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In mammalian muscle, SIRT3 is present in mitochondria and not in the nucleus; and SIRT3 is upregulated by chronic muscle contraction in an adenosine monophosphate-activated protein kinase–independent manner

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ABSTRACT

In selected cell lines, it appears (*a*) that metabolic stressors induce the translocation of SIRT3 from the nucleus to mitochondria and (*b*) that SIRT3 may contribute to the regulation of mitochondrial biogenesis and/or fatty acid utilization. We have examined in mammalian muscle (1) the association between SIRT3 protein content and muscle oxidative capacity and mitochondrial fatty acid oxidation, (2) the subcellular location of SIRT3, (3) whether exercise induces the translocation of SIRT3 from the nucleus to the mitochondria, and (4) the response of SIRT3 protein to stressors known to induce mitochondrial biogenesis (chronic muscle stimulation and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside administration). SIRT3 protein displayed hierarchical expression based on oxidative potential of muscle tissues (heart >> red >> white). In contrast to studies in some cell lines, metabolic stress (exercise) did not induce the translocation of SIRT3 from the nucleus to mitochondria, as SIRT3 was only present in subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria, not in the nucleus. Chronic stimulation increased muscle mitochondrial content and SIRT3 protein in SS (+33%) and IMF (+27%) mitochondria ($P < .05$). In contrast, chronic 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside administration, while inducing mitochondrial biogenesis, did not alter SS or IMF mitochondrial SIRT3 protein content. These studies have shown that, in muscle, SIRT3 (*a*) scales with muscle oxidative capacity and with enzymes regulating fatty acid oxidation, (*b*) in resting muscle is localized to SS and IMF mitochondria and not nuclei, (*c*) in contracting muscle is not acutely translocated to mitochondria, and (*d*) is upregulated with chronic stimulation in an adenosine monophosphate–activated protein kinase–independent manner.

Author contributions: BG, GH, YY, and AB all contributed to study design, data collection, data analysis, interpretation, and manuscript writing for the current study.

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1. Introduction

The sirtuins are a family of class III histone deacetylases that have been implicated in a wide range of cellular functions [1,2]. There are 7 sirtuins in mammals (SIRT1-7) whose functions are postulated to be largely determined by their intracellular localization. One of these seven, SIRT3, is generally believed to be a mitochondrial protein [3] and is thought to primarily function as a regulator of cellular metabolism [4], both acutely through mitochondrial activation [5] and chronically via regulation of gene expression [6]. Skeletal muscle is a metabolically active tissue that can undergo large changes in metabolic rate and plays an important role in maintenance of whole-body metabolic homeostasis. Although SIRT3 is known to be expressed in skeletal muscle [7,8], its intracellular location and metabolic stimuli regulating SIRT3 protein content in this metabolically important tissue are largely unknown.

The subcellular localization of SIRT3 has been studied in a variety of cell lines; and although there is some controversy, most of this work indicates that SIRT3 is localized to the mitochondria. For example, works in HeLa, COS7, NIH3T3, and U2OS cells have all shown that SIRT3 is targeted to the mitochondria [6,9,10], where it is truncated and activated [11]. However, SIRT3 may also be located within the nucleus until cellular stress induces the translocation of SIRT3 to the mitochondria [12,13]. Whether a similar process occurs in mammalian skeletal muscle remains to be determined.

There is considerable evidence linking SIRT3 to metabolic function and mitochondrial density in selected cell lines and in mammalian tissues. SIRT3 deacetylates and activates several enzymes involved in acetyl-CoA production and metabolism, specifically acetyl-CoA synthetase [14,15], isocitrate dehydrogenase, and glutamate dehydrogenase [5]. SIRT3 also deacetylates complex I of the electron transport chain and contributes to the regulation of cellular adenosine triphosphate levels in mice [16]. Presumably, there are additional SIRT3 mitochondrial targets, as upwards of 20% of mitochondrial protein are potentially regulated by lysine acetylation [17,18]. SIRT3 also contributes to the regulation of mitochondrial content and substrate utilization, as in brown adipose tissue SIRT3 increased peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) [6], an inducer of mitochondrial biogenesis. Conversely, in SIRT3 knockout mice, fatty acid oxidation is decreased in many tissues including skeletal muscle [19], possibly as a result of increased acetylation of mitochondrial proteins and/or a reduced mitochondrial content. Indeed, in diabetic [20] and obese [6] murine tissue, SIRT3 reductions were associated with reduced mitochondrial content. Conversely, in brown adipose tissue, several metabolic stressors (caloric restriction and cold exposure) increase mitochondrial content and also SIRT3 [6]. These results suggest that SIRT3 may contribute to the regulation of mitochondrial biogenesis and metabolic function, including fatty acid metabolism.

