closed bar) and soleus muscle (SOL; open bar). (A) Protocol I (normalization for muscle mass), (B) Protocol I (normalization for CS activity), (C) Protocol IIa (normalization for muscle mass), (D) Protocol IIa (normalization for CS activity), (E) Protocol IIb (normalization for muscle mass), (F) Protocol IIb (normalization for CS activity). Data are presented as means ± SEM (n=14–15). Asterisks show statistical differences (P<0.05) between oxidative muscles. G, glutamate; M, malate; S, succinate; AA, antimycin A; TMPD, N,N,N′,N′-tetramethyl-p-phenyldiamine; Asc, ascorbate; Oc, octanoyl-l-carnitine; Pa, palmitoyl-l-carnitine; Rot, rotenone; GM, resting $LEAK_N$ respiration with GM; GM3, state 3 respiration with GM; GMS3, state 3 respiration with GMS; 4o, $LEAK_{omy}$ respiration with oligomycin; MOc, resting $LEAK_N$ respiration with MOc; MOc3, state 3 respiration with MOc; MOc (Rot) S3, state 3 respiration with MOc (Rot) S; MPa, resting $LEAK_N$ respiration with MPa; MPa3, state 3 respiration with MPa; MPa (Rot) S3, state 3 respiration with MPa (Rot) S.

In protocol II, mass-specific MOc and MPa were significantly higher in cardiac than soleus muscles, however, mass-specific MOc3 and MPa3 were comparable between the two types of muscle (Fig. 2C and 2E). Moreover, mass-specific MOc (Rot) S3 and MPa (Rot) S3 were increased in cardiac muscle compared with soleus muscle, indicating greater mass-specific complex II-linked state 3 respiration in the heart (Fig. 2C and 2E). However, when normalized

for CS activity, MOc3 and MPa3 were significantly higher in the soleus muscle than in the heart (Fig. 2D and 2F). Similarly, MPa (Rot) S3, but not MOc (Rot) S3, was significantly higher in the soleus muscle than in cardiac muscle when normalized for CS activity (Fig. 2D and 2F). We also calculated respiratory control ratio (RCR; state 3/$LEAK_N$), which is known as a parameter for overall mitochondrial respiratory capacity independent of mitochondrial content [29]. RCR with malate and each of the fatty acids was higher in the soleus muscle than in the heart (RCR with MOc: 5.7 ± 0.5 *vs*. 3.0 ± 0.2, $P<0.0001$; RCR with MPa: 3.0 ± 0.2 *vs*. 2.0 ± 0.1, $P<0.001$), which was similar to MOc3 and MPa3 normalized for CS activity. There was no additional increase in respiration after addition of FCCP in both cardiac and skeletal muscles (Fig. 2C and 2E).

Furthermore, there were no apparent differences in mitochondrial OXPHOS capacity between young and aged cats and between female and male cats in any of the measured substrate states.

DISCUSSION

This study is the first to report the data on mitochondrial respirometric measurements in permeabilized cardiac and skeletal muscle fibers from cats. We compared mitochondrial OXPHOS capacity with non-fatty-acid and fatty-acid substrates between two functionally different types of muscles: the left ventricular cardiac muscle, with extremely high density of mitochondria, and the soleus muscle, a postural skeletal muscle with a relatively lower density of mitochondria when compared to cardiac muscle. Both muscles are oxidative and capable of utilizing fatty acids as well as non-fatty-acid substrates for ATP production. With complex I-linked and complex I+II-linked substrates, mass-specific mitochondrial OXPHOS capacity was higher in the cardiac muscle than in the soleus muscle. When normalized for mitochondrial content, mitochondrial OXPHOS capacity with these non-fatty-acid substrates was comparable between the two types of muscles. On the contrary, with fatty-acid substrates, there was no difference in mass-specific mitochondrial OXPHOS capacity between the cardiac muscle and the soleus muscle. Moreover, intriguingly, mitochondrial OXPHOS capacity with fatty-acid substrates was higher in the soleus muscle than in the cardiac muscle when it was normalized for mitochondrial content.

The differences in mass-specific state 3 respiration with complex I-linked and complex I+II-linked substrates, respectively, between cardiac muscle and soleus muscle correspond well to the previous studies of rodents in which two- to five-fold higher respiratory rates have been reported in the heart compared with the soleus muscle [7, 32]. In contrast, when normalized for CS activity, these differences in mitochondrial state 3 respiration did not persist, which raises the possibility that greater, mass-specific mitochondrial OXPHOS capacity with non-fatty-acid substrates in cardiac muscle solely depends on higher mitochondrial content rather than on qualitative differences between cardiac and oxidative skeletal muscles in cats.

Mass-specific mitochondrial state 3 respiration with fatty-acid substrates was comparable between cardiac muscle and soleus muscle from cats. However, enzymatic activity of HAD normalized for muscle mass was higher in the heart compared with the soleus muscle, suggesting that β-oxidation is not a rate-limiting factor for mitochondrial OXPHOS capacity with fatty acids. Moreover, HAD regulates only a single step in β-oxidation and may not reflect overall β-oxidation activity. In contrast, when normalized for CS activity, qualitative tissue-specific differences in mitochondrial OXPHOS capacity with fatty acids *do* exist between feline cardiac and soleus muscles. MOc3 and MPa3 were higher in the soleus muscle than in cardiac muscle, suggesting a higher maximal capacity to oxidize fatty acids per mitochondrion in the oxidative skeletal muscle. However, in the healthy heart, 90% of ATP is generated via mitochondrial OXPHOS with 60–70% of that energy being derived from fatty-acid oxidation [23]. To meet larger energetic demand in the heart, higher affinity for fatty acids including fatty acid uptake into the cell and fatty acid delivery in the cytosol as well as greater mitochondrial content of the left ventricle may contribute to increase in mitochondrial fatty-acid oxidation in the heart [22].