There is also evidence that chronic contractile activity (ie, exercise training) also induces concomitant increases in mitochondrial content and SIRT3. Treadmill running increases both SIRT3 protein content and markers of mitochondrial content in skeletal muscle of both rats and mice [7,8], whereas trained humans demonstrate greater SIRT3 protein and mitochondrial content than sedentary subjects [21]. At present, no study has examined the impact of either acute or chronic contractile activity on mitochondrial localization of SIRT3. Thus, it is not clear whether changes in whole muscle SIRT3 accompanying exercise training are reflected in mitochondrial pools. It is also not clear what intracellular signals contribute to the exercise-induced upregulation of SIRT3.

Therefore, in the present study, we have examined in rodent skeletal muscle (1) the association between SIRT3 protein content and muscle oxidative capacity and mitochondrial fatty acid oxidation and (2) the subcellular location of SIRT3 and (3) determined whether exercise induces the translocation of SIRT3 to the mitochondrion. We also examined (4) the response of SIRT3 protein to stressors known to induce mitochondrial biogenesis, namely, chronic muscle stimulation and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) administration. These studies have revealed a number of novel observations, primarily that, in mammalian muscle, (*a*) SIRT3 is only present in mitochondria and not the nucleus and (*b*) chronic contraction-induced upregulation of SIRT3 is localized to mitochondria and occurs in an adenosine monophosphate–activated protein kinase (AMPK)–independent manner.

2. Methods

Experiments were performed on Sprague-Dawley rats (female, 3-6 months, 250-300 g) that were bred on site and housed at 22.5°C on a 12-hour light (7:00 AM to 7:00 PM) and 12-hour dark (7:00 PM to 7:00 AM) cycle. Animals were randomly assigned to 1 of 4 experimental conditions: (1) resting comparisons in red, white, and heart muscles; (2) acute treadmill running and recovery; (3) chronic muscle contraction; or (4) chronic AICAR treatment. At the end of each experiment, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg); and the selected tissues were harvested and either processed immediately for mitochondrial extraction or flash frozen (liquid nitrogen) and stored at −80°C until analyzed. Immediately after the harvesting of tissues, the animals were killed with an overdose of sodium pentobarbital. All procedures for this study were approved by the animal care committee at the University of Guelph.

2.1. *Tissue harvesting from animals at rest*

The relationships between SIRT3 and both oxidative capacity of skeletal and heart muscle and mitochondrial function were examined in tissues harvested from animals at rest (n = 6). Following anesthetization, the selected tissues were harvested and either processed immediately for nuclear or mitochondrial extraction or flash frozen (liquid nitrogen) and stored at −80°C until analyzed.

2.2. *Acute exercise protocol*

To examine the acute effects of exercise on mitochondrial SIRT3 protein content, rats ran on a rodent treadmill for 2 hours at 15 m/min followed by an increase in speed of 5 m/min every 5 minutes until volitional cessation of exercise.

Before the exercise protocol, animals were familiarized to the treadmill at slow speeds (5-10 m/min) for 3 consecutive days followed by a full 24-hour rest before the start of the exercise bout. Muscles were harvested from animals without exercise (control), immediately following exercise to volitional cessation (t = 0 hour), and following 3 hours of recovery after exercise (t = 3 hours). At the appropriate time point, animals were anesthetized; and the red vastus muscles were removed (n = 6 for each condition).

2.3. Chronic muscle stimulation

To examine the changes in SIRT3 protein that accompany contraction-induced mitochondrial biogenesis in skeletal muscle, the red and white tibialis muscles from rats (n = 6) were chronically stimulated in 1 hind limb as described previously [22-24]. Briefly, stainless steel electrodes were sutured to muscles on either side of the peroneal nerve and passed subcutaneously from the thigh to the back of the neck where they were attached to an external electronic stimulator. Animals recovered from surgery for 7 days before stimulation (12 Hz, 50-millisecond duration) was initiated. Stimulation of the peroneal nerve was administered 24 hours a day for 7 days. Twenty-four hours following cessation of the stimulation, chronically contracting muscles (red tibialis anterior [RTA] and white tibialis anterior [WTA]) were removed. Muscles from the sham-operated contralateral limb were also removed and used as control.

2.4. AICAR treatment

Mitochondrial biogenesis was also induced via chronic AICAR administration as described previously [24]. Briefly, rats were injected subcutaneously with a bolus of AICAR (1 mg/g body weight) dissolved in saline solution (4.5% saline) for 5 days. Control animals received an equivalent volume of saline solution alone (subcutaneously). Twenty-four hours after the final AICAR or saline injection, the RTA and WTA muscles were removed.

2.5. Nuclear and mitochondrial extraction

Nuclear extraction was performed according to the manufacturer's specifications using a Pierce nuclear extraction kit (Pierce Biotechnology, Rockford, IL). Harvested muscles were immediately placed in 750 μL of phosphate-buffered saline (PBS), minced with scissors, and briefly homogenized (~3 seconds at 24 000 rpm). Cytosolic and nuclear extraction was performed using the cytosolic and nuclear extraction reagents supplemented with 1 mmol/L sodium orthovanadate; 1 mmol/L phenylmethylsulfonyl fluoride; and 10 μg/mL of pepstatin A, aprotinin, and leupeptin. Isolated nuclei were washed 15× in PBS and PBS supplemented with 0.1% Nonidet P-40 alternatively before nuclear extraction.

In experiments in which mitochondria were isolated, differential centrifugation was used to obtain both subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondrial fractions from the heart and the red and/or white portions of the tibialis anterior, as we have previously published [23]. Briefly, tissue was minced and homogenized; and SS was separated from IMF by centrifugation at 800*g* and pelleted from the supernatant at 9000*g*. Intermyofibrillar mitochondria were obtained from the initial pellet following a series of washes and spins [23]. Mitochondria were further purified using a Percoll gradient for Western blotting analysis [23,25].

2.6. Western blotting

Protein contents were determined on isolated nuclei, mitochondria, or a whole muscle lysate isolated from the tissue as described previously [26]. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis using a 7.5% (SIRT2, PGC-1α) or 12.5% (SIRT3, cytochrome oxidase IV [COXIV]) polyacrylamide gel and were subsequently transferred to a polyvinylidene difluoride membrane. For the detection of proteins, commercially available antibodies were used for SIRT2 (Cell Signaling Technologies, Boston, MA), PGC-1α (Calbiochem, San Diego, CA), SIRT3 (Cell Signaling Technologies), and COXIV (Molecular Probes, Carlsbad, CA). Equal amounts of protein were loaded to all wells for each gel, whereas equal loading for all Western blots was confirmed for all blots using ponceau staining. In addition, α-tubulin was used as a loading control for all whole muscle blots, whereas COXIV was used for all experiments with isolated mitochondria (data not shown). Proteins were visualized by chemiluminescence detection according to the manufacturer's instructions (Perkin Elmer Life Sciences, Boston, MA). Blots were quantified using the ChemiGenius 2 Bioimaging system (Syngene, Cambridge, UK).

2.7. Mitochondrial palmitate oxidation

Palmitate oxidation was measured in a sealed system, as previously described by our laboratory [27]. Briefly, mitochondria (100 μL) were added to a system containing a pregassed modified Krebs Ringer buffer; and the reaction was initiated by a 6:1 palmitate/bovine serum albumin complex (containing 10 μCi of [1-[^{14}C] palmitate, for a final palmitate concentration of 77 μmol/L). The reaction continued for 30 minutes at 37°C and was terminated with the addition of ice-cold 12 N perchloric acid.

2.8. ß-Hydroxyacyl-CoA dehydrogenase and citrate synthase activity

A small portion of frozen muscle or fresh mitochondria from the heart, RTA, and WTA muscle was used for determination of whole muscle and mitochondrial β-hydroxyacyl-CoA dehydrogenase (β-HAD) and citrate synthase (CS) activity. The β-HAD reaction was started by the addition of 100 μmol acetoacetyl-CoA (37°C) [28]. Total CS activity was started by the addition of 10 mmol/L oxaloacetate, and activity was measured spectrophotometrically at 37°C by measuring the disappearance of nicotinamide adenine dinucleotide [29].

2.9. Carnitine palmitoyltransferase I activity

The forward radioisotope assay was used for the determination of carnitine palmitoyltransferase 1 (CPT-1) activity as described by McGarry et al [27] with minor modifications as we have previously reported [30,31]. Briefly, the assay was

conducted at 37°C and initiated by the addition of 10 μL mitochondria (~0.75 μg/μL) to a standard reaction medium containing 75 μmol/L palmitoyl-CoA and L-[^{3}H] carnitine (Amersham Bioscience, Buckinghamshire, England). Palmitoyl-[^{3}H] carnitine was extracted in water-saturated butanol in a process involving 3 washes with distilled water and subsequent recentrifugation steps to separate the butanol phase, in which the radioactivity was counted.

2.10. Statistics

Analyses of variance were used to compare the effects of muscle type, acute exercise, chronic stimulation, AICAR treatment, and protein expression. Post hoc tests were conducted using the Bonferroni test. Correlations between protein expressions were determined using least squares linear regression. Significance was accepted at a $P < .05$.

3. Results

3.1. SIRT3 in muscle and in mitochondria, and relationship with CS activity and PGC-1α

As a first step, we examined SIRT3 in muscle and heart homogenates. SIRT3 protein content scaled with the oxidative capacities of these tissues (heart >> red > white) (Fig. 1A) and was highly correlated with total cellular CS activity (Fig. 1B, r^2 = 0.96) and PGC-1α protein content (Fig. 1C, r^2 = 0.99), an index of mitochondrial content and an inducer of mitochondrial biogenesis, respectively.