Intriguingly, tissue-specific quantitative and qualitative differences in mitochondrial OXPHOS capacity were dependent on types of substrates. Although we could not identify these substrate-dependent mechanisms, one possible explanation is that there may be a difference in the capacity of the electron transfer flavoprotein (ETF) between mitochondria from feline cardiac muscle and feline soleus muscle. ETF is an acceptor for reducing equivalents from the acyl-CoA dehydrogenase, one of the β-oxidation enzymes, and plays a role in electron transfer at the level of coenzyme Q via ETF oxidoreductase (ETF:QO) [5]. In fatty-acid linked mitochondrial OXPHOS, ETF and complex I are the main acceptors for reducing equivalents. Because mitochondrial OXPHOS capacity with non-fatty-acid complex I-linked substrates was comparable between the two types of muscles when normalized for CS activity, a higher ETF capacity may contribute to the increased mitochondrial OXPHOS capacity with fatty-acid substrates in the soleus muscle when normalized for CS activity. However, the precise mechanisms responsible for the substrate-dependent differences in mitochondrial OXPHOS capacity between feline oxidative muscles are unknown.

In our study, two kinds of fatty acids, octanoyl-l-carnitine (medium-chain fatty-acid) and palmitoyl-l-carnitine (long-chain fatty-acid), were applied to measure mitochondrial OXPHOS capacity with fatty-acid substrates. For the uptake of these fatty-acid-substrates into the mitochondria, palmitoyl-l-carnitine, but not octanoyl-l-carnitine, is dependent on the carnitine palmitoyl transferase I (CPT-I) and carnitine palmitoyl tranferase II (CPT-II), located on the outer and the inner mitochondrial membranes, respectively. In our study, when normalized for CS activity, MPa (Rot) S3, but not MOc (Rot) S3, was higher in the soleus muscle than in the cardiac muscle. This may indicate an increase in the activity of CPT-I and/or of CPT-II per mitochondrion in the soleus muscle

from cats, although we did not measure these activities.

The optimal concentration of each fatty acid necessary to reach maximal respiration was determined in the heart and soleus muscle in advance. When octanoyl-l-carnitine was used as a fatty-acid substrate, higher maximal respiration rates and RCRs were obtained than with palmitoyl-l-carnitine in permeabilized cardiac and skeletal muscle fibers. The same has been reported in human permeabilized cardiac muscle fibers [21]. Therefore, when designing SUIT protocols, octanoyl-l-carnitine may be the preferred choice over palmitoyl-l-carnitine to measure maximal oxidative capacity of fatty acids in permeabilized muscle fibers.

There was no additional increase in O_2 flux after adding FCCP in feline cardiac muscle and soleus muscle, which is not usually observed in human muscles [4, 14, 21, 26]. The non-coupled state of maximal respiration can be experimentally induced by FCCP to collapse the proton gradient across the mitochondrial inner membrane and measure excess capacity of the ETS [25]. The absence of enhanced respiratory capacity following uncoupling by FCCP suggests that no limitation of mitochondrial OXPHOS by the phosphorylation system is present in feline muscle [8]. However, it has been reported that excess capacity of ETS over OXPHOS is lowered in the presence of complex II-linked substrates compared to complex I-linked or complex I+II-linked substrates [8]. Because FCCP was added in the presence of complex II-linked substrates in our study, maximal ETS capacity may be underestimated. Therefore, we cannot completely exclude a possible excess capacity of the ETS over OXPHOS in cat muscles when applying different SUIT protocols.

The absolute maximal oxidative capacity was reached with Asc/TMPD in the heart and soleus muscle. Excess capacity of cytochrome *c* oxidase over complex I, II and III has previously been shown to exist in isolated mitochondria from the soleus muscle in cats [33] and other species [9]. In human vastus lateralis muscle, a strong correlation between mitochondrial content and complex IV capacity in permeabilized muscle fibers has been shown to exist [20]. When normalized for mitochondrial content, complex IV capacity was two-fold higher in the soleus muscle than in cardiac muscle, indicating that a higher excess capacity of cytochrome *c* oxidase is present in the soleus muscle compared to cardiac muscle. These qualitative measures of complex IV capacity suggest tissue-specific factors other than mitochondrial content as determining for the capacity of cytochrome *c* oxidase. Similar findings were observed in previous studies of other species [9, 24]. One possible determinant of the tissue-specific differences observed in the capacity of cytochrome *c* oxidase is O_2 affinity to the mitochondria [9]. It has been reported that mitochondrial O_2 affinity correlates with cytochrome *c* oxidase capacity [19].

In conclusion, we demonstrated that mitochondrial respiratory measurements of permeabilized muscle fibers with various SUIT protocols were applicable to cats, and intriguingly, there were quantitative and qualitative tissue-specific and substrate-specific differences in mitochondrial respiratory capacity between the cardiac muscle and oxidative skeletal muscle. Our findings may provide the basis of mitochondrial bioenergetics in permeabilized muscle fibers from cats and may support future research to clarify the pathophysiology of mitochondria-related disease, such as cardiomyopathy, in cats and other species including humans.

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