3.2. Subcellular localization of SIRT3

To determine the subcellular localization of SIRT3 in mammalian skeletal muscle, we examined SIRT3 in the nucleus and in isolated SS and IMF mitochondria. When nuclear and mitochondrial extracts were probed, SIRT2, a known nuclear protein, was located in nuclei but not in SS or IMF mitochondria. In contrast, SIRT3 was only present in SS and IMF mitochondria but not in the nucleus (Fig. 2).

The purity of nuclear extracts was confirmed via the absence of the highly abundant cytosolic protein lactate dehydrogenase in the purified nuclei (<2% contamination, data not shown), as we have done previously [26]. The purity of nuclear and mitochondrial extracts was confirmed via the presence of the nuclear proteins histone 2B and SIRT2 in nucleus, whereas they were absent in SS and IMF mitochondria (Fig. 2). For each experiment in the present study, we routinely confirmed the purity of our preparations.

3.3. SIRT3 and mitochondrial palmitate oxidation

SIRT3 has also recently been proposed as an important regulator of mitochondrial fatty acid oxidation in a variety of tissues [19]. Therefore, we have examined the relationship between mitochondrial SIRT3 protein, the activities of several mitochondrial enzymes involved in fatty acid oxidation, and mitochondrial palmitate oxidation in skeletal muscle.

SIRT3 protein content in isolated SS and IMF mitochondria scaled with the oxidative capacities of muscle (heart >> red >> white) (Fig. 3A). The CPT-1 (Fig. 3B) and β-HAD (Fig. 3C) activities followed a similar hierarchy (heart > red > white) and were positively correlated with SIRT3 in isolated SS (CPT-1, r = 0.96 and β-HAD, r = 0.95) and IMF (CPT-1, r = 0.98 and β-HAD, r = 0.96) mitochondria. In contrast, the Krebs cycle enzyme CS did not display a similar hierarchy (heart = red = white) and therefore was not correlated with SIRT3 in isolated mitochondria. Palmitate oxidation rates, while displaying a similar hierarchy to SIRT3 (Fig. 3D), did not correlate with SIRT3 either in isolated SS or IMF mitochondria.

3.4. Effects of acute exercise and recovery on mitochondrial SIRT3

Previous work had shown that SIRT3 was induced to translocate from the nucleus to the mitochondria in HeLa cells [13]. We therefore sought to observe changes in mitochondrial SIRT3 protein content when the metabolic demand in this tissue was increased by an acute bout of exercise. We observe that mitochondrial content of SIRT3 (SS

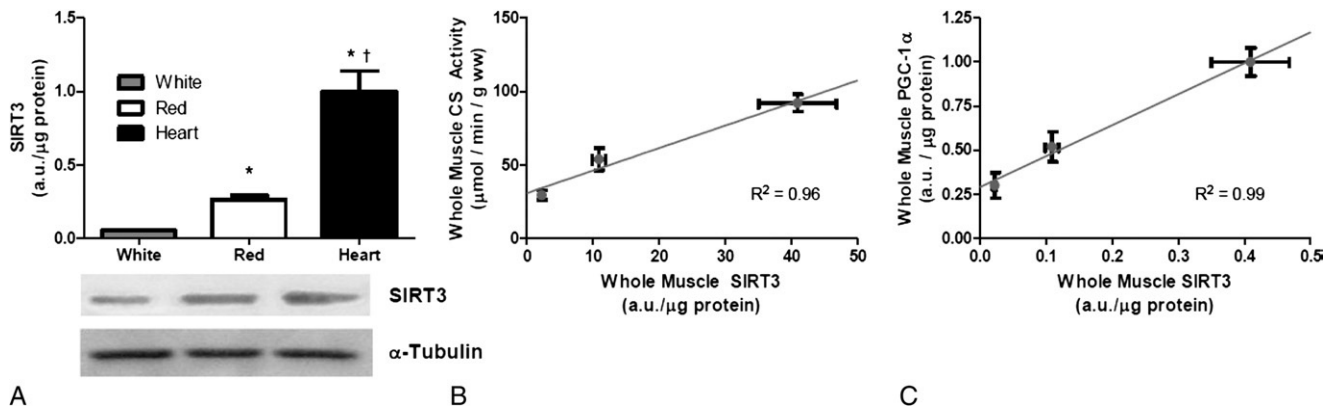

Fig. 1 – SIRT3 is correlated with oxidative capacity of muscle. Whole muscle SIRT3 (A) was positively correlated with whole muscle CS activity (B) and PGC-1α (C) protein in red, white, and heart muscle. *Significantly ($P < .05$) different from white muscle. †Significantly different from red muscle.

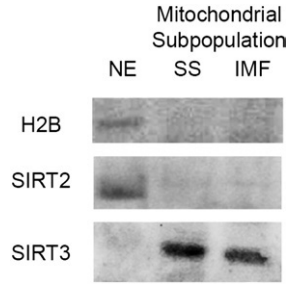

Fig. 2 – SIRT3 is localized to the mitochondria in skeletal muscle. Histone 2B protein was detected in nuclear extract (NE) but not in either mitochondrial subpopulation. SIRT2 protein was detected in NE but not in either mitochondrial subpopulation, whereas SIRT3 was absent from the NE but present in both the SS and IMF subpopulations of mitochondria.

and IMF) was not altered immediately following exercise, or following exercise and 3 hours of passive recovery (Fig. 4). This indicates that SIRT3 is not translocated to the mitochondria from the nucleus or from other unknown intracellular depots during this time frame.

3.5. *Response of SIRT3 protein to chronic contractile activity*

As our initial experiments indicated a positive relationship between SIRT3 and mitochondrial content (Fig. 1), we sought to examine whether this relationship held following an intervention that is known to induce mitochondrial proliferation, namely, 7 days of chronic muscle contraction [24]. As expected from our previous work [23,24], this stimulus increased whole muscle mitochondrial content as indicated by the upregulation of COXIV protein in red (+23%) and white (+71%) (data not shown) muscle. Concomitantly, chronic muscle stimulation also increased whole muscle PGC-1α (white, +11%; red, +7%; Fig. 5A) and SIRT3 protein contents (white, +25%; red, 32%; Fig. 5B). There were also increases in SIRT3 in SS mitochondria in red muscle (+33%) and in IMF mitochondria in white (+40%) and red (+25) muscle (Fig. 5C-D).

3.6. *Response of SIRT3 protein to chronic AICAR administration*

To examine if the observed increase in SIRT3 protein in red muscle following chronic contractile activity was AMPK mediated, we also examined the whole muscle and mitochondrial

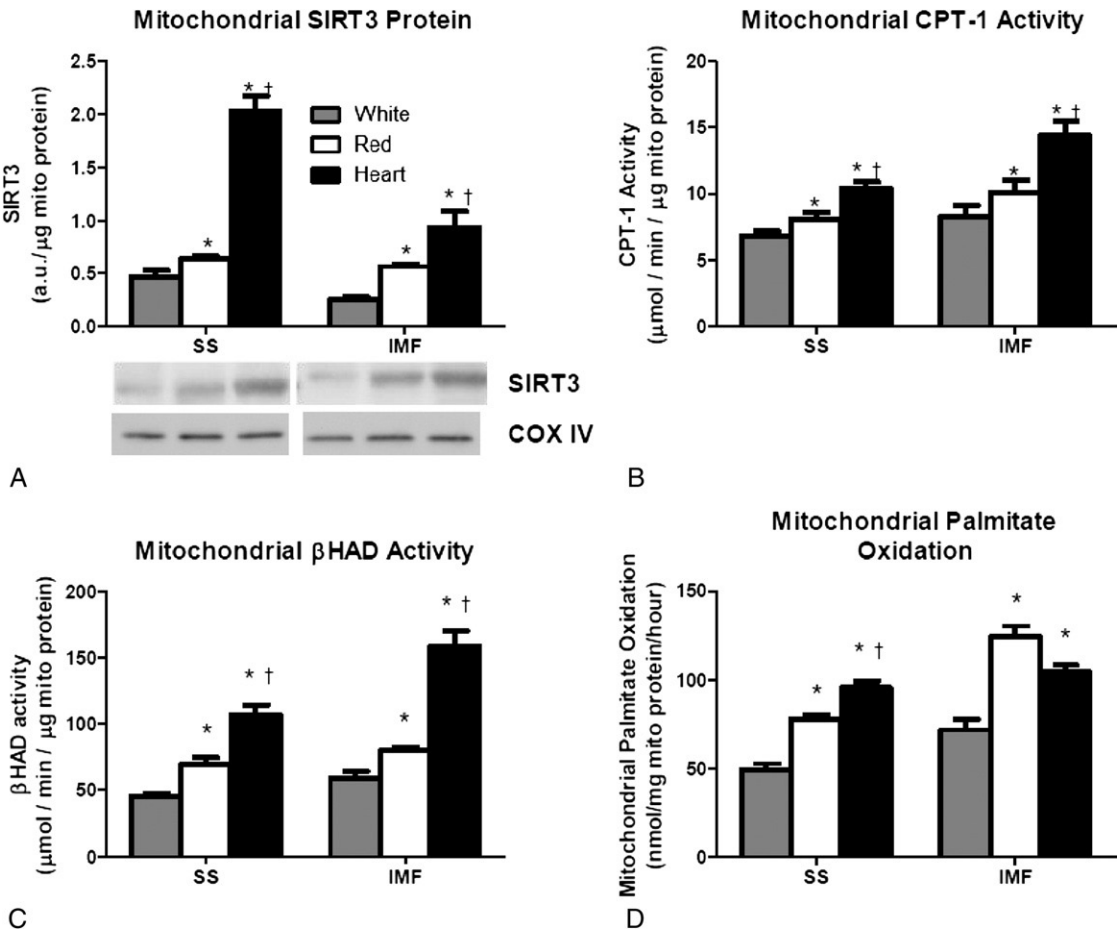

Fig. 3 – Mitochondrial SIRT3 is not correlated with mitochondrial fatty acid oxidation. Mitochondrial SIRT3 (A), CPT-1 activity (B), ß-HAD activity (C), and palmitate oxidation (D) in red, white, and heart muscle. *Significantly ($P < .05$) different from white muscle. †Significantly different from red muscle.

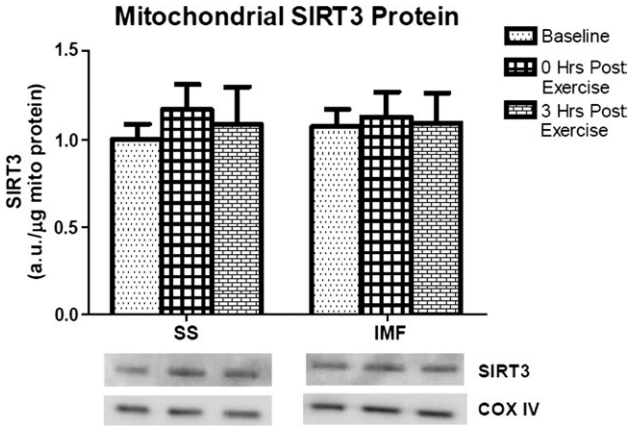

Fig. 4 – Mitochondrial SIRT3 is not increased in SS or IMF mitochondria following acute exercise. Immediately (0 hour) and 3 hours following exhaustive exercise in rats, the mitochondrial content of SIRT3 was not elevated in either SS or IMF mitochondria.

contents of SIRT3 following 5 days of AICAR injections (Fig. 6). As expected, this treatment increased whole muscle COXIV (white, +14%; red, 16%; data not shown) and PGC-1α (Fig. 6A) protein content in red (+16%) and white (+43%) muscle. However, AICAR did not increase either whole muscle (Fig. 6B) or mitochondrial SIRT3 protein contents in red or white (Fig. 6C-D) skeletal muscles.

4. Discussion

The current studies have revealed a number of novel observations: (1) Among metabolically heterogeneous muscle tissues, SIRT3 protein scales with PGC-1α protein and with the oxidative capacity (heart > red > white muscle) of muscle. (2) In contrast to some reports in HeLa and 293T cells [13], SIRT3 is not present in the nucleus of skeletal muscle. Moreover, (3) SIRT3 translocation to mitochondria does not occur in mammalian muscle in response to an acute metabolic stress (exercise). Finally, (4) consistent with previous reports, chronic muscle contraction (7 days) induces SIRT3 upregulation. Importantly, (5) an increase in SIRT3 protein is not required for an increase in PGC-1α or mitochondrial content, as PGC-1α is upregulated in the absence of changes in SIRT3 following AICAR treatment. This later results also suggests that SIRT3 protein content is regulated in an AMPK-independent manor.

4.1. *SIRT3 in skeletal muscle and heart*

SIRT3 protein was examined in the heart and in red and white muscle because these tissues are well known to have marked differences in mitochondrial content. Given that SIRT3 was only present in mitochondria, it is not surprising that the SIRT3 protein content scaled directly with the oxidative capacities of these tissues as well as with CS and PGC-1α (Fig. 1), markers of mitochondrial density and biogenesis, respectively [30,32]. Although studies in SIRT3 knockout mice have shown that fatty acid utilization is downregulated in a number of tissues, the differences in palmitate oxidation between red and white skeletal muscle and heart muscle observed in the current study were not associated with differences in SIRT3 protein in SS and IMF mitochondria (Fig. 3).

These results suggest that if SIRT3 contributes to the regulation of fatty acid oxidation in muscle, this contribution appears not to be determined by protein content per se. Interestingly, several targets of SIRT3 have already been identified including acetyl-CoA synthetase, isocitrate dehydrogenase, glutamate dehydrogenase, and complex I of the

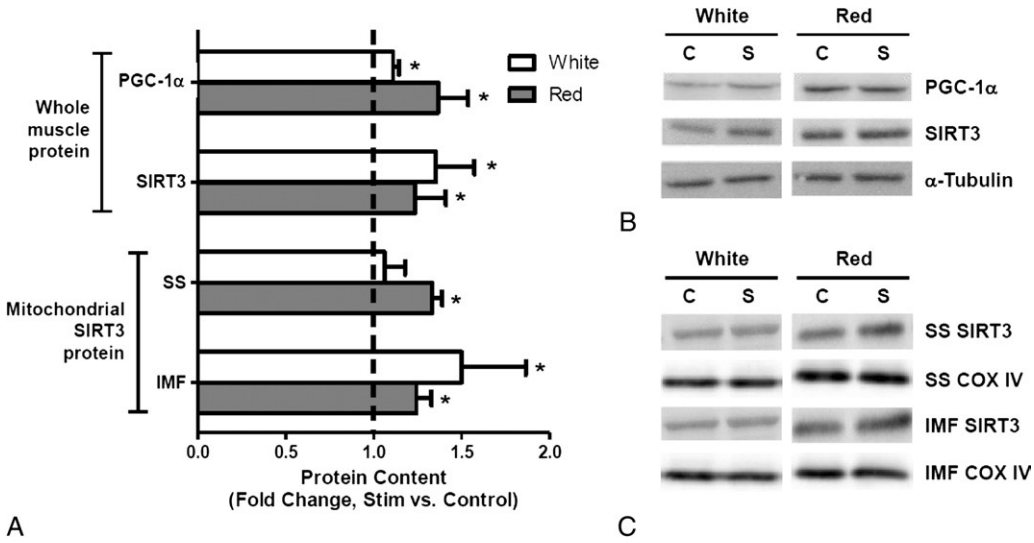

Fig. 5 – Mitochondrial SIRT3 protein content is increased by chronic muscle contraction. Whole muscle PGC-1α and SIRT3 protein contents were increased relative to control following 7 days of electrical stimulation in red and white skeletal muscle (A). This increase was reflected by a corresponding increase in SIRT3 protein in the IMF fraction of white muscle and both the SS and IMF mitochondrial fractions in red muscle (A). Representative blots are shown for all protein from both whole muscle (B) and mitochondrial (C) extracts. *Significantly ($P < .05$) different from control.

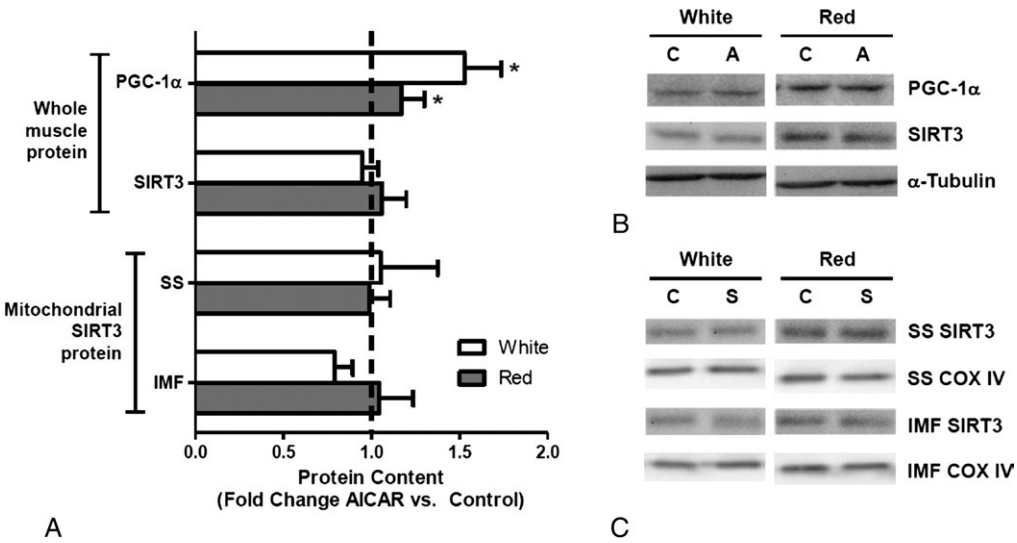

Fig. 6 – Mitochondrial SIRT3 is not altered by chronic AICAR administration. Whole muscle PGC-1*α* protein content was increased whereas SIRT3 protein content was not altered in either red or white muscles following 7 days of AICAR treatment (A). SIRT3 protein in isolated SS IMF mitochondria was also not altered by AICAR treatment in either white or red muscles (A). Representative blots are shown for all protein from both whole muscle (B) and mitochondrial (C) extracts. *Significantly ($P < .05$) different from control.

electron transport chain [5,14-16]. All of these enzymes play a role in the regulation of oxidative adenosine triphosphate production, and all are deacetylated and activated by SIRT3. Thus, despite the absence of a relationship between mitochondrial SIRT3 protein content and fatty acid oxidation in the present study, there appears to be the potential for posttranslational activation of SIRT3 activation status, rather than protein content, to play an acute regulatory role in oxidative metabolism in skeletal and heart muscle in vivo. In addition, all of the experiments in the current study (including isolated mitochondrial palmitate oxidation) were carried out under resting conditions; thus, it remains possible that, in muscle, there may be a relationship between the metabolically stimulated capacity for fatty acid oxidation and SIRT3 protein content (ie, the capacity to deacetylate mitochondrial protein).

4.2. *Subcellular location of SIRT3 in skeletal muscle*

Studies in HeLa and 293T cells have shown that SIRT3 is present in the nucleus and in mitochondria and that, upon providing a metabolic stress, SIRT3 is translocated to the mitochondria [13], raising the potential for this to occur during muscle contraction. However, in skeletal muscle, we found that SIRT3 protein was detected in both SS and IMF mitochondria, but was not present in the nucleus of the same muscle (Fig. 2). In addition, there was no increase in mitochondrial SIRT3 with the metabolic stimulus of a single exercise bout, a stressor known to increase fatty acid oxidation [33]. Thus, our results indicate that, in skeletal muscle, (1) there is no protein to be translocated from the nucleus to the mitochondria and (2) there is no SIRT3 translocation to the mitochondria from other unknown intracellular depots. Clearly, the mechanisms regulating the subcellular distribution of SIRT3 in HeLa and 293T cells [13] compared with skeletal muscle differ substantially. Our results in muscle are however in line with several other studies in cell lines (HeLa, COS7, NIH3T3, and U2OS) that have also shown that SIRT3 is only present in mitochondria [6,9-11]. Hence, any acute SIRT3-mediated effects on mitochondrial metabolism likely involve the acute activation of SIRT3 rather than an upregulation or translocation of SIRT3 protein.

The studies mentioned above in HeLa and 293T cells had shown that the full-length 44-kd SIRT3 protein was present in the nucleus of these cells, whereas the truncated 28-kd SIRT3 protein was present in mitochondria [13]. Interestingly, when human SIRT3 was expressed in *Escherichia coli*, only the truncated mitochondrial form demonstrated deacetylase activity [11]. We only probed for the truncated 28-kd form of SIRT3 in the nucleus and therefore cannot exclude the possibility that the full-length 44-kd SIRT3 protein is present in the nucleus of skeletal muscle. This however would seem unlikely because exercise did not induce the translocation of the truncated 28-kd SIRT3 protein (ie, derived from the 44-kd SIRT3 protein) to the mitochondria. Based on the present observations in muscle and others in selected cell lines [6,9-11], we conclude that SIRT3 protein is confined to mitochondria in skeletal muscle.

We have also observed an increase in both whole muscle SIRT3 protein and the content of SIRT3 in isolated mitochondria following chronic contractile activity. Although this evidence suggests that increased SIRT3 following contractile activity is targeted to mitochondria, because we have only demonstrated that SIRT3 is not present in nuclei from skeletal muscle, we cannot conclude with certainty that there were no increases in SIRT3 in other organelles other than nuclei and mitochondria.

4.3. *Upregulating the oxidative capacity of muscle*

In addition to a role in the acute activation of mitochondrial metabolism, SIRT3 has also been postulated as a regulator of

mitochondrial content via an increase in PGC-1α expression [6]. In the present study, SIRT3 protein was positively correlated with PGC-1α protein in resting muscle (Fig. 1). We also observed a marked increase in SIRT3 and PGC-1α proteins following a period of chronic muscle contraction (7 days; Fig. 5). These latter findings are consistent with previous reports from both human and murine muscle that exercise training increases SIRT3 and mitochondrial content [7,8,21]. Our observation that increases in whole muscle SIRT3 following chronic muscle contraction are reflected in mitochondria suggests that SIRT3 protein, synthesized in response to exercise training, is targeted to the mitochondria.

Concurrent changes in SIRT3 and PGC-1α (chronic muscle contraction experiment) cannot be interpreted to indicate that SIRT3 has a regulatory role in inducing PGC-1α expression. Furthermore, previous studies have not examined the mechanisms underlying the exercise-induced upregulation of SIRT3. The failure to observe an increase in muscle SIRT3 after chronically activating AMPK by AICAR would suggest strongly that the increased content of PGC-1α and the increase in mitochondrial biogenesis in AICAR-treated muscles (Fig. 6) are not SIRT3 dependent. Importantly, our observations also indicate that mitochondrial SIRT3 upregulation observed in chronically contracting muscle occurs in an AMPK-independent manner. The mechanisms regulating SIRT3 protein expression in skeletal muscle represent an interesting area for future study.

5. Conclusion

We have found that, in mammalian skeletal muscle, SIRT3 is not present in the nucleus and therefore is not induced to translocate to mitochondria by a metabolic stimulus as has been suggested elsewhere in various cell lines [12,13]. SIRT3 in muscle scales with the oxidative capacities of muscle tissues (heart >> red > white muscle), but does not correlate with rates of fatty acid oxidation in isolated mitochondria. In addition, we provide novel evidence that chronic muscle contraction upregulates SIRT3 protein in an AMPK-independent manner. Finally, our results show that increases in PGC-1α and oxidative capacity of skeletal muscle can occur without concomitant increases in SIRT3.

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Conflict of Interest

There are no conflicts of interest associated with the current work.

